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# An Analysis of Polymorphism for the *ABO* Blood Group Genes in a Japanese Population Based on Polymerase Chain Reaction

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**Abstract** The allele frequencies and the polymorphism in the *ABO* blood group gene of 340 individuals living in the central part of Japan near Tokyo were analyzed using polymerase chain reaction (PCR) based techniques. We analyzed the known polymorphic sites (at positions 297, 526, 657, 703, 796, 803 and 930) between *A* and *B* alleles, and the single deletion at position 261 in *O* alleles using PCR-RFLP (restriction fragment length polymorphism), PCR-SSCP (single strand conformation polymorphism) and sequencing methods. We found two new allele subtypes in the *A* and *B* alleles. A new *A* allele subtype showed four nucleotide differences at positions 297 (adenine versus guanine), 526 (cytosine versus guanine), 657 (cytosine versus thymine) and 703 (guanine versus adenine), and the allele frequency was 0.0044. This *A* allele subtype was probably generated by a crossing-over of *B* allele and *A* or *O* allele. Second new *B* allele subtype had a single nucleotide difference at position 467 (cytosine versus thymine) and the frequency was 0.0015. The ratio of a mutant *O* allele (the nucleotide differences at positions 646, 681, 771 and 826) to the common *O* allele in the central part of Japan was low in the western part (Osaka region) and higher in the northern part (Yamagata region). There may be a geographical cline for these two allele frequencies in Japan. There was no difference in the transferase activities between common alleles and rare alleles.

**Keywords:** ABO blood group, PCR-RFLP, polymorphism, allele frequency, central Japanese population

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## Introduction

The ABO system, discovered by Landsteiner (1900), is without doubt the most important of the blood group systems as far as the blood transfusion is concerned. In the ABO system there are three major alleles, *A*, *B* and *O*, any one of which may occupy the *ABO* locus on each of the paired chromosomes. The *O* gene does not produce any demonstrable red cell antigen, thus it is an amorphism. The structure of the antigen determinants was established by Watkins and Morgan (1959, 1969) and Kabat (1973). The determinant for the A antigen is *N*-acetyl-D-galactosamine added by α-*N*-acetylgalactosaminyl transferase, and for the B antigen, D-galactose by α-galactosyl transferase. The A and B gene-specified transferases add their specific sugars to an oligosaccharide chain called the H chain.

In 1990, Yamamoto and others (1990a, 1990b) cloned cDNA encoding A and B transferases and determined the nucleotide sequences of the cDNA. They found seven nucleotide differences (nucleotide positions 297, 526, 657, 703, 796, 803 and 930) and four amino acid differences (amino acid positions 176, 235, 266, 268) between the coding regions of cDNA for A and B transferases, and that *O* allele is specified by the 261 deletion of the sequence of the *A* allele. The nucleotide differences between *A*, *B* and *O* alleles have provided a means for direct ABO genotyping.

Recently, DNA typing methods for the *ABO* blood group genes based on polymerase chain reaction (PCR) were demonstrated by Lee and Chang (1992), Ugozzoli and Wallace (1992), Johnson and Hopkinson (1992), Iwasaki and others (1993a, 1993b), Drouse and Vincek (1995), Fukumori and others (1995) and Herrin (1996). Analysis of the polymorphism has been investigated by Yamamoto and others (1992, 1993a, 1993b, 1993c, 1993d), Kobayashi and others (1994), Iwasaki and others (1996), and Ogasawara and others (1996). The presence of the polymorphism of the *ABO* genes, especially *A* and *O* alleles, have been proved.

In the present study, we analyzed polymorphism of *ABO* blood group gene and surveyed the distribution of each allele in a Japanese population.

