DETECTION OF THE ABO, GC, ACP and HLA-DQA1 POLYMORPHISMS AT THE DNA LEVEL USING PCR AND SSCP

J. Dissing and D. Christiansen

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark.

INTRODUCTION

The formal genetics of the classical polymorphisms such as the ABO marker are well established via extensive World wide population studies, and gene frequencies are known for vast numbers of populations and subpopulation. The use of these polymorphisms as evidence in paternity testing and criminal case work has been accepted for decades by courts throughout the World. In step with the attainment of sequence information on the genes encoding classical markers it has become possible to design methods for genotyping based on DNA technology. We have developed methods for the detection of three of these markers, ABO, GC and ACP1, as well as for HLA-DQA1. The methods are based on PCR amplification of gene segments containing allele specific mutation sites followed by electrophoretic characterization of the separated DNA strands at non denaturing condition allowing the strands to attain sequence specific conformations (single strand conformation polymorphism, SSCP (Orita et al. 1989)).

MATERIALS AND METHODS

PCR amplification:
PCR reactions (50μl) were performed in 0.01M Tris pH 8.3, 50mM KCl, 1.5 - 2.5mM $MgCl_2$, 0.01% gelatine containing 2 U of TAQ polymerase (Perkin-Elmer Cetus), 0.2mM of each dNTP and 20 pmol of each primer using a Perkin-Elmer Cetus 480 thermocycler. A 404nt segment at the ACP1 locus was amplified using primers 10 and 16 as previously described (Lazaruk et al. 1993); a 186nt segment at the GC locus was amplified using primers flanking exon 11 (Witke et al. 1993, and Dissing et al. unpubl. results); a 198nt segment at the ABO locus was amplified using primers flanking positions 258 and 293 (Yamamoto and Hakomori, 1990; Yamamoto et al. 1990, and Dissing et al. unpubl. results); 239/242nt segments at the HLA DQα locus was amplified according to Saiki et al. (1989).

SSCP analysis:
SSCP analysis was performed using precast 20% polyacrylamide gels (PhastGels) and a semi automatic electrophoresis and staining system (PhastSystem, Pharmacia). Running conditions for ACP1/GC, ABO and DQα were 600Vh at 13°C, 400Vh at 14°C and 450Vh at 15°C, respectively. Silver staining was performed according to the recommendations of the manufacturer.

RESULTS AND DISCUSSION

GC and ACP1:
Codon 416 and 420 in exon 11 of the GC locus contain two mutational sites, and three combinations of base substitutions at these codons distinguish the GC*1F, *1S and *2 alleles (Reynolds and Sensabaugh, 1990; Witke et al. 1993). Primers flanking this region were used

for amplification of the GC locus. At the ACP1 locus a segment spanning two mutational sites in exon 3F and 3S, respectively, at which base differences distinguish the ACP1* A, *B and *C alleles was amplified (Lazaruk et al. 1993). Initially GC and ACP1 were analyzed separately and primer positions, PCR and electrophoretic conditions were optimized to obtain unequivocal genotypic SSCP patterns (results not shown). However, it was also possible to define conditions allowing multiplexing and SSCP analysis of these two markers in the same lane (Fig. 1).

ABO:

The ABO*O allele is characterized by a single-base deletion (nt 258) as detected in cDNA cloned from the corresponding glycosyltranferase gene (Yamamoto and Hakomori, 1990; Yamamoto et al. 1990). By analysis of a 250 nt segment spanning this site using denaturing gradient gel electrophoresis Johnson and Hopkinson (1992) detected four different O alleles, two B alleles and one A allele, which were assumed to be the result of combinations of different point mutations flanking nt 258. Using PCR and SSCP of a 198 nt segment spanning the same region we detected three O alleles, one A and one B allele in blood samples from 100 unrelated Danes (Fig. 2). The allele frequencies observed were: ABO*O1, 42%; *A, 27%; *O2, 19%; *B, 10%; *O3, 2%. Combined with the ability to detect heterozygosity with respect to the ABO*O alleles this greatly increases the informative value of the ABO polymorphism, thus the power of discrimination is increased from 62 to 86% and the theoretical rate of exclusion of non-fathers from 17 to 46%.

