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Genotyping of ABO blood groups by PCR and RFLP analysis of 5 nucleotide positions

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Abstract The genotyping of ABO blood groups was performed using the polymerase chain reaction (PCR) method. The 4 DNA fragments containing the nucleotide position 261, 526, 703 and 796 of cDNA from A-transferase were amplified by PCR, and the amplified DNA subjected to restriction fragment length polymorphism (RFLP) analysis. The different nucleotide at position 803 was clearly distinguished by electrophoresis of the PCR products amplified with allele-specific primers. By analyzing the electrophoresis patterns, ABO genotyping was conclusively accomplished. The frequencies of ABO genotypes found in Japanese blood donors with A and B phenotypes were as follows: in the phenotype A group, AA = 19.8% and AO = 80.2%; and in the phenotype B group, BB = 12.8% and BO = 87.2%.

Key words ABO blood group · Genotyping · PCR · RFLPs · Allele-specific primers

Zusammenfassung Die Bestimmung der ABO-Blutgruppen wurde mittels Polymerase-Kettenreaktion (PCR) durchgeführt. Die vier DNA-Fragmente, die Nukleotidpositionen 261, 526, 703 und 796 von der cDNA der A-Transferase enthielten, wurden mittels PCR amplifiziert. Die amplifizierte DNA wurde einer Restriktionsfragmentlängen-Analyse (RFLP) unterzogen. Nach Amplifikation mit allelspezifischen Primern konnte der Nukleotidunterschied an Position 803 durch Elektrophorese der PCR-Produkte klar getrennt werden. Die Bestimmung der ABO-Genotypen war durch die Analyse der eletrophoretischen Muster eindeutig durchführbar. Folgende Frequenzen der ABO-Genotypen von japanischen Blutspendern wurden für die Phänotypen A und B gefunden: In der Phänotypgruppe A, AA = 19,8% und AO = 80,2%; Phänotypgruppe B, BB = 12,8% und BO = 87,2%.

Schlüsselwörter ABO-Blutgruppen · Bestimmung der Genotypen · PCR · RFLPs · Allelspezifische Primer

Introduction

The ABO system is one of the most important blood groups for transfusion medicine, forensic medicine and anthropology. A, B and O alloantigens are coded by the A, B and O genes respectively on the human 9th chromosome. In 1990 Yamamoto et al. [1, 2] cloned and sequenced a complementary DNA (cDNA) encoding the A_1 transferase, and subsequently cloned and analysed both B and O allelic cDNAs. They found a single-base deletion (nucleotide position 261; tentatively named as point 1 in the present study) in the O gene, and 4 single-base substitutions (nucleotide positions 526, 703, 796 and 803; designated as points 2, 3, 4 and 5, respectively) between the A and B genes. They also reported a PCR-RFLP method for analyzing the nucleotides on points 1, 2 and 3 to identify the ABO genotypes [2, 3].

We describe here the genotyping of ABO blood group by analyzing the 5 nucleotide positions. The PCR and RFLP methods were carried out, and allele-specific primers were used for the analysis of point 5. A population study is also reported.

Materials and methods

DNA samples. Blood samples were collected from healthy individuals with 6 different ABO genotypes (AA, AO, BB, BO, OO and AB) which had been determined serologically in family studies. The blood samples for the population study were taken from 200 randomly selected blood donors living in the Osaka district. The preparation of genomic DNA was performed using the phenol-chloroform extraction method as described by Maniatis et al. [4].