## Materials and Methods

*DNA samples*

Peripheral blood was collected from 340 healthy volunteers. All subjects in this study were of Japanese ancestry and lived in the central part of Japan. Peripheral blood was hemolyzed in 10 mM Tris-HCl, pH 7.6, 10 mM NaCl and 5 mM $MgCl_2$, and then the hemolyzed red cells were removed by aspirating. The isolated leukocytes were then washed with PBS. Genomic DNA was isolated from the leukocytes by solubilizing the cells overnight in 10 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10 mM EDTA, pH 8.0, 0.1% SDS, and 100 μg/mℓ proteinase K (Boehringer Mannheim GmbH, Germany) at 42℃. High molecular weight DNA was extracted with phenol, chloroform, isoamyl alcohol (25:24:1), and recovered by isopropyl alcohol precipitation.

*Amplification of the* ABO *blood group transferase genes*

A total of 200 ng of genomic DNA were subjected to amplification of *ABO* transferase genes using PCR procedure. DNA was amplified using the synthetic oligonucleotide primers listed in Table 1. Amplifications were performed in a 50 $\mu\ell$ reaction mixture containing 1 unit of *Taq* DNA polymerase (Boehringer Mannheim GmbH, Germany) in 67 mM Tris-HCl, pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 200 $\mu$g/m$\ell$ gelatin, 1.5 mM $MgCl_2$, 200 $\mu$M of each deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP; Toyobo, Osaka, Japan), and 0.5 $\mu$M of each primer. Thirty cycles, each containing a denaturation for 30 seconds at 95°C, annealing for 30 seconds at 62.5°C for primers ABO56-ABO36 and at 60°C for primers ABO52N-ABO312, primers ABO52N-ABO313 and primers ABO57-ABO312, and extension for 30 seconds at 72°C, were performed on an automated thermal controller (PC-800; Astec Inc, Fukuoka, Japan). Primers, ABO52N and ABO312 or ABO313, were designed to detect

**Table 1** The oligonucleotide sequences for polymerase chain reaction

| Name | Sequence | Nucleotide Position | |
|---|---|---|---|
| ABO56 | 5'-CACCTGCAGATGTGGGTGGCACCCTGCCA-3' | Intron 5 | 500 - 519 |
| ABO36 | 5'-GTGGAATTCACTCGCCACTGCCTGGGTCTC-3' | Intron 6 | 27 - 46 |
| ABO52N | 5'-GTGGCTTTCCTGAAGCTGTTC-3' | Exon 7 | 349 - 399 |
| ABO57 | 5'-TTCCTCAGCGAGGTGGATTAC-3' | Exon 7 | 598 - 618 |
| ABO312 | 5'-GATGCCGTTGGCCTGGTCGAC-3' | Exon 7 | 868 - 888 |
| ABO313 | 5'-CGTTCTGCTAAAACCAAGGGCGG-3' | 3'-UTR | 49 - 71 |
| ABO5ABs | 5'-AGGAAGGATGTCCTCGTGGTG-3' | Exon 6 | 241 - 261 |
| ABO5Os | 5'-AGGAAGGATGTCCTCGTGGTA-3' | Exon 6 | 241 - 262 |
| ABO5AOs | 5'-CCATTGTCTGGGAGGGCACA-3' | Exon 6 | 278 - 297 |

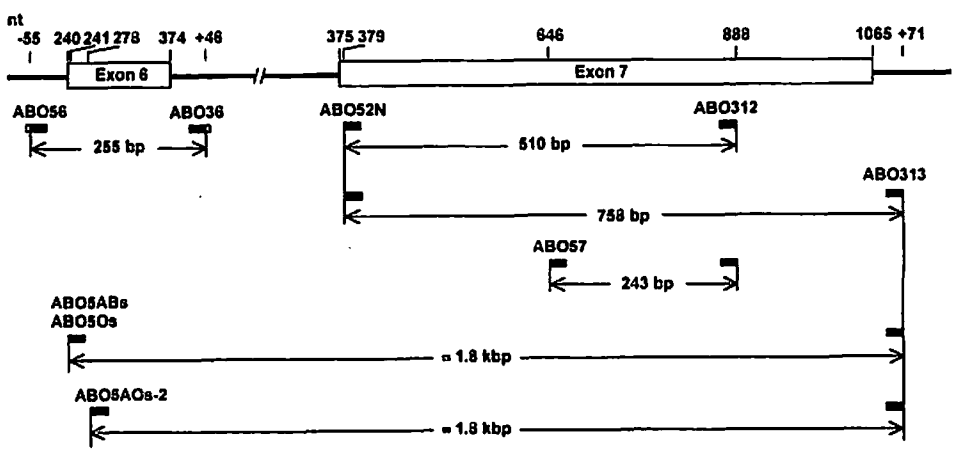


**Figure 1** Positions of primers and amplified fragments in the *ABO* blood group gene. Numbers above the top line indicate nucleotide positions of the last two exons.