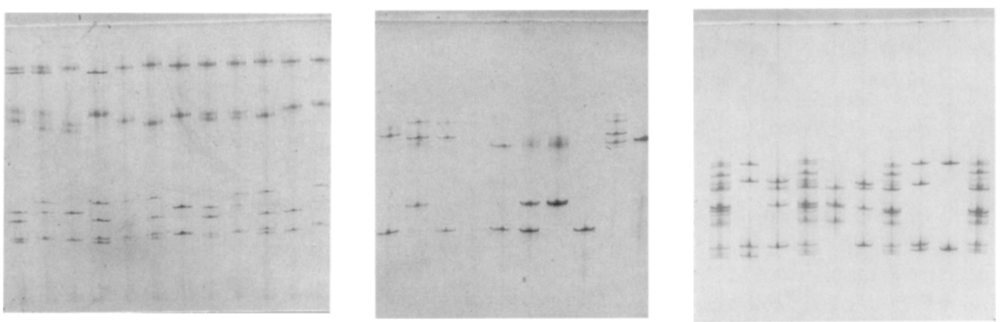

Fig. 1 (left). SSCP patterns of GC and ACP1 phenotypes using multiplexing and electrophoretic separation in 20% polyacrylamide gel for 600Vh at 13°C. GC (bottom) and ACP1 (top) phenotypes are from left to right (lanes 1-12): 1S,2/B,C; 1F,2/A,C; 1S/A,B; 1F,1S/C; 1F/B; 1F,1S/A; 2/B; 1S,2/A,B; 1F,2/A,B; 1F,1S/A; 1F/B.

Fig. 2 (middle). SSCP patterns of ABO phenotypes after electrophoretic separation in 20% polyacrylamide gel for 400Vh at 14°C. The phenotypes are from left to right (lanes 1-10): O1,O3; B,O2; B,O1; (the pattern in lane 4 does not reproduce well on the photograph); A,O1; O1,O2; O2; O1; A,B; A.

Fig. 3 (right). SSCP patterns of HLA-DQA1 phenotypes after electrophoretic separation in 20% polyacrylamide gel for 450Vh at 15°C. The patterns are from left to right (lanes 1-10): allelic ladder; 0101,0301; 0301,0501; allelic ladder; 0201,0501; 0301,0501; allelic ladder; 0102,0301; 0102; allelic ladder.

DQA1:

The HLA-DQA1 polymorphism is commonly detected by PCR and a reversed dot blot format as developed by Perkin-Elmer Cetus (Saiki et al. 1989). This method allows the detection of 6 alleles. Using a combination of PCR-RFLPand allele-specific amplification Cowland et al. (1995) were able to discriminate between 8 alleles, however, this procedure is rather laborious. Barros et al. (1994) have previously shown that DQA1 alleles are detectable using PCR and SSCP. We investigated this approach and conditions were determined for the unequivocal detection of 8 alleles (HLA-DQA1*0101, *0102, *0103, *0201, *0301, *0401, *0501, *0601) in a single electrophoretic run (Fig. 3).

CONCLUSIONS

Genotyping of classical genetic markers by PCR and SSCP is simple and fast and much less labour-intensive than classical phenotyping. The technique allows multiplexing. Except for the components of the PCR reaction the only other reagents needed are simple chemicals for silver staining. With the Pharmacia PhastSystem electrophoretic separation and staining can be accomplished in 2-3 hours. The cumulative power of discrimination of the GC/ACP1, ABO and DQA1 polymorphisms as described above is 99.93% and the theoretical chance of exclusion of non-fathers in paternity testing is 90%. Thus genotyping of "classical" structural loci by PCR and SSCP offers a valuable supplement to mutation prone VNTR and STR loci.

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