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Graphic SSCP analysis of ABO genotypes for forensic application

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Abstract. Nowadays, many of the DNA sequences of ABO blood group system have been disclosed to perform DNA testing, and one-base mutations or variations among different ABO genotypes could be detected for genotyping. For this purpose, single-strand conformation polymorphism (SSCP), which detects only one-base difference between different genotypes, is applied to detect ABO genotypes and is one of the choices among ABO genotyping technology. For practical application in forensic DNA testing, we tried to apply SSCP method using high-resolution ALF express DNA sequencer (Pharmacia Biotech). High-resolution SSCP is extremely sensitive and reliable that almost all cases of trace evidence obtained from criminal investigation were able to be genotyped visually. The denatured single-stranded amplicons were electrophoresed in sequencing gel, analyzed by laser detector, and visualized the peak patterns of chromatogram. With this method, we are able to classify ABO genotypes into 15 groups and additional subtypes. In addition, analysis of ABO genotypes of 400 DNA samples extracted from individuals living in Japan, Mongolia, and Colombia revealed a remarkable regional difference in allele frequency of A101 versus A102, and O^A vs. O^G.

Keywords: ABO genotype; SSCP; DNA polymorphism; Allele frequency

1. Introduction

The greatest advantage of using ABO blood group system in forensic identification is that ABO blood type to be screened is almost always known beforehand [1 2]. Therefore, we can narrow down the focus of crime investigation from the very beginning. The gene of transfer enzyme, which encodes ABO blood group was identified on the long arm of ninth chromosome (9q34) and composed of seven exons. Various genotypes based on a lot of

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nucleotide substitutions have been reported in exon6 and exon7 so far. Until now, 5 major alleles and more than 70 subtypes including rare ones were discovered [3]. Although several methods of detecting single-base difference in ABO genotypes have been developed [4 5], Multiplex primer extension, their procedures are usually complicated, and we must perform the many steps to obtain the clear results. Therefore, we developed PCR-SSCP analysis using ALF express DNA sequencer (Pharmacia Biotech) for clear, easy, and quick analysis of trace/insufficient amount of DNA samples, which are either trace or insufficient in amount.

2. Materials and methods

The DNA samples of this study were collected from 686 Japanese, 155 Mongolian, and 117 Colombian in South America.

2.1. Polymerase chain reaction

In this method, three sets of fluorescent labeled primers were used in PCR
For exon6 (fragment I)

Primer1-F Cy5′ -TGCAGTAGGAAGGATGTCCTC-3′
Primer2-R Cy5′ -AATGTCCACAGTCACTCGCC-3′

For exon7 (fragments II and III)

Primer3-F Cy5′ -GTGGCTTTCCTGAAGCTGTT-3′
Primer4-R Cy5′ -AAGTCACTGATCATCTCCATG-3′
Primer5-F Cy5′ -TGGAGATCCTGACTCCGCTG-3′
Primer6-R Cy5′ -GTAGAAATCGCCCTCGTCCTT-3′

2.2. Single-strand conformational change polymorphism

After amplifying exon6 and exon7, SSCP was conducted by using a DNA sequencer (ALF Express, Amersham Pharmacia Biotech) equipped with a short gel (173 × 317 × 0.5 mm thick). The data were then analyzed after visualization of peak patterns of chromatogram. This method is rapid and economic. We can perform electrophoresis simultaneously within 4 h, 40 samples at the most can be analyzed at the same time, and we can use the old gel for more than three times.

3. Results and discussion

Ten kinds of fundamental genotypes (AA, AO^A, AO^G, BB, BO^A, BO^G, O^AO^A, O^AO^G, O^GO^G, and AB) by the combination of a base substitution of np261 (G/del) and np297 (A/G) were detected (Fig. 1). These genotype patterns result from the difference in the mobility shift of sense chain and anti-sense chain based on one base substitution or deletion.

In addition, examination of 400 DNA samples from Japan, Mongolia, and Columbia revealed a remarkable regional deviation in allele frequency of A101 versus A102, and O^A

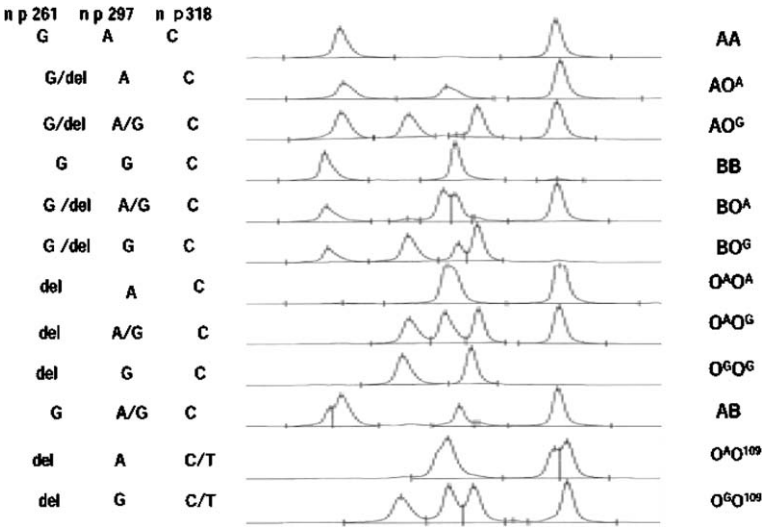

Fig. 1. SSCP pattern of fragment I and their corresponding nucleotide substitution and defined genomic types.

vs. O^G. In Japanese, although the frequency of O^A allele and O^G allele were almost the same in general (51% vs. 49%), the difference was seen between Kagoshima prefecture and Gifu prefecture (Kagoshima: $O^A/O^G = 47:53\%$, Gifu: $O^A/O^G = 57:43\%$). When compared to other population, O^G allele is more frequent than O^A allele in Mongolian ($O^A/O^G = 43:57\%$) and in Colombian ($O^A/O^G = 39:61\%$) by this study. After examination of exon6 and in exon7 including nps 703, we can classified ABO genotype in 15 groups and additional subtypes; that is, three types of AA (A101/A101, A101/A102, A102/A102), four types of AO (A101O^A, A101O^G, A102O^A, A102O^G), BB, two types of BO (BO^A, BO^G), three types of O (O^A O^A, O^G O^G, O^A O^G), two types of AB (A101B, A102B).

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