the six nucleotide differences (nucleotide position 526, 657, 703, 796, 803 and 930) between the coding regions of cDNA for A and B transferases, primers, ABO56 and ABO36, to detect the single base deletion in *O* alleles (nucleotide position 261 and 297) (Figure 1). Amplification efficiency was checked by electrophoresis of 5 $\mu\ell$ of the sample in 1.5% agarose gel. The reaction product was purified by centrifuge filtration (Ultrafree C3LTK; Nihon Millipore Ltd., Tokyo, Japan).

*Digestion and electrophoresis*

After amplification, an aliquot (5 $\mu\ell$) of reaction mixture was digested with the restriction endonucleases, *Bst*EII, *Kpn*I, *Mae*II, *Msp*I, *Alu*I, *Ban*I, *Bss*HII, *Nla*III, *Mva*I, *Tse*I, and *Sau*3AI (Boehringer Mannheim GmbH, Germany and New England Bio Labs, USA). This treatment was carried out overnight at a concentration of 2 units restriction enzyme at 37°C. The samples of cleaved DNA were subjected to electrophoresis on 4% NuSieve 3:1 agarose gel (FMC BioProducts, USA) in a mini-gel apparatus (Mupid-2; Cosmo Bio Co. Ltd., Tokyo, Japan). Restriction fragments were detected by staining with ethidium bromide.

*Single strand conformation polymorphism (SSCP) analysis*

PCR-SSCP fragments were compared with homozygous reference samples to screen mutant allele. A mixture of 1 $\mu\ell$ of amplified DNA sample (primers ABO56-ABO36 and ABO57-ABO312) and 6 $\mu\ell$ of loading buffer (95% deionized formamide, 20 mM EDTA (pH 8.0), 0.05% bromophenol blue, and 0.05% xylene cyanol FF) was heated for 5 minutes, and immediately chilled on ice. One microliter of denatured solution was subjected to 12% polyacrylamide gel (acrylamide: bisacrylamide=49:1) with 5% glycerol cooled continuously at 10°C. The separated DNA fragments in the gel were visualized by silver staining (Daiichi Pure Chemicals, Tokyo, Japan).

*DNA sequencing*

After group-specific amplification (ABO5ABs and ABO313 for groups A and B, ABO5Os and ABO313 for group O, and ABO5AOs and ABO312 for groups A and O), the samples were amplified with a combination of primers ABO52N and ABO313 (758 bp), or ABO5Os or ABO5ABs and ABO36 (181 bp or 182 bp) and were purified by centrifuge filtration (Ultrafree C3HK and C3LTK, Nihon Millipore Ltd., Tokyo, Japan). The purified DNA was sequenced by the cycle-sequencing method with the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems Division, Perkin-Elmer Japan, Japan) containing forward primer (ABO52N and ABO57, or ABO5Os or ABO5ABs). The sequencing procedure was performed essentially according to the manufacturer's recommendations. DNA sequencings were performed using an ABI 373A automated DNA sequencer (Applied Biosystems Division, Perkin-Elmer Japan, Japan). A reverse sequencing primer (ABO32N and ABO313, or ABO36) was also used and sequences were determined for both strands.

*Transferase activity*

Plasma was separated from fresh blood sample collected in EDTA. The transferase activity was tested using Galserve AB (Sumitomo Seika Chemicals, Tokyo, Japan). The activity titers were compared with those from normal controls. The procedure was performed according to the manufacture's recommendations. Transferase activity measurement was based on the principle that transferase from group B (group A) plasma can catalyse the transfer of *N*-acetylgalactosamine (galactose) from UDP-*N*-acetylgalactosamine (UDP-galactose) to 2'-fucosyllactose (a low molecular weight analogue of H) to form an A-active (a B-active) structure.

