

Comparison of two methods for HLA-DQA1 typing

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INTRODUCTION

PCR-based typing for the HLA-DQA1 gene is widely used in cases of disputed paternity and for forensic purposes. With the introduction of the AmpliType™ HLA DQ α Forensic DNA Amplification and Typing Kit (1,2) this method of HLA-DQA1 typing has been adapted by most forensic laboratories. This analysis relies on the hybridisation of the PCR product to fixed oligonucleotide probes by the reverse dot-blot principle. As is the case for any typing method which relies on hybridisation the outcome of the reverse dot-blot method is sensitive to small changes in the temperatures and salt concentrations of the hybridisation and stringency washing buffers. A second typing method, which relies on an entirely different principle of analysis, may therefore be advantageous and may help to increase the overall level of certainty about the typing result. Here, we present a modification of the PCR-RFLP method published by Ota and coworkers (3) which in combination with allele-specific amplification (ASA) gives even more information about the HLA-DQA1 alleles than the AmpliType kit. The two methods of HLA-DQA1 typing were compared and found equally suited for paternity testing.

MATERIALS AND METHODS

Genomic DNA from 109 healthy Danes was purified as described (4). For the reverse dot-blot method, 50 ng of genomic DNA was amplified and analyzed according to the manufacturers instructions (2). For the PCR-RFLP method, 50 ng of DNA was amplified with the primers GH26 and GH27 (3) in a total volume of 100 μ l. The amplification conditions were: 2 min at 94°C, 32 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and finally 5 min at 72°C. Seven μ l of the amplification mixture was digested with each of the enzymes ApaI, BsaI, FokI, HphI, and MboII and analyzed on a 4% MetaPhor™ (FMC) agarose gel alongside 7 μ l of undigested DNA (fig 1). The HLA-DQA1 phenotypes were assigned as described (3). In order to discern between the HLA-DQA1*0101 and -0102 subtypes, two allele-specific amplification reactions were set up: One which amplify the HLA-DQA1*0101 allele (with the primers DQA1.1-5' and DQA1-3'), and another which amplify the HLA-DQA1*0102 allele (with the primers DQA1.2-5' and DQA1-3'). The DNA was amplified in a volume of 100 μ l using a hot-start technique. The amplification conditions were as for GH26 and GH27 except for the initial denaturation step being extended from 2 to 4 min. The primers DQA5'-int3 and DQA3'-int3, which amplify intron 3 of the HLA-DQA1 gene, was included as internal controls of the reaction. The amplification product was analyzed on a 4% NuSieve® 3:1 (FMC) gel (fig 2).

RESULTS AND DISCUSSION

Complete concordance of the typing results was found for all 109 persons tested. Every third person was typed a second time by both methods and again complete concordance was observed, both between the first and second typing by each of the two methods and when comparing the typing results of the two methods to one another. The PCR-RFLP/ASA method was found to be as reliable and easy to handle as the AmpliType kit. As the AmpliType method relies on the principle of hybridisation for the detection of the HLA-DQA1 phenotypes and the PCR-RFLP/ASA method on restriction analysis of the PCR product and selective amplification of a subset of HLA-DQA1 alleles, a higher level of certainty about the typing result is expected if the two methods are used in conjunction compared to using only one of the two typing techniques. The PCR-RFLP/ASA method is well suited for paternity testing but problems may arise if it is used for forensic investigations of mixed samples. In such instances, more than two HLA-DQA1 alleles may be present in the amplification product and the restriction pattern might be too complex for reliable deduction of the HLA-DQA1 types.

REFERENCES

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2. The AmpliType™ package insert and AmpliType™ User Guide, version 2, Cetus Corporation.
3. Ota M, Seki T, Nomura N, Sugimura K, Mizuki N, Fukushima H, Tsuji K, Inoko H. Modified PCR-RFLP method for HLA-DPB1 and -DQA1 genotyping. *Tissue Antigens* (1991) **38**, 60-71.
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Table 1. PCR primers for allele-specific amplification

Primers	Sequences
DQA1.1-5'	5'-ATGAATTTGATGGAGATGAGG-3'
DQA1.2-5'	5'-ATGAATTTGATGGAGATGAGC-3'
DQA1-3'	5'-CTGCGGGTCAAAACCTCC-3'
DQA5'-int3	5'-AGCCTCTTCTGAAACACTGGG-3'
DQA3'-int3	5'-TCTCTGTGAGCTCTGACATAGG-3'

