Forensic application of SSCP in the analysis of HLA-DQ, which was amplified by PCR.

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Introduction

Although various method have been developed for the confirmation of various alleles belonging to HLA class II genetic loci, SSO bot hybridization (1), RFLP (2), SSCP (3), SSCP/ARMS (4), some difficults are encountered always in forensic practice. Resently, we have succeeded in establishing method for diagnosis of HLA-DQA1 alleles by appling PCR-amplification and simple SSCP using a covenient primer set, which is recommend by International Histocompatibility Workshop(1991)(5). Based on the experience, we have examined the DNA analysis of alleles belonging to HLA- DQB and found the experimental results give comformity diagnosis of alleles belonging, not only to HLA-DQB1, but also -DQB2. This method can be used for the routine forensic test in determining identical identification.

Materials and methods

The experimental conditions were as follows: 1) A primer set for HLA-DQB1 amplification and isotope labeling (DQAAMP-A:5'-ATGGTGTAAACTTGTACCAGT-3', and DQAAMP-B: 5'-TTGGTAGCAGCGGTAGAGTTG-3') and (DQBAMP-A:5'-CATGTGCTACTTCACCAACGG-3', and DQBAMP-B: 5'-CTGGTAGTTGTGTCTGCACAC-3') recommended by the 11th International Histocompatibility Workshop (1991) was used in this experiment.

2)DNA samples(1μg) were mixed with 10 $\mu\ell$ of the primer set (25 pmol of the respective primers), 2 mM dNTP, 10 $\mu\ell$ of 25 mM $MgCl_2$(15 mM for DQA), 10X PCR reaction buffer (Biotech.International Ltd., Australia),

and 2 units of Taq polymerase(Biotech) and brought to 100 μℓ by adding distilled water. Temperature cycling was carried out in a Program Temperature Control System PC-700(Astec) as follows: 30 cycles of 1 min at 94℃, 1 min at 55 ℃, and 2 min at 72 ℃.

3) The amplified DNA sample after PCR was diluted to the optical concentration (100 ng/μℓ) by adding TE solution using electrophoresis of a 2% agarose gel.

4) Isotope labelling of the amplified DNA for SSCP analysis was performed by mixing 1 μℓ of DNA, 2μℓ of 2 mM dNTPs, 2μℓ of the same primer set for DQA1 and DQB1, 2 μℓ of α^{32}P-dCTP, (9.25 Mbq/ 25 μℓ:Amersham Co., Ltd.,England), 2μℓ of 25 mM $MgCl_2$, 2μℓ of 10X reaction buffer and 1.5units of Taq Polymerase to a final volume of 20μℓ. The PCR was carried out with 15 of the same temperature cycles described the PCR section.

5) The DNA fragments after isotope labelling for SSCP were diluted 1:20 by adding conformation solution containing 10% glycerol, 0.05% bromophenolblue and 0.05% xylenecyanol. The conformation was obtained by heating at 94 ℃ for 2 min, and then placing the mixture on ice.

6) Electrophoresis was performed under the a condition of 12-18 mAmp (1700 V) for 3 hours at 4℃ on a 30X40-cm gel plate (Nihon Eido Co.,Ltd., Japan) containing 7.5% bisacrylamide-0.5XTBE(Tris-borate buffer: 0.04 M boric acid, and 0.002 M EDTA) with glycerol in a final concentration of 10%.

Results and Discussion

From the results, it is possible to make a distinct determination of the HLA-DQA1 and DQB1 types, when the standards including DNA fragments from various common DQA1 and DQB1 alleles are inserted on the same gel plate. Figure 1 is DQA1 genotypes by the SSCP analysis. As shown in Fig.2, it could also be shown arrows in the same figure, that the SSCP bands of amplipfied DNA include another types

