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DNA analysis of ABO blood group system detected by single-base nucleotide substitutions in a paternity case

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Abstract

DNA analysis of ABO blood group system detected by single-base nucleotide substitutions in a paternity case is reported. The ABO phenotypes of the mother, child and putative father were typed as A with the agglutination test. The ABO genotyping was performed at nucleotide positions 261 and 703 by PCR-RFLP as described Lee and Chang. The genotypes of the trio were AB, BO and AO, respectively. In this case there was a discrepancy between the serological determination of the blood group and the PCR ABO genotyping, so that a sequence determination was additionally carried out. The nucleotide sequence of the mother had six nucleotide differences, at positions 297, 526, 657, 703, 771 and 829, compared with A1. The mother and child possessed each of these differences. These nucleotide differences coincided with that of the ABO * R101 allele previously reported by Ogasawara et al. detected in an A2 individual. As the nucleotide (np703) of the * R101 allele is the same as that of B allele, the analysis of at least three nucleotide positions, i.e. nps 261, 703 and 796, is necessary to avoid the mistyping of the ABO genotype.

Keywords: ABO blood group system; ABO genotyping; PCR-RFLP; Paternity testing

1. Introduction

The ABO blood system is one of the major blood group systems in man and is important in transfusion medicine, forensic serology and anthropological genetics. With the establishment of the molecular genetic basis of the ABO grouping by Yamamoto et al.

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[1], it became possible to determine the ABO blood groups at the DNA level. The A1 allele (*A101) differs from the B allele (*B101) in seven single-base nucleotide substitutions at positions 297, 526, 657, 703, 796, 803 and 930, in which the four nucleotides at positions 526, 703, 796 and 803 lead to amino acid changes in the final transferase protein. A2 allele (*A105) differs from A1 allele by a single-base substitution (np 467) and a single-base deletion (np 1060). The O allele is identical to the A allele except for a single-base deletion at nucleotide position (np) 261. In subgroup 01 and 02, two common alleles, *0101 and *0201, were observed, *0101 was different from *0201 by five nucleotide substitutions at positions 297, 646, 681, 771 and 829. In the present investigation, the DNA analysis of ABO blood group system detected by single-base nucleotide substitutions in a paternity case is reported.

2. Materials and methods

2.1. DNA samples

EDTA-treated blood samples were collected from the trio (mother, child and putative father), and genomic DNA was isolated by proteinase K/phenol/chloroform extraction.

2.2. ABO blood group system

The ABO phenotype of the trio was determined by agglutination test. The ABO genotyping was performed at nucleotide positions 261 and 703 by PCR-RFLP described by Lee and Chang [2]. The sequencing analysis was done using BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (PE Biosystems) according to the user's manual provided by the manufacturer. Two sets of primers, ABO-1/ABO-2 and ABO-3/ABO-4, used in this study were as follows. Sense primers: ABO-1: 5′-CACCGTGGAAG-GATGTCCTC-3′, ABO-3: 5′-TGTCTTCACCGACCAGCTGG-3′, antisense primers: ABO-2: 5′-AATGTCCACAGTCACTCGCC-3′, ABO-4: 5′-CTGGTCGACCAT-CATGGCCT-3′. The PCR cycles consisted of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1.5 min. An amplification of 30 cycles and a final extension at 72 °C for 5 min was carried out. The PCR products were electrophoresed by ABI 310 genetic analyzer.

3. Results and discussion

The ABO phenotypes of the mother, child and putative father were typed as A with the agglutination test. By using PCR-RFLP described by Lee and Chang [2], Kpn I restriction of the PCR product is diagnostic for the 261G deletion of the 0 allele, whereas Alu I digests the PCR product at 703 A, which is specific to the B allele. The RFLP patterns of the mother were non-digestively homozygous at the Kpn I site, and heterozygous at the Alu I site, indicating that the genotype of the mother is AB. The child showed heterozygous restriction patterns for the two enzymes, and is thus heterozygous for the

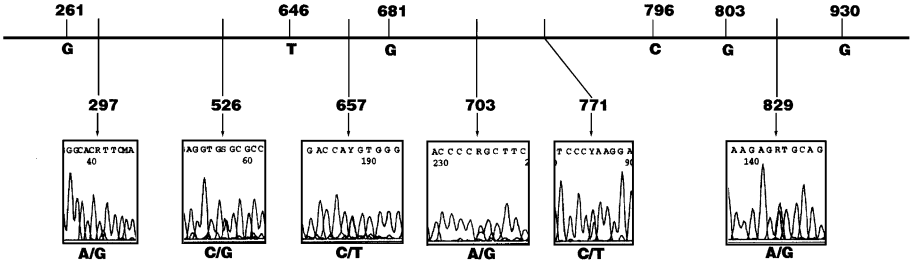

Fig. 1. Partial coding sequences of ABO alleles detected in the mother.

B and 0. The genotype of the putative father was found to be A0 because of the heterozygous pattern at the Kpn I site, and the non-digestively homozygous pattern at the Alu I site. In this case there was a discrepancy between the serological determination of the blood group and the PCR ABO genotyping, so that a sequence determination was additionally carried out. The nucleotide sequence of the mother had six nucleotide differences, at positions 297, 526, 657, 703, 771 and 829, compared with the common A1 transferase encoded by *A101 (Fig. 1). The mother and child each possessed these differences. These nucleotide differences were coincided with that of *R101 allele previously reported by Ogasawara et al. [3]. The nucleotide sequence of *R101 is identical to that of *B101 upstream of nucleotide position 703 and to that of *0201 downstream of nucleotide position 771. *R101 may have originated by a recombination between *B101 and *0201 at around positions 703–771. From these results, the genotypes of the mother, child and putative father were determined as A1/R101, R101/0 and A1/0, respectively. As the nucleotide (np 703) of the *R101 allele is the same as that of B allele, the analysis of at least three nucleotide positions, i.e. nps 261, 703 and 796, is necessary to avoid the mistyping of the ABO genotype.

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