TECHNICAL NOTE

Jan Kreike,[1] *Ph.D. and Angelika Lehner,*[1] *Ph.D.*

Rapid Screening of Multiple Forensic Stains by SSCP Analysis of HLA-DQα Amplification Products

REFERENCE: Kreike J, Lehner A. Rapid screening of multiple forensic stains by SSCP analysis of HLA-DQα amplification products. J Forensic Sci 1997;42(4):715–717.

ABSTRACT: Single strand conformation polymorphism (SSCP) analysis was performed with HLA-DQα amplification products. The various HLA-DQα genotypes reveal different SSCP patterns. These are reproducible in DNA preparations from unrelated persons with identical HLA-DQα genotype. However, in one of the more frequent HLA-DQα genotypes (1.1/4) two variant SSCP patterns were observed. SSCP analysis allows rapid low-cost screening of many specimens and is an alternative discrimination test of stains beside reverse dot blot hybridization.

KEYWORDS: forensic science, DNA typing, single strand conformation polymorphism, HLA-DQA1, heteroduplex analysis, polymerase chain reaction

In many forensic cases, a large number of biological stains has to be analyzed, of which only a small number may be informative for identification of the offender(s). The others originate from the victim(s) or from other known persons not involved in the crime. HLA-DQα analysis is a sensitive method for typing DNA prepared from such forensic specimens. HLA-DQα typing of DNA specimens can be done by reverse dot blot hybridization after PCR amplification (1) or, alternatively, by restriction digest of the amplified product (2). Routinely, we perform HLA-DQα PCR amplification with subsequent hybridization to the test strips. We also perform HLA-DQα PCR amplification without subsequent hybridization in order to estimate the amount and quality of the purified DNA. Single strand conformation polymorphism (SSCP) is a rapid way to detect or to search for mutations in PCR fragments amplified from the human nuclear or organellar genomes in diagnosis of inherited disease (3,4) as well as DNA typing in forensic medicine (5,6). Here we show that all 21 different HLA-DQα genotypes can be discriminated by the SSCP technique, prior to standard typing of a selected number of informative DNAs using the test strips available in the kit. Therefore, SSCP is a rapid and cheap first screening of many forensic specimens. Because in one of the more frequent HLA-DQα genotypes (1.1/4) two different SSCP patterns were observed, SSCP allows further discrimination of the stains after reverse dot blot hybridization to HLA-DQα test strips.

[1]Institut für Gerichtliche Medizin, Universität Wien, Sensengasse 2, A-1090 Wien Austria.

Received 9 April 1996; and in revised form 2 Aug. 1996 and 14 Nov. 1996; accepted 18 Nov. 1996.

Materials and Methods

DNA is prepared from stain specimens using standard techniques and HLA-DQα amplification is performed as described by the manufacturer (Perkin Elmer/Roche) with the exception that the volume of the PCR assay is reduced to 25 or 50 μL. After amplification, the products are analyzed in a 2% agarose gel. For SSCP analysis 4 μL are mixed with the same volume of loading buffer containing 95% formamide. The samples are denatured at 95°C for 2 min and rapidly cooled to 0°C. The various DNA conformations are separated in a Cleangel 36S or 48S gel (Pharmacia Biotech) on a Pharmacia/LKB Multiphor II electrophoresis unit for 20 min at 120 V, followed by 60 min at 600 V at 15°C. The gel is stained with the silver sequence staining kit (Promega). After SSCP analysis the remaining aliquot of the PCR assay is sufficient for further analysis using the reverse dot blot strips.

Results

Motivated by a number of criminal cases in which many stains had to be evaluated, we have tried SSCP analysis of HLA-DQα amplification products to predict the genotype. HLA-DQα amplification is performed in 50 μL assays, of which a 10 μL aliquot is separated in a 2% agarose gel to check for successful amplification. From 21 selected DNA preparations with different HLA-DQα genotypes, we denatured the amplification products, separated them in a Cleangel 36 S gel and visualized the DNA fragments by silver staining. Most genotypes can easily be recognized directly by the different mobility of homoduplex, heteroduplex, and single strand conformations (Fig. 1), only few genotypes would be distinguished unequivocally when run in adjacent lanes, e.g., types (2/2) and (4/4) (data not shown). Some of the allele specific presumably homoduplex bands as well as heteroduplex bands (which do not exactly comigrate) are indicated in Fig. 1.