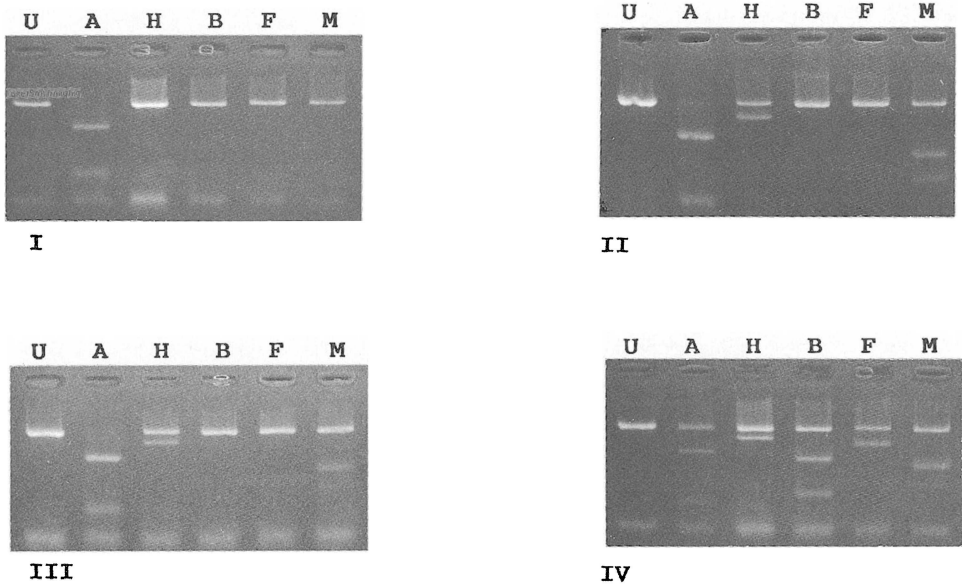


Figure 1. Restriction patterns of the PCR-amplified HLA-DQA1 exon 2 region from persons with the phenotypes HLA-DQA1*0101,0102 (I), HLA-DQA1*0101,0103 (II), HLA-DQA1*0102,0103 (III) and, HLA-DQA1*0103,0401 (IV) when digested with the 5 enzymes ApaLI (A), HphI (H), BsaJI (B), FokI (F), and MboII (M); U denotes undigested DNA. Digestion with these 5 enzymes allowed for the discrimination of all phenotype combinations of the HLA-DQA1 alleles (0101/0102), 0103, 0201, 0301, 0401, 0501, and 0601. Discrimination between the 0101 and 0102 alleles was achieved by the ASA technique.

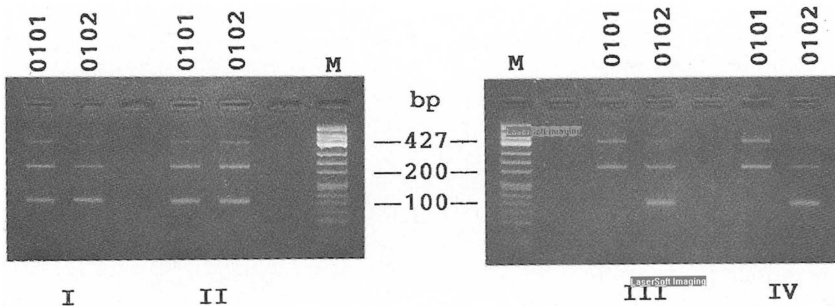


Figure 2. Allele-specific amplification bands from persons with the phenotypes HLA-DQA1*0101,0102 (I), HLA-DQA1*0101,0103 (II), HLA-DQA1*0102,0103 (III), and HLA-DQA1*0103,0401 (IV). The two bands of 441 and 230 bp are internal controls of the reaction which derive from the intron 3 region of the HLA-DQA1 gene and HLA-DQA2 pseudogene, respectively. The 0101/0102 allele sub-type was determined from the appearance of an 101 bp exon 2-specific band in the 0101 and/or 0102 reactions. The 0103 allele was also amplified in the 0102 reaction, but as the existence of this allele is known from the PCR-RFLP data this did not influence the interpretation of the results. The molecular weight marker (M) was ϕ X174/HinfI.