TECHNICAL NOTE

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ABO Genotyping Following a Single PCR Amplification

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ABSTRACT: Using primers designed by Lee and Chang, 200 base-pair (bp) fragment of ABO locus was amplified by PCR, which spans the site of the single nucleotide deletion associated with O allele. O allele could be identified by *Kpn* I digestion of the PCR product as reported. A and B alleles were also distinguishable by *Mae* II digestion of the product. Thus restriction digestion by *Kpn* I and *Mae* II could genotype ABO blood group following the single amplification. The nucleotide substitution in the 200-bp product between A and B alleles was also found in O allele, resulting in 2 different suballeles O^A and O^G. The single-strand conformational polymorphism of the PCR product was also investigated for ABO genotyping following the single amplification.

KEYWORDS: forensic science, DNA, ABO blood group system, PCR, restriction endonuclease, single-strand conformational polymorphism (SSCP)

Since 1990 when the nucleotide sequences of ABO gene were reported (1,2), direct genotyping of ABO blood group system has been explored using polymerase chain reaction (PCR) combined with diagnostic restriction enzyme digestion (2–4) or with the use of allele-specific primers (5,6), based on several single base substitutions between A and B alleles and the single nucleotide deletion in O allele (261th G: This location was first numbered 258th (1,2), but the deletion of three nucleotides has been found in the first-sequenced clone (7). Thus the accurate location is 261th, and the numbering through this manuscript is according to reference (7)). In these methods, at least two other sequences were amplified for the genotyping, since the substitutions between A and B alleles reported primarily (526th, 703th, 796th and 803th nucleotides in the cDNA clone (2)) are located far from the 261th nucleotide. Further sequencing of the alleles has shown the changes of 297th, 657th and 930th nucleotides between A and B alleles (7–9), and Johnson and Hopkinson found other base changes near the base deletion by denaturing gradient gel electrophoresis (10). Based on these findings, the conventional A, B and O alleles should be divided into many suballeles. We however investigated a simple method identifying the conventional alleles by restriction digestion or single-strand conformational polymorphism (SSCP) analysis (11) following a single PCR amplification at the ABO locus.

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Materials and Methods

DNA was isolated from peripheral blood leukocytes of 24 unrelated Japanese individuals as described previously (12). ABO phenotypes of these samples were identified by serological methods. Primers 5′-ACACCGTGGAAGGATGTCCTC-3′ and 5′-AATGTCCACAGTCACTCGCC-3′ designed by Lee and Chang (3) were used for amplification of 200 base-pair (bp) sequence which spans 261th and 297th nucleotides. The PCR reaction mixture (100 μL) consisted of 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of deoxyribonucleoside-5′-triphosphates (dNTP), 0.5 μM each of primers and 2.5 units of Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany). Following a preheating at 95°C for 2 min, 30 cycles of consecutive incubations at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min were carried out, followed by post-incubation at 72°C for 7 min.

The PCR products were purified by Microcon® 100 microconcentrators (Amicon, Danvers, CT) and digested by a restriction endonuclease *Kpn* I (Boehringer Mannheim) at 37°C. Some of them were purified by Microcon 100 and further digested by a restriction enzyme *Mae* II (Boehringer Mannheim) at 50°C. These samples and 100-bp ladder molecular weight standard (Pharmacia, Uppsala, Sweden) were electrophoresed in 2% NuSieve® GTG® Agarose gel (FMC, Rockland, ME) stained by ethidium bromide.

SSCP was also analyzed using an automated electrophoresis system PhastSystem™ (Pharmacia) with PhastGel Homogeneous 20 (4.5 × 5.0 cm, 20% polyacrylamide gel, Pharmacia). The PCR products were mixed with an equal volume of 98% formamide-2% glycerol, boiled for 3 min and chilled rapidly in ice-water. Following pre-electrophoresis at 400 V, 10 mA, 2.5 W, 14°C for 50 Vh (run time), 4 μL each of the samples were applied and electrophoresed at 400 V, 10 mA, 2.5 W, 14°C for 300 Vh. Then the gel was stained using PhastGel Silver Staining Kit (Pharmacia).

Results and Discussion

When 200-bp sequence of ABO locus was amplified by PCR, less-intense bands much longer than 200-bp were sometimes detected in the gel. However, these minor fragments did not affect

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the ABO genotyping described below. Following the amplification, *Kpn* I digestion revealed O allele as 172-bp fragment (2,3). Although Lee and Chang reported that 199-bp O allele is cut to 171- and 28-bp fragments (3), the nucleotide deletion is located within the shorter fragment so that 172- and 27-bp fragments [more accurately, 172-bp double-strand (ds) fragment with a 4 nucleotide (nt) single-strand (ss) tailing and 23-bp ds fragment with a 4-nt ss tailing] should be produced. As shown in Fig. 1, B allele was cut by *Mae* II, producing 140- and 60-bp fragments (accurately, 138-bp ds fragment with a 2-nt ss tailing and 60-bp ds fragment with a 2-nt ss tailing). This result supported the base substitution of 297th A in A allele to G in B allele (4). Some O alleles were also digested by the enzyme producing 140-bp fragments while other O alleles were not. Repeated experiments excluded the possibility of partial digestion, suggesting the existence of two O suballeles, O^A with 297th A and O^G with 297th G (8,9).