In order to investigate the reproducibility of the method, we compared the SSCP patterns of HLA-DQα products from unrelated individuals showing the same HLA-DQα type. DNAs with the same HLA-DQα type produced identical patterns (Fig. 2). During this study, however, we observed two different but reproducible patterns (arbitrarily named type *a* and type *b* in Fig. 2) in DNAs with HLA-DQα type (1.1/4), suggesting an additional allele not detected by reverse dot blot hybridization.

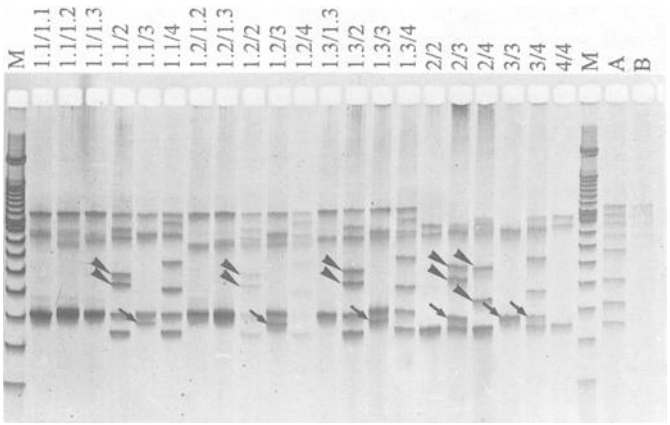

FIG. 1—*SSCP analysis of HLA-DQα amplification products in a Cleangel 36S gel, visualized by silver staining. HLA-DQα genotypes are indicated on top. Lanes M: 100 bp ladder (Gibco-BRL); Lanes A and B: PCR product (1.2/4) undiluted and 10× diluted, resp. Allele (3) specific homoduplex bands are indicated by arrows; allele 2 specific heteroduplex bands (which are absent in the 2/2 homozygote) by arrowheads.*

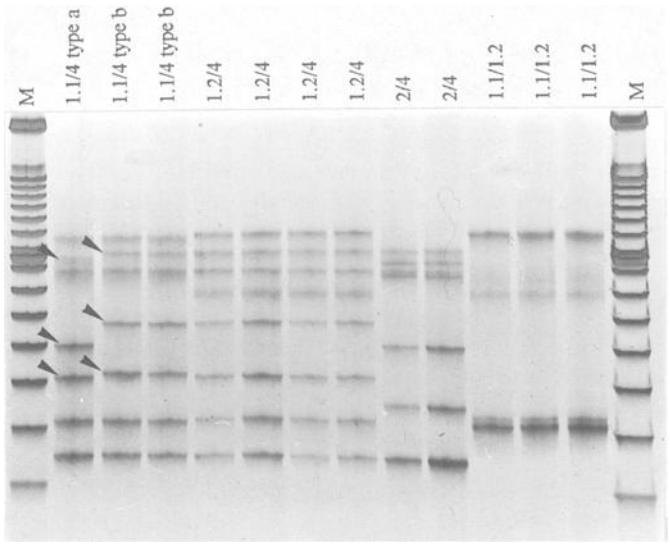

FIG. 2—*SSCP analysis of HLA-DQα amplification products from unrelated individuals. HLA-DQα genotypes are indicated on top. Lanes M: 100 bp ladder (Gibco-BRL). Variant bands are indicated by the arrowheads.*

Comparison of 20 DNAs of type (1.1/4) confirmed this result and showed that five of them are of type *a* and 15 of type *b* (data not shown). Subsequent hybridization to HLA-DQA1 test strips, discriminating HLA-DQα allele (4) into (4.1) and (4.2 or 4.3), all 5 DNAs of type *a* had allele (4.2) or (4.3) and all 15 DNAs of type *b* had allele (4.1). This allele ratio is in line with the allele frequencies provided by the manufacturer. Also the homozygous (4.1) genotype is easily distinguished from the (4.1/4.2 or 4.3) heterozygote (not shown). These results show that SSCP analysis is a valuable alternative to reverse dot blot hybridization. SSCP analysis of 48 different HLA-DQα genotypes, including denaturation, running, and silver staining of the gel is completed within 4 h and is approximately ten times less expensive than the reverse dot blot hybridization. Screening multiple DNA samples first by SSCP analysis and selecting only informative ones for hybridization, therefore, may reduce time and costs significantly.