## Results

We studied the genotypes of the ABO blood group using the differences between *A* and *B* alleles at nucleotide positions 297, 526, 657, 703, 793, 803 and 930, and a single nucleotide deletion site at position 261 in *O* allele. Five polymorphic positions 467, 646, 681, 771 and 826 were also investigated. The results of genotyping by PCR-RFLP method perfectly coincided with the serological typing results in all subjects. The serologically determined phenotype frequencies of 340 Japanese individuals were $A_1$; 0.3588 ($n$=122), B; 0.2324 ($n$=79), O; 0.2912, ($n$=99) and $A_1B$; 0.1176 ($n$=40), respectively. The $A_2$ phenotype was not observed in this study. The genotype frequencies obtained from PCR-RFLP analysis were *AA*; 0.0647 ($n$=22), *AO*; 0.2941 ($n$=100) *BB*; 0.0294 ($n$=10), *BO*; 0.2029 ($n$=69), *OO*; 0.2912 ($n$=99), and *AB*; 0.1176 ($n$=40), respectively (Table 2). The allele frequencies estimated from the phenotype frequencies of the ABO blood group were *A*; 0.2738, *B*; 0.1919, and *O*; 0.5343, respectively. The observed allele frequencies from genotype data obtained by the PCR-RFLP method were *A*; 0.2706, *B*; 0.1897 and *O*; 0.5397, respectively. There was no significance ($\chi^2$=0.7476) between the estimated allele frequencies from serological data and those from the PCR-RFLP (Table 2). The *O* allele with no nucleotide deletion at position 261 was not found in this study.

**Table 2** The phenotype and genotype frequencies in a Japanese population

| Phenotype | Observed number | Frequency | Expected number | $\chi^2$ | Genotype | Observed number | Frequency |
|---|---|---|---|---|---|---|---|
| A | 122 | 0.3588 | 124.9553 | 0.0699 | *AA* | 22 | 0.0647 |
| | | | | | *AO* | 100 | 0.2941 |
| B | 79 | 0.2324 | 82.2520 | 0.1286 | *BB* | 10 | 0.0294 |
| | | | | | *BO* | 69 | 0.2030 |
| O | 99 | 0.2912 | 97.0635 | 0.0386 | *OO* | 99 | 0.2912 |
| AB | 40 | 0.1176 | 35.7292 | 0.5105 | *AB* | 40 | 0.1176 |
| Total | 340 | 1.0000 | 340.0000 | 0.7476 | | 340 | 1.0000 |

In the blood group $A_1$, three alleles, *A0101*, *A0102* and *A0103*, were observed in this study (Figure 2). We tentatively named the allele with cytosine at nucleotide position 467 (proline at amino acid residue 156) as the *A0101* allele and that with thymine (leucine at amino acid residue 156) as the *A0102* allele. The *A0101* allele sequence was identical to that of the cDNA clone FY-66-1 (Yamamoto and others, 1990a), the *ABO*A101* allele (Ogasawara and others, 1996) and *ABO*A(Pro)* (Fukumori and others, 1996). We consider the sequence of this allele (*A0101*) as the reference, and the difference from this sequence will be mentioned for the remaining alleles. The *A0102* had an identical sequence to that of the cDNA clone FY-59-5 (Yamamoto and others, 1990a), the