Amplification of DNA fragments. The PCR method [5] was carried out in a reaction mixture of 50 µl containing 0.1–0.5 µg genomic

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Table 1 Primers used for PCR amplification of ABO genes

Point	Primer	
1	GA16	5′ AGAAGCTGAGTGGAGTTCCAGGTG 3′
	GA17	5′ TGATGGCAAACACAGTTAACCC 3′
2	GA01N	5′ TCCTGGAGACGGCGGAGAAGCA 3′
	GA08N	5′ ACAGCGGGAGTCAGGATCTCCAT 3′
3	fy-31	5′ GAAATCGCCCTCGTCCTT 3′ (ref. [2])
	fy-47	5′ TGCTGGAGGTGCGCGCCTAC 3′ (ref. [2])
4	GA07	5′ AGCCGGGAGGCCTTCACCTA 3′
	GA12	5′′TGAGCCGCTGCACCTCTTGCA 3′
5	GA07	5′ AGCCGGGAGGCCTTCACCTA 3′
	GA13*	5′ ACCGACCCCCCGAAGAACG 3′
	GA07	5′ AGCCGGGAGGCCTTCACCTA 3′
	GA14**	5′ ACCGACCCCCCCGAAGAACC 3′

* GA13 = B-gene specific; ** GA14 = A- and O-gene specific

DNA, 50 mM KCl, 2 mM Tris-HCl (pH 9.0), 1 mM DTT, 200 µM each dNTP (Takara Shuzo, Kyoto, Japan), 0.2 µM each primer, and 1 U Taq polymerase (Wako Pure Chem., Osaka, Japan). Primers for the PCR amplification were synthesized with the Gene Assembler Plus (Pharmacia-LKB, Uppsala, Sweden). The primers and their sequences are shown in Table 1. The PCR cycles consisted of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 2 min. An amplification of 30 cycles and a final extension at 72°C for 3 min was carried out with a DNA Thermal Cycler (Perkin-Elmer Cetus Instruments, Norwalk, CT.).

RFLP analysis. Aliquots (4 µl) of the amplified fragments containing the nucleotides at points 1, 2, 3 and 4 were digested with the following restriction enzymes; Bst PI and Kpn I for point 1, Bst U1 and Nar I for point 2, Hap II and Alu I for point 3, and Mva I and Nla III for point 4. Each mixture was made up to 10°µl with digestion buffer and distilled water, and the mixture was incubated at the optimum temperature as stated in the instruction manual at least 3 h before electrophoresis. The digested (points 1, 2, 3 and 4) and amplified (point 5) fragments were subjected to electrophoresis in 10% polyacrylamide gels (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) using a MUPID Mini-Gel Electrophoresis System (Cosmo Bio., Tokyo, Japan). øX174/Hinf I digest (Marker 9™; Nippon Gene, Tokyo, Japan) was used as a size marker. The electrophoresis patterns were visualized by staining with ethidium bromide.

The expected digestion and amplification profiles of DNA fragments at the ABO locus are shown in Table 2. An alphabetical-numerical code, i.e., 1a, 1b, 2a, 2b, etc., was assigned to each band pattern of points 1, 2, 3 and 4.

Results

RFLPs at points 1, 2, 3 and 4. Figure 1 shows the electrophoresis patterns of digested DNA from the 6 individuals with different ABO phenotypes. An alphabetical-numerical code is given to each band pattern. These DNA fragment patterns were consistent with the expected profiles as shown in Table 2.