Discussion

The HLA-DQα amplification and detection kit is a sensitive system in forensic stain analysis. It is an easy and sensitive method allowing DNA typing using less than 0.5 ng of genomic DNA (7). Because of its sensitivity even a minor amount of DNA in mixed stains is easily detected and any contamination during DNA preparation uncovered. However, several disadvantages make the system less attractive: (i) the relative low number of alleles, (ii) high frequency of alleles 1.2 and 4 in the population, and (iii) its high price. Reducing the volume of the PCR assay allows the investigator to use HLA-DQα amplification for additional purposes without using the test strips. HLA-DQα amplification with different quantities of the same genomic DNA followed by agarose gel electrophoresis only, enables estimation of the amount of DNA, and the quality of the preparation with respect to substances inhibitory to PCR and facilitates finding optimal conditions for amplification. Here, we describe SSCP analysis of HLA-DQα amplification products as an alternative method for selection of informative stains. These may, e.g., represent stains originating from the unknown offender among many stains originating from the victim or from known persons not involved in the crime.

Using the Cleangel rehydrated gels and silver stain detection SSCP analysis of HLA-DQα amplification products is rapid, easy, highly reproducible and low in costs. No special equipment is required because the LKB/Multiphor II electrophoresis unit is present in all serological departments and, due to the tendency to shift from classical serological methods to the DNA approach, readily available. In contrary to restriction digestion of the amplified products as described by Limm et al. (2), no additional reagents are required because HLA-DQα amplification in an 50–60 μL assay is sufficient for: (i) a first screening for successful amplification in an agarose gel, followed by (ii) a second screening for informative stains by SSCP and, finally, (iii) reverse dot blot hybridization for HLA-DQα typing.

The SSCP patterns depend on the HLA-DQα type but are rather independent of the amount of DNA loaded on the gel. With lower amounts of PCR product, showing weaker bands in the agarose gel, the pattern is unchanged but faster migrating bands disappear preferentially (Fig. 2, lanes A and B). This is in line with the assumption that these represent the homo- and heteroduplexes, whereas the slower migrating bands may represent the single-stranded conformations.

We have tested the reliability of SSCP analysis of HLA-DQα amplification products in several cases, e.g., identification of body limbs of four persons after aircraft crash, murder cases with more than one suspect and GEDNAP (*german DNA profiling group*) stains and consider it a rapid and reliable method for screening multiple stains prior to reverse dot blot hybridization.

References

1. Erlich HA, Bugawan TL. HLA class II gene polymorphism: DNA typing, evolution, and relationship to disease susceptibility. In: Erlich HA, editor. PCR technology, principles and applications for DNA amplification. New York: Stockton, 1989:193–208.
2. Limm TM, Ashdown ML, Naughton MJ, McGinnis MD, Simons MJ. HLA-DQA1 allele and suballele typing using noncoding sequence polymorphisms: application to 4 AOHW cell panel typing. Human Immunol 1993;38:57–68.
3. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Nat Acad Sci USA 1989;86:2766–70.

4. Hayashi K. PCR-SSCP: A simple and sensitive method for detection of mutations in the genomic DNA. PCR Meth Appl 1991;1:34–8.
5. Lin GP, Luo CQ, Yang YH, Zhang QX, Liu XW. HLA-DRB genotyping with PCR-SSCP. In: Jacob B, Bonte W, editors. Advances in forensic sciences, forensic haemogenetics. Berlin: Köster, 1995;6:248–50.
6. Szibor R, Plate I, Kirches E, Krause D. Mixing and thermal treatment of mitochondrial PCR fragments reveal sequence differences by heteroduplex formation—a rapid method for forensic identify testing. Personal communication at the 16th International Congress of the International Society for Forensic Haemogenetics; 1995 Sept 12–16; Santiago de Compostela, Spain.
7. Kreike J, Lehner A, Bauer G. Sensitivity and discriminative power of HLA-DQ-Alpha- and D1S80-amplification by the polymerase chain reaction (PCR) in the analysis of forensic mixed stains. In: Jacob B, Bonte W, editors. Advances in forensic sciences, forensic haemogenetics. Berlin: Köster, 1995;6:167–71.

Additional information and reprint requests:
Dr. Jan Kreike
Institut für Gerichtliche Medizin
Universität Wien
Sensengasse 2, A-1090 Wien
Austria