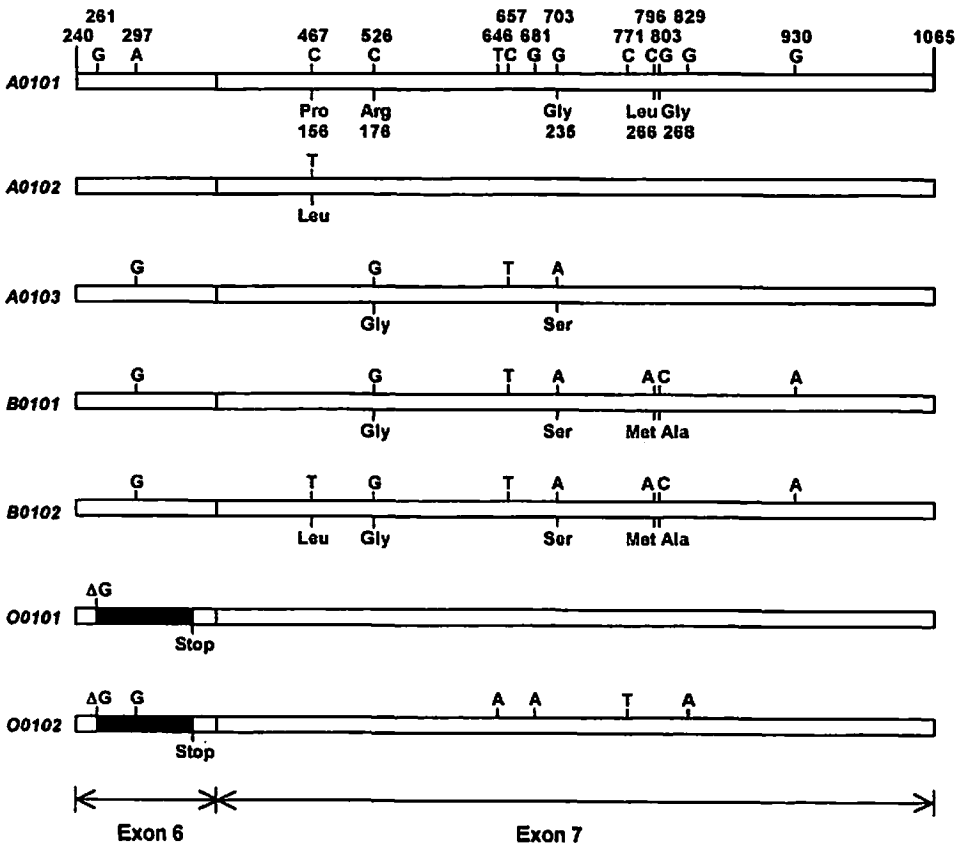


**Figure 2** Schematic comparison of nucleotide and deduced amino acid sequences of the *ABO* alleles found in this study. The adenine residue and the methionine residue of the initiation codon of *A0101* transferase are numbered 1 for nucleotide and amino acid sequences, respectively. Only the differences from the nucleotide and amino acid sequence of *A0101* allele are indicated. An entirely different deduced amino acid sequence in *O* alleles are due to frame-shifting cased by single-base deletion, indicated by black bars.

*ABO*A102* allele (Ogasawara and others, 1996) and *ABO*A(Leu)* (Fukumori and others, 1996). The *A0102* allele was found in 73.0% of the phenotype $A_1$ (89/122) and the allele frequency was 0.1971, and the *A0101* allele was found in 25.4% of $A_1$ (31/122) and the allele frequency was 0.0691 (Table 3). In the *B* and *O* alleles, the sequences of cytosine type at nucleotide position 467 were found in 98.7% of the phenotype B (78/79) and 100% of the O (99/99). Therfore, cytosine type was common in *B* and *O* alleles.

In an *A* allele, four nucleotide differences at nucleotide positions 297 (adenine versus guanine), 526 (cytosine versus guanine), 657 (cytosine versus thymine) and 703 (guanine versus adenine) were confirmed in this study (Figure 2). Those differences correspond to two nonsynonymous differences (residues 176, leucine versus proline and 253, glycine versus serine) and two synonymous differences (at nucleotide positions 297 and 657). This *A* allele (*A0103* allele) that showed the B allelic pattern at these nucleotide positions was found in 0.0164 of A individuals (2/122) and 0.0256 of AB (1/39). The allele frequency of the *A0103* was 0.0044 and it is rare allele in Japanese population (Table 3).