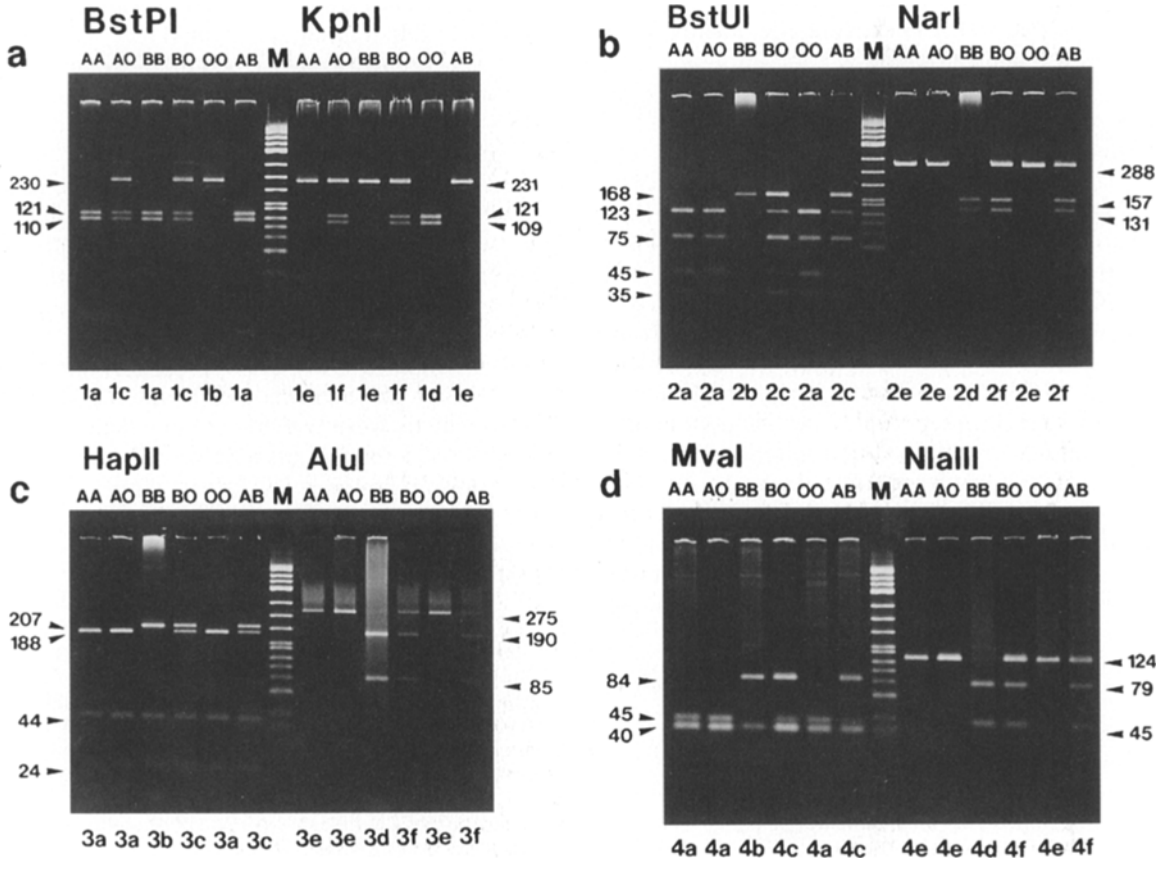

Fig. 1a–d RFLP patterns of DNA after amplification by PCR. BstPI, KpnI, etc. are the restriction enzymes used. **a, b, c** and **d** show the results of the analyses of nucleotide changes at points 1, 2, 3 and 4, respectively. **1a, 1b, 1c**, etc. = pattern code; M = size marker

Table 2 Expected digestion (points 1, 2, 3 and 4) and amplification (point 5) patterns of the DNA fragments

Point	bp	AA	AO	BB	BO	OO	AB
1 BstPI	230		+		+	+	
	121	+	+	+	+		+
	110	+	+	+	+		+
		1a	1c	1a	1c	1b	1a
KpnI	231	+	+	+	+		+
	121		+		+	+	
	109		+		+	+	
		1e	1f	1e	1f	1d	1e
2 BstUI	168			+	+		+
	123	+	+		+	+	+
	75	+	+	+	+	+	+
	45	+	+		+	+	+
	35	+	+	+	+	+	+
	10	+	+	+	+	+	+
		2a	2a	2b	2c	2a	2c
NarI	288	+	+		+	+	+
	157			+	+		+
	131			+	+		+
		2e	2e	2d	2f	2e	2f
3 HapII	207			+	+		+
	188	+	+		+	+	+
	44	+	+	+	+	+	+
	24	+	+	+	+	+	+
	19	+	+		+	+	+
		3a	3a	3b	3c	3a	3c
AluI	275	+	+		+	+	+
	190			+	+		+
	85			+	+		+
		3e	3e	3d	3f	3e	3f
4 MvaI	84			+	+		+
	45	+	+		+	+	+
	40	+	+	+	+	+	+
	39	+	+		+	+	+
		4a	4a	4b	4c	4a	4c
NlaIII	124	+	+		+	+	+
	79			+	+		+
	45			+	+		+
		4e	4e	4d	4f	4e	4f
5 GA07/GA13	104			+	+		+
GA07/GA14	104	+	+		+	+	+

1a, 1b, 1c, etc. = pattern code

Analysis of point 5. As shown in Fig. 2, the presence (+) or absence (–) of the 104 bp band was demonstrated on the gel. DNA from individuals with AA, AO and OO genotypes was not amplified by PCR with primer GA13 (specific for B gene), and that from the BB genotype was not amplified with primer GA14 (specific for A and O genes).

