HLA-DQA1 and HLA-DPB1 gene polymorphisms in the Japanese populations

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INTRODUCTION

Highly polymorphic HLA systems have greatly contributed to paternity testing and personal identification in forensic work. Recently HLA alleles in the class II region has been identified easily at the nucleotide level using PCR method. The PCR procedure involves the PCR-SSO (sequence specific oligonucleotide) method and the modified PCR-RFLP method (1). Those two methods permit precise and direct analysis of allelic variations by using less than 1 ng of DNA. The modified PCR-RFLP method provides a simpler and more rapid technique for accurate definition of the HLA-class II alleles. In this study, HLA-DQA1 and -DPB1 alleles were defined in 150 unrelated healthy Japanese individuals using the modified PCR-RFLP method. Exclusion probability (EP) was calculated from the allele frequencies of DQA1 and DPB1 obtained in this population.

MATERIAL AND METHOD

DNA samples : Genomic DNAs from 150 healthy Japanese volunteers' whole blood were isolated by phenol extraction of sodium dodecyl sulfate (SDS)-lysed and proteinase K-treated cells.

PCR amplification: Genomic DNA (1 μg) was amplified by the PCR procedure with 2.5 units of the Taq DNA polymerase (Perkin Elmer Cetus Inc.). The reaction mixture was subjected to 30 cycles of 1 min at 94 °C, 1 min at 62 °C, and 2 min at 72 °C by automated PCR thermal sequencer (Iwaki Glass Inc.). The second exon of the DQA1 gene was amplified by using PCR primers, GH26 and GH27 at 1 μM and the second exon of the DPB1 gene was amplified by using the PCR primers, DPB101 and DPB201 at 1 μM (Table 1).

Digestion with restriction endonucleases and acrylamide gel electrophoresis: Restriction enzymes of ApaLI, HphI, BsaJI, FokI, MboII and MnlI were used for digestion of the amplified DQA1 gene and Bsp1286I, FokI, DdeI, BsaJI, Cfr13I, RsaI, EcoNI and AvaII were used for digestion of the amplified DPB1 gene. Samples of the restriction enzyme-cleaved amplified DNAs were subjected to electrophoresis in 12% polyacrylamide gels in a horizontal mini-gel apparatus (Mupid, Cosmo Bio Co.). Cleavage or no cleavage of amplified fragments was detected by staining with ethidium bromide.

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CONCLUSION

For the DQA1 alleles, 36 combinations including 8 homozygotes and 28 heterozygotes can be unequivocally determined by the modified PCR-RFLP method (1). DQA1 gene frequencies are shown in Table 2. Cleavage patterns of DQA1 genes are shown in Fig.1. DQA1*0301 was the most frequent (37.8%) allele. EP of the DQA1 genotype was 0.51. For the DPB1 alleles, 190 combinations including 19 homozygotes and 171 heterozygotes could be determined. DPB1 gene frequencies are shown in Table 3. Cleavage patterns of DPB1 gnes are shown in Fig.2. EP of the DPB1 genotype was 0.57. The HLA-DQA1 and -DPB1 polymorphisms are useful for paternity test and personal identification.

REFERENCE

1) Ota M, Seki T, Nomura N, Sugimura K, Mizuki N, Fukushima H, Tsuji K, Inoko H. (1991) Modified PCR-RFLP method for HLA-DPB1 and DQA1 genotyping. Tissue Antigens 38:60-71

Table 1. PCR primers for amplification of DQA1 and DPB1 genes

Gene	Primers	Sequences (5' to 3')
DQA1	GH26	GTGCTGCAGGTGTAAACTTGTACCAG
	GH27	CACGGATCCGGTAGCAGCGGTAGAGTTG
DPB1	DPB101	GTGAAGCTTTCCCCGCAGAGAATTAC
	DPB201	CACCTGCAGTCACTCACCTCGGCGCTG