One case of *B* allele ($n$=129) showed a single nucleotide difference at position 467 (cytosine versus thymine) and we tentatively named it as *B0102* allele (Figure 2). The allele frequency of *B0102* was 0.0015 in Japanese population used in this study (Table 3).

Four nucleotide differences were reported at positions 646 (thymine versus adenine), 681 (guanine versus adenine), 771 (cytosine versus thymine) and 829 (guanine versus adenine) in an *O* allele (Ogasawara and others, 1996; they named it as *ABO*O201*) and we tentatively named it as *O0102* allele (Figure 2). This *O0102* allele was found in 0. 520 of the *AO* (52/100), 0.3623 of the *BO* (25/69) and 0.7374 of the *OO* (73/99) genotypes, respectively and the allele frequency was 0.2471 in Japanese population (Table 3).

The restriction maps of new alleles reported in this paper are illustrated in Figure 3.

**Table 3** The allele frequencies of ABO gene in a Japanese population

| Allele | *n* | Frequency |
|---|---|---|
| *A0101* | 47 | 0.0691 |
| *A0102* | 134 | 0.1971 |
| *A0103* | 3 | 0.0044 |
| Subtotal | 184 | 0.2706 |
| *B0101* | 128 | 0.1882 |
| *B0102* | 1 | 0.0015 |
| Subtotal | 129 | 0.1897 |
| *O0101* | 199 | 0.2926 |
| *O0102* | 168 | 0.2471 |
| Subtotal | 367 | 0.5397 |
| Grand total | 680 | 1.0000 |

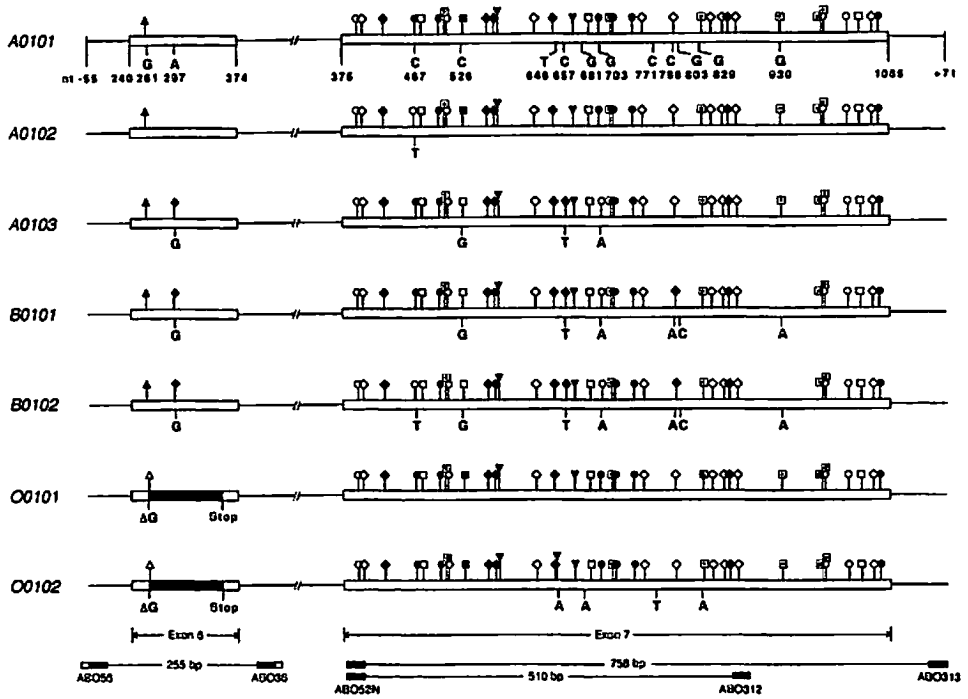


**Figure 3** Restriction enzyme maps of seven *ABO* alleles for exons 6 and 7. *Bst*EII (▲), *Kpn*I (△), and *Mae*II (◈) restriction enzyme site are illustrated in exon 6, and *Ban*I (□), *Bss*HII (■), *Alu*I (○), *Msp*I (●), *Mva*I (◇), *Nla*III (◆), *Sau*3AI (▼), and *Tse*I (▣) sites are in exon 7. Nucleotide differences are also indicated.