Frequencies of AA, AO, BB and BO genotypes in Japanese. The ABO genotypes of 133 blood samples from Osaka blood donors with A and B phenotypes determined serologically were examined by the present method. From 86 individuals with phenotype A, 17 (19.8%) were AA and 69 (80.2%) were AO, and from 47 with phenotype B, 6 (12.8%) were BB and 41 (87.2%) were BO. These data are in good agreement with the ratio calculated from the data obtained serologically.

Discussion

We designed new PCR primers based on the report of Yamamoto et al. [1]. Prior to ABO genotype analyses, we

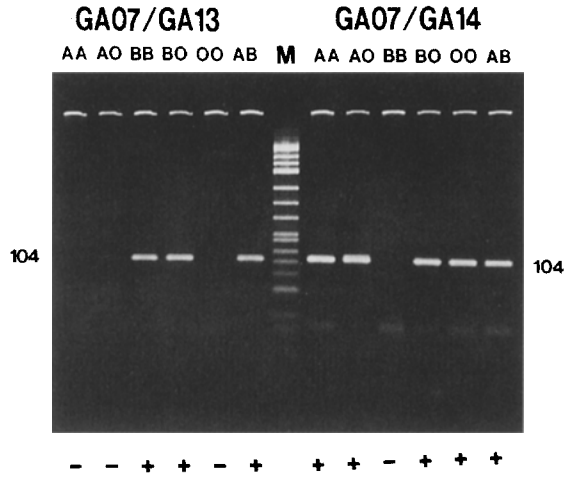

Fig. 2 Fragments amplified by PCR with 2 pairs of allele-specific primers, GA07/GA13 and GA7/GA14. +=presence of amplified fragment; −=absence of amplified fragment; M=size marker

checked the purity of the products amplified by PCR with the new primers by polyacrylamide gel electrophoresis followed by ethidium bromide staining, and rarely observed non-specific products in the gel. There is no restriction enzyme for analyzing point 5, therefore, we designed 2 allele-specific primers, GA13 and GA14, to discriminate the A/O and B genes. It was possible to classify the genotypes into 3 groups: (1) AA, AO and OO, (2) BB, and (3) BO and AB. The new primers are very useful for ABO genotyping.

The results obtained here are in good agreement with the common ABO types, reported by Yamamoto et al. [2]. The genotyping of common types can be performed by analyzing point 1 and one of the other 4 points. On the other hand, several variant types, such as Aint, A_2 and Cis AB, may result from nucleotide substitutions at points 2, 3, 4 and 5, therefore, the analysis of all 5 points is necessary to detect the rare ABO variants. We have found some variants with recombinant A and B genes in a Japanese population which will be described elsewhere.

The present method has several advantages. The ABO genotypes of the samples with A and B phenotypes cannot be determined from an individual blood sample by serological methods, however, they can be done correctly by the present method. Furthermore, genotyping is accomplished using a small quantity of DNA. In our preliminary study, at least 5 ng of template DNA was required for the standard PCR procedure (30 cycles), and 5 pg of template DNA was required when PCR of 60 cycles (30 cycles × 2 times) was carried out. Kaneshige et al. [6] analysed the D-loop region of mtDNA, the HLA DPB1 gene and the MCT118 locus using fingernail DNA. We also purified DNA from fingernails and toenails and successfully demonstrated the ABO genotype. ABO genotyping using DNA samples from fingernails, bloodstains, and the other forensic biological materials are now in progress, and the results will be described elsewhere. The present method may be very useful for personal identification as well as for paternity testing in forensic science.

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