These suballeles could explain the SSCP patterns of the PCR products (Fig. 2). SSCP analysis is known to detect one nucleotide

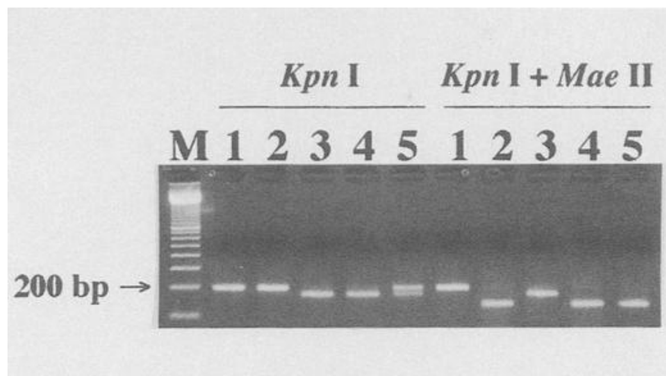

FIG. 1—*Kpn I digestion and Kpn I and Mae II double-digestion patterns of the PCR products. Lanes 1–5 represent ABO genotypes of AA, BB, O^AO^A, O^GO^G and BO^G, respectively. Lane M is 100-bp ladder molecular weight standard marker (Pharmacia).*

FIG. 2—*SSCP patterns of the PCR products. Lanes 1–5 represent ABO genotypes of AA, BB, O^AO^A, O^GO^G and BO^G, respectively.*

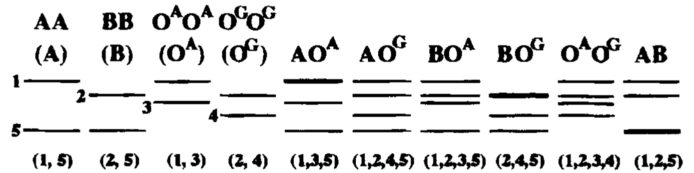

FIG. 3—*Schematic SSCP patterns of the PCR products corresponding to alleles and heterozygous genotypes of ABO system. The detected bands were arbitrarily numbered in parentheses.*

substitution as difference in the electrophoretic mobilities of two ss fragments of each PCR product (11). A, B, O^A and O^G alleles could be thus identified by the method as shown in Fig. 2. Above the allele-specific fragments of each sample, there was a less-intense band, which was likely to be denatured (non-conformational) single-strands. From these results, each of 10 genotypes consisted of the four alleles can be determined following the single PCR amplification by the SSCP analysis (Fig. 3), or, alternatively, by the combination of the single-digestion (*Kpn* I) and double-digestion (*Kpn* I and *Mae* II) patterns (Table 1). Instead of *Kpn* I, a restriction enzyme *Bst* EII can be used for the genotyping by the restriction digestion; *Bst* EII does not cut 199-bp O suballeles but 200-bp A and B alleles producing 177- and 23-bp fragments (accurately, 172-bp ds fragment with a 5-nt ss tailing and 23-bp ds fragment with a 5-nt ss tailing). Frequencies of O^A and O^G suballeles in 36 unrelated Japanese individuals were 0.389 and 0.154, respectively, and the sum 0.543 was equal to allele frequency of O allele in Japanese population (0.5407).

Johnson and Hopkinson reported two B alleles and four O alleles in 250-bp sequence which spans 261th nucleotide (10). The four O alleles may be derived from two nucleotide substitutions, one of which is likely identical with 297th A to G. Other base changes in B and O alleles, which were not found in the SSCP of the 36 individuals, may be located out of the 200-bp sequence amplified in this study. Otherwise, frequencies of the base changes may be very low (10). A large number of nucleotide substitutions have been found in ABO locus (1,2,7–9) so that the three conventional alleles will be divided into numbers of suballeles. Complicated polymorphism in the locus is quite informative for personal identification. However, ABO blood group has been usually typed by more simple serological method and will be. So the simple method for ABO genotyping as demonstrated herein will be useful in several cases.

TABLE 1—*Restriction pattern of the PCR product of ABO locus using primers designed by Lee and Chang.*

		Restriction fragment length (bp)	
Phenotype	Genotype	*Kpn* I digestion	(*Kpn* I + *Mae* II) digestion
A	AA	200	200
	AO^A	200 + 172	200 + 172
	AO^G	200 + 172	200 + 140
B	BB	200	140
	BO^A	200 + 172	172 + 140
	BO^G	200 + 172	140
AB	AB	200	200 + 140
O	O^AO^A	172	172
	O^AO^G	172	172 + 140
	O^GO^G	172	140

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