*Transferase activity*

We compared the transferase activity titers with the common alleles (*A0101* and *B0101*) and the rare alleles (*A0102*, *A0103* and *B0102*). These transferase titers were $2^4$ or $2^5$ in the plasmas from *A0102* allele ($n$=10) and from *A0103* allele ($n$=3) as well as $2^4$ or $2^5$ in the plasmas from *A0101* allele ($n$=20). The titer of the transferase from *B0102* ($2^6$) was the same as *B0101* allele ($2^5$ to $2^7$, $n$=5).

## Discussion

Since Yamamoto and others (1990a) reported the sequences of the *ABO* alleles, analyses for the genotype have been investigated (Ugozzoli and Wallace, 1992; O'Keefe and Dobrovic, 1993; Crouse and Vincek, 1995; Fukumori and others, 1995; Stroncek and others 1995). It has become possible to examine whether the estimated frequencies calculated from the ABO phenotypes agreed with the observed allele frequencies from the genotypes. Recently, the evolution and the presence of the polymorphism of the *ABO* genes have been investigated (Yamamoto and others, 1992, 1993a, 1993b, 1993c;

**Table 4** The ABO allele frequencies in Japanese, Korean, Thai and German populations

| Population | ABO allele | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *A0101* | *A0102* | *A0103* | $A_1$ (Total) | *B* | *O0101* | *O0102* | *O0201* | *O* (Total) |
| Japanese (Tokyo) (n=340) | 0.069[a,d] | 0.197[c,d] | 0.004 | 0.271[c] | 0.190[d] | 0.293[b,d] | 0.247 | 0.000[d] | 0.540[d] |
| Japanese (Osaka) (n=520) | 0.071 | 0.216 | 0.000 | 0.277 | 0.178 | 0.273 | 0.262 | 0.000 | 0.535 |
| Japanese (Yamagata) (n=104) | | 0.289* | | 0.289 | 0.178 | 0.303 | 0.231 | 0.000 | 0.534 |
| Korean (n=253) | 0.022 | 0.209 | 0.000 | 0.231 | 0.209 | 0.360 | 0.201 | 0.000 | 0.561 |
| Thai (n=114) | 0.066 | 0.118 | 0.000 | 0.184 | 0.206 | 0.338 | 0.272 | 0.000 | 0.610 |
| German (n=169) | 0.213 | 0.077 | 0.000 | 0.290 | 0.047 | 0.426 | 0.216 | 0.021 | 0.663 |

* These three alleles were not distinguishable in that study
[a] Japanese (Tokyo) versus Korean, $p<0.001$
[b] Japanese (Tokyo) versus Korean, $p<0.05$
[c] Japanese (Tokyo) versus Thai, $p<0.01$
[d] Japanese (Tokyo) versus German, $p<0.001$

Grunnet and others, 1994; Yamamoto and others, 1994; Clausen and others, 1994; Ogasawara and others, 1996; Saitou and Yamamoto, 1997). The present study is an attempt to elucidate the polymorphism of the *ABO* gene in a population in Japan.

The observed allele frequencies from genotype data obtained by the PCR-RFLP method were *A*; 0.2706, *B*; 0.1897 and *O*; 0.5397, respectively. There was no significant difference ($\chi^2$=0.7476) between the expected and the observed data. The present allele frequencies did not have significant difference from those of Fukumori and others (1996) and Watanabe and others (1997) (Table 4). Phenotype frequencies fitted to the Hardy-Weinberg expectation.

In the present analysis of the polymorphism, four mutant alleles were found in the *ABO* locus. The glycosyl transferase activities were not different between common and rare alleles.

