DISTRIBUTION OF HLA CLASS II GENES IN A CAUCASIAN POPULATION AS DETERMINED BY PCR AND REVERSED-DOT-BLOT TYPING

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INTRODUCTION

The human leucocyte antigens (HLA) loci are extremely polymorphic and are, therefore, of interest for identity testing and paternity determinations. The advent of the polymerase chain reaction (PCR) has made it possible to determine in more detail the different alleles at the nucleotide level. Analysis of the PCR products can be performed either by sequence analysis or by hybridization with sequence-specific-oligonucleotides (SSO's). In order to identify the existing alleles, a large panel of SSO's has to be used which makes hybridisation with individual SSO's a tremendous task. The development of the reversed-dot-blot technique, however, has made it possible to identify each allele in a minimum of time by hybridization of the amplified product to a membrane on which all the different SSO's bound are bound (Saiki et al., 1989).
We have developed a reversed-dot-blot assay (Buyse et al., 1993) for all the polymorphic HLA Class II genes (DQA1, DQB1, DPB1 and DRB1) and have applied this method for the determination of the allele and genotype frequency in the Belgian population.

MATERIALS AND METHODS

A panel of 200 DNA samples was obtained from random blood donors while additional DNA samples were from blood samples received for paternity determinations. All individuals were Caucasians of Belgian descent.
The polymorphic second exon of the HLA Class II genes was amplified by PCR and analyzed by hybridisation to sequence-specific-oligonucleotides bound to membranes as described by Buyse et al. (1993). Detection of the positive spots was done either by chemiluminescence (AMPPD, Tropix, Bedford, MA) or by a colorimetric assay (Inno-Lipa, Innogenetics, Gent, Belgium).

RESULTS AND DISCUSSION

In this study, we determined the allele frequency for all HLA class II loci in a random sample of the Belgian population. The method applied, PCR in combination with reversed-dot-blot hybridization, is able to identify 89.2% of the defined alleles from the latest HLA workshop (1992). Only for 5.4% of the total number of possible genotypes, it is not always possible to define exactly the genotype because of the complex pattern generated by the different SSO's which could give identical patterns for different genotypes.
The number of alleles that were observed in the population sample ranged between 8 for DQA1 and 28 for DRB1 (Table 1). The lowest heterozygosity was seen for DPB1 (79.7%) although it had the second largest number of alleles among the HLA class II loci. The reason can be found in the presence of one dominant allele (0401: 41.3%) in the population while for the other loci the maximum allele frequency is not more than 27.3% (DQA1: 0501) with the majority of the alleles below 20% (Table 2).
The observed genotype distributions were tested for Hardy-Weinberg (HW) equilibrium by two different methods: (1) excess of homozygosity was determined by comparing the number of observed and expected homozygotes (Wahlund, 1928) and (2) an exact test of HW proportion for multiple alleles based on Monte Carlo methods (Guo and Thompson, 1992). Both tests could not show any departure from Hardy-Weinberg equilibrium in the genotype distributions for all the HLA class II loci in the Belgian population sample.

Table 1: Summary of the characteristics for the different HLA Class II loci determined in this study.

Locus	Observed no. of alleles[1]	Heterozygosity (%)[2]	Power of discrimination	Power of paternity exclusion
DQA1	8 (218)	82.0 ± 0.7	0.942	0.639
DQB1	13 (205)	87.1 ± 0.6	0.970	0.739
DPB1	19 (202)	77.8 ± 1.6	0.930	0.604
DRB1	28 (251)	90.1 ± 0.4	0.981	0.796

[1] In parenthesis are the number of individuals
[2] Expected heterozygosity and the standard error calculated according to Nei (1978)

A comparison with other Caucasian population frequencies for the DQA1 locus (Helmuth et al., 1990; Sajantila et al., 1991; Kloosterman et al., 1993; Allen et al., 1993) showed that there was no significant difference for the different statistics (heterozygosity, power of exclusion and power of discrimination) with our population and only slight differences are seen between individual alleles. However, the DQA1 system developed by us, discriminates between three alleles which are typed as one allele in the AmpliType HLA-DQα Kit (Perkin-Elmer/Cetus) which was used for determination of the population frequencies in the other populations. As a consequence, with the commercial kit, a false inclusion could be obtained if the second allele (0401, 0501 or 0601) in the genotype would be different between the reference sample (e.g. 0101/0401) and the evidence sample (e.g. 0101/0501).
The reversed dot blot method, as applied in this study, has been extensively used for HLA association studies (Insulin Dependent Diabetes Melitis, rheumatoid arthritis..), corneatransplants and paternity determinations. Currently, these methods are evaluated for application in forensic cases. The most polymorphic locus (DRB1) can be used without any problem of interpretation for forensic samples which are known to originate from one person. In sexual assault cases, however, mixtures of vaginal cells from the victim and semen from the assailant can not always be completely separated by differential lysis and therefore mixed DNA samples are obtained. Due to the complexity of the typing - a pattern which is a combination of several SSO's - problems of interpretation could be foreseen. Preliminary experiments with the DPB1 and DRB1 system indicate that in those samples the DRB1 system is favourable over the DPB1 system. Moreover, a combination of the two systems could increase the informativity in those cases where both victim and suspect do have the same genotype for one system.

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Table 2: Allele frequency distribution of the different HLA class II loci in the Belgian population

DQA1	Freq.	DQB1	Freq.	DPB1	Freq.	DRB1	Freq.
0101	0.154	0201	0.200	0101	0.084	0101	0.092
0102	0.204	0302	0.083	0201	0.155	0102	0.014
0103	0.092	0501	0.132	0202	0.003	0103	0.012
0201	0.131	0502	0.027	0301	0.084	0301	0.119
0301	0.128	0604	0.049	0401	0.413	0400	0.036
0401	0.016	0605	0.017	0402	0.109	0401	0.068
0501	0.273	1301	0.198	0501	0.027	0402	0.008
0601	0.002	1303	0.051	0601	0.003	0403	0.002
		1402	0.012	0901	0.005	0407	0.002
		1503	0.019	1001	0.030	0700	0.137
		1601	0.012	1101	0.015	0801	0.012
		1602	0.119	1301	0.017	0802	0.004
		1603	0.081	1401	0.005	0803	0.006
				1501	0.007	0804	0.002
				1601	0.005	0901	0.006
				1701	0.012	1001	0.004
				1901	0.020	1101	0.113
				2001	0.003	1102	0.008
				2301	0.003	1103	0.004
						1201	0.018
						1202	0.002
						1301	0.137
						1303	0.006
						1305	0.002
						1401	0.020
						1404	0.002
						1500	0.141
						1601	0.023

DQA1*0101 contains the alleles DQA1*0101 and DQA1*0104. Alleles DQA1*0301 and DQA1*0302 can not be discriminated and are typed as DQA1*0301. DRB1*1500 contain the alleles DRB1*1501 and DRB1*1503. Allele DRB1*1401 includes DRB1*1407. Allele DRB1*1101 includes the alleles DRB1*1104 and DRB1*1105. Allele DRB1*1305 includes allele DRB1*1306. Allele DRB1*0400 contains the alleles DRB1*0404, DRB1*0405, DRB1*0408 and DRB1*0410. Alleles DRB1*0701 and DRB1*0702 can not be discriminated and are typed as DRB1*0700. DRB1*1600 contain the alleles DRB1*1601 and DRB1*1602.