Table 2. DQA1 gene frequency

Type	n	P.F.	G.F.
DQA1*0101	45	0.300	0.163
DQA1*0102	48	0.320	0.175
DQA1*0103	56	0.373	0.208
DQA1*0201	1	0.007	0.004
DQA1*0301	92	0.613	0.378
DQA1*0401	1	0.007	0.004
DQA1*0501	22	0.147	0.076
DQA1*0601	4	0.027	0.014

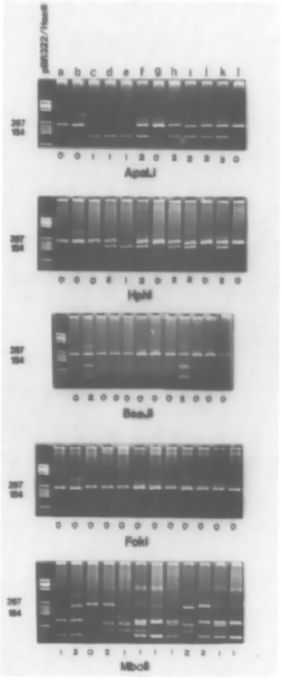
P.F.: Phenotype Frequency
G.F.: Gene Frequency
Total number examined=150

Table 3. DPB1 gene frequency

Type	n	P.F.	G.F.
DPB1*0101	0	0.000	0.000
DPB1*0201	48	0.320	0.175
DPB1*0202	14	0.093	0.048
DPB1*0301	10	0.067	0.034
DPB1*0401	14	0.093	0.048
DPB1*0402	35	0.233	0.124
DPB1*0501	83	0.553	0.331
DPB1*0601	1	0.007	0.004
DPB1*0801	0	0.000	0.000
DPB1*0901	29	0.193	0.102
DPB1*1001	0	0.000	0.000
DPB1*1101	0	0.000	0.000
DPB1*1301	4	0.027	0.014
DPB1*1401	3	0.020	0.010
DPB1*1501	0	0.000	0.000
DPB1*1601	2	0.013	0.007
DPB1*1701	0	0.000	0.000
DPB1*1801	0	0.000	0.000
DPB1*1901	0	0.000	0.000

P.F.: Phenotype Frequency
G.F.: Gene Frequency
Total number examined=150

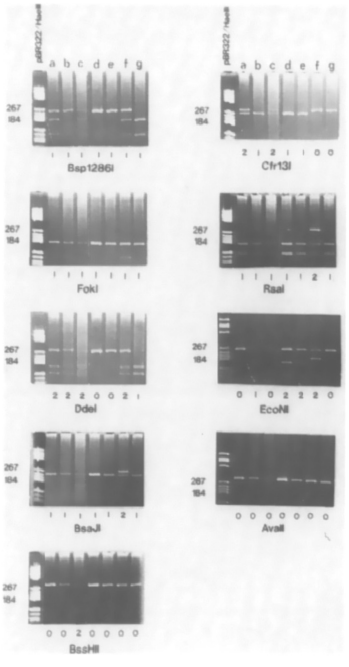
Fig.1 Cleavage patterns of polymorphic restriction fragments in the PCR-amplified DQA1 genes obtained from DNAs of 12 normal individuals after digestion with 5 restriction enzymes



DQA1 genotype

a:DQA1*0301/0301
b:DQA1*0301/0501
c:DQA1*0101/0102
d:DQA1*0102/0103
e:DQA1*0103/0103
f:DQA1*0103/0301
g:DQA1*0301/0301
h:DQA1*0103/0301
i:DQA1*0103/0501
j:DQA1*0102/0301
k:DQA1*0103/0301
l:DQA1*0301/0301

Fig.2 Cleavage patterns of polymorphic restriction fragments in the PCR-amplified DPB1 genes obtained from DNAs of 7 normal individuals after digestion with 9 restriction enzymes



DPB1 genotype

a:DPB1*0402/0501
b:DPB1*0201/0202
c:DPB1*0401/0501
d:DPB1*0201/0402
e:DPB1*0201/0402
f:DPB1*0501/0901
g:DPB1*0501/0501