The *A0102* (position 467 is thymine) allele was found in 89 of 122 individuals of blood type A (0.730) and the allele frequency was 0.1971 in Japanese population. The ratio of the *A0101* (nucleotide position 467 is cytosine) and *A0102* alleles almost agreed with the data reported by Fukumori and others (1996). In 169 unrelated German the frequency of *A0101* allele was higher than *A0102* allele (Kang and others, 1997). On the other hand, Fukumori and others (1997) have proved the presence of high frequency of the *A0102* allele in Korean and Thai populations (Table 4). Consequently, it is suggested that *A0102* in the *A* type alleles is a common allele in Asian populations. The *A0102* allele frequency in northern Asia is higher than that of the Southeast Asia, and the *A0101* allele frequency in Korean was lower than those of the other Asian populations. We conjecture that the *A0102* allele originated in the northern Asia migrated to Japan through Korea peninsula and to Europe through the Silk Road, respectively. On the other

hand, the *A0101* allele in Europe was introduced to Japan through the Southeast Asia.

Three *O* alleles have been identified previously. The first *O* allele (tentatively named as *O0101*) is characterized by a single nucleotide deletion at 261G in exon 6 of the sequence of the *A* allele (Yamamoto and others, 1990a, 1993d; Grunnet and others, 1994). The second has the same deletion and four nucleotide differences at positions 646, 681, 771 and 829 (Olsson and Chester, 1996; Ogasawara and others, 1996), and we tentatively named it as *O0102*. The final *O* allele is specified by no deletion at nucleotide position 261G and has a nucleotide difference at 802 (Yamamoto and others, 1993c; Grunnet and others, 1994; Olsson and Chester, 1995). The *O0102* allele was found in 0.520 of the *AO* (52/100), 0.3623 of the *BO* (25/69) and 0.7374 of the *OO* (73/99) genotypes, respectively, and the allele frequency was 0.2471 in Japanese population. The ratio of the *O0102* allele to *O0101* allele in our data of the central Japan (*O0101*:*O0102* =1:0.845) was lower than in the western Japan (Osaka, 1:0.950) (Fukumori and others, 1996) and higher than in the northern Japan (Yamagata, 1:0.778) (Watanabe and others, 1997). There may be a geographical cline for these two allele frequencies in Japan. However, there was no significant difference among three regions in Japan in all *ABO* alleles (Table 3). Non-deletion type *O* allele is reported in European population, but it was not detected in Japanese population in this study.

Furthermore, we observed two new alleles, tentatively named as *A0103* and *B0102*. The *A0103* allele which had four nucleotide differences at positions 297 (adenine versus guanine), 526 (cytosine versus guanine), 657 (cytosine versus thymine) and 703 (guanine versus adenine) was identified in three Japanese individuals. This *A0103* allele was not found in previous studies (Watanabe and others, 1997; Ogasawara and others, 1998). The DNA sequence of this *A0103* allele from nucleotide positions 297 to 703 were same as the *B* type alleles. The allele frequency of the *A0103* was 0.0044. Fukumori and others (1996) have described that the *A0103* allele has not been confirmed in Osaka region (western Japan). They have also reported that the allele has not been found in Korean, Thai nor German populations (Fukumori and others, 1997; Kang and others, 1997) (Table 4). Consequently, the *A0103* allele is truly a new allele in the *ABO* blood group locus.

The new *A0103* allele may be generated by a recombination of two segments originated from *B* allele and *A* or *O* allele, as in the case of a recombinant allele reported by Suzuki and others (1997). They described that a child having *A* allele was born from mother of *BO* genotype and father of *OO* genotype, and concluded that the recombinant allele encodes a transferase with $A_1$ specificity, resulting from de novo recombination between the *B* and *O* allele of the mother during meiosis. They also reported that such recombinant alleles were found in 0.009 of a Japanese population. The transferase activity derived from the *A0103* allele was the same as that of the common A transeferase.

Second new allele (*B0102*) showed a nucleotide difference (cytosine versus thymine) at position 467. This allele was confirmed by sequencing that the remaining nucleotides

were same as those of common *B* allele. The frequency of *B0102* allele was 0.0015 in this study. This *B* allele have not been previously reported in Japanese and Caucasian. The transeferase activitiy of this allele was the same as that of the common *B* allele.

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