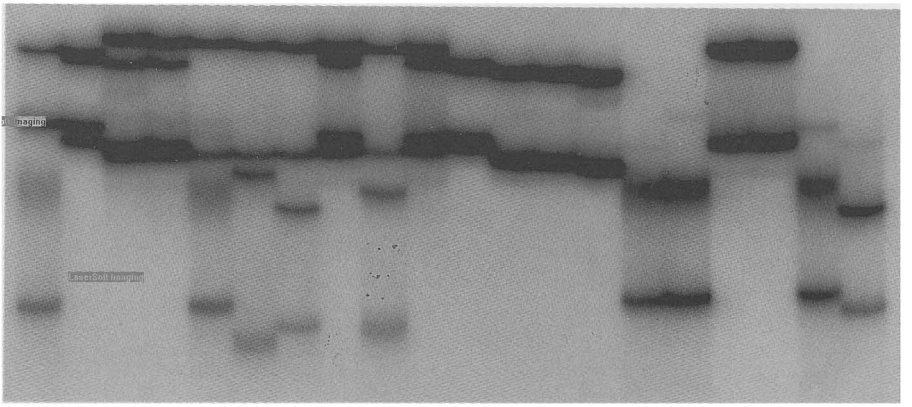

Fig.1. PCR-SSCP analysis of HLA-DQA1 genotypes. Each of the genotypes from left to right;1)0101/0501,2)0101/0102, 3)0103/3011,4)0103/3011,5)3011/0501,6)3011/0601,7)3011/0201,8)3011/0101,9)3011/0401,10)3011/0101.11)0101,12)0102,13)0102,14)0103,15)0501,16)0501,17)3011,18)3011.

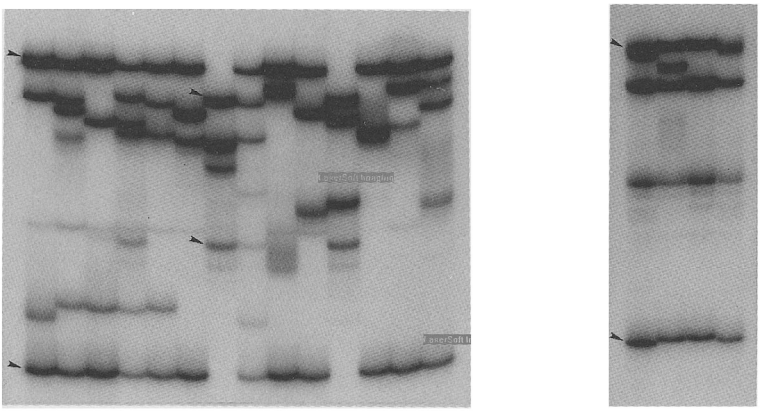

Fig.2.PCR-SSCP analysis of HLA-DQB genotypes using a comformation solution containing 10% glycerol followed by electrophoresis using a gel containing 10% glycerol. An arrowheads are DQB2 of DQB subregion.
Each of the genotypes from left to right;1)B1*0501,B2*1.2. 2)B1*0502/0601,B2*1.2. 3)B1*5031,B2*1.2.4)B1*3032/5031, B2*1.1/1.2. 5)B1*3032/5032,B2*1.2. 6)B1*0601,B2*1.2. 7)B1*0602,B2*1.1. 8)B1*0604/0501,B2*1.1/1.2. 9)B1*0201, B2*1.2. 10)B1*0301,B2*1.2. 11)B1*0302,B2*1.1. 12)B1*3032, B2*1.2. 13)B1*0401,B2*1.2. 14)B1*0402/0301,B2*1.2.
Fig.3. The HLA-DQB2*2.1 genotype of DQB1 subregion. 1) DQB1*3031, DQB2*2.1. 2)DQB1*0301/0402, DQB2*1.2. 3)DQB1*0301, DQB2*1.2. 4)DQB1*0301, DQB2*2.1.

of DNA strand, which are different from those of HLA-DQB1. The cloning (TA cloning kit; Invitrogen Corp.USA) of these strands and successive sequencing (Sequenase Version Ⅱ;United States Biochemical Corp.) revealed that they are identical with those of DQB2*1.1,DQB2*1.2 and DQB2*2.1. Fig.3 represents the SSCP images of the DQB2*2.1 allele belong to HLA-DQB2.

It is evident from this result that complete dissolution of DQB1 and DQB2, can be achieved , simultaneously, without any devices.

Reference

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