Recombination and gene conversion-like events may contribute to \textit{ABO} gene diversity causing various phenotypes

\textbf{Abstract} We identified five different alleles, tentatively named \textit{ABO}*-O301, *O302, *R102, *R103, and *A110, in Japanese individuals possessing the blood group O phenotype. These alleles lack the guanine deletion at nucleotide position 261 which is shared by a majority of O alleles. Nucleotide sequence analysis revealed that *O301 and *O302 had single nonsynonymous substitutions compared with *A101 or *A102 responsible for the A\textsubscript{1} phenotype. Analysis of intron 6 at the \textit{ABO} gene by polymerase chain reaction-single-strand conformation polymorphism and direct sequencing revealed that *R102 and *R103 had chimeric sequences of A-O2 and B-O2, respectively, from exons 6 to 7. In the analysis of five other chimeric alleles detected in the same manner, we identified a total of four different recombination-breakpoints within or near intron 6. When 510 unrelated Japanese were examined, the frequency of the chimeric alleles generated by recombination in intron 6 or exon 7 was estimated to be 1.7\%. In addition, we found that *O301, *A110, *C101, *A111, and 35\% of *A102 had a unique A-B-A chimeric sequence at intron 6, presumed to originate from a gene conversion-like event. We had previously established that *A110 also had an A-O2-A chimeric sequence around nucleotide position 646 in exon 7. Thus this allele has an A-B-A-O2-A chimeric sequence from intron 6 to exon 7 probably generated by two different gene conversions. Similar patchwork sequences around nucleotide position 646 in exon 7 were observed in two other new alleles responsible for the A\textsubscript{2} and B\textsubscript{2} phenotypes. Thus, the site is presumably a hotspot for gene conversion. These results indicate that both recombination and gene conversion-like events play important roles in generating \textit{ABO} gene diversity.

\textbf{Keywords} \textit{ABO} · Chimeric allele · Polymorphism · Recombination · Gene conversion

\textbf{Introduction}

Since the genes encoding transferases synthesizing ABO antigens were cloned and sequenced (Yamamoto et al. 1990a, 1990b), several ABO alleles have been identified (Olsson and Chester 1996; Olsson et al. 1995; Yamamoto et al. 1992, 1993a, 1993b, 1993c, 1993d). With the accumulation of different ABO alleles, the allele-phenotype relationship as well as population-specific alleles have been elucidated (Franco et al. 1994; Grunnet et al. 1994; Kang et al. 1997). To date, we have identified 27 different ABO alleles in healthy Japanese individuals possessing the common and variant ABO phenotypes (Ogasawara et al. 1996a, 1996b, 1998). Based on these findings, we proposed a numerical nomenclature system for the ABO alleles, according to the guidelines for human gene nomenclature (Shows et al. 1987) in order to avoid confusion. In the Japanese population, *A101,
*A102, *Q101, *Q201, and *B101 are the common alleles and most of the other alleles have only one or two nucleotide differences, such as a substitution, deletion, or insertion, in one of these five alleles. On the other hand, we were the first to discover putative hybrid ABO alleles named *R101 and *A110 (Ogasawara et al. 1996b). *R101 has a chimeric sequence that may have originated from recombination between *B101 and *Q201 around nucleotide positions 703–771 in exon 7. This allele is responsible for the A2 phenotype in heterozygotes with B alleles, and for the A1 phenotype in heterozygotes with O alleles (Ogasawara et al. 1998). The other chimeric allele, *A110, has a sequence identical to that of *A102 except for the replacement of the nucleotide sequence around 646–681 in exon 7 by that of *Q201. Thus, *A110 likely originated from a gene conversion-like event. Subsequently, Olsson and co-workers (1997) identified five different chimeric alleles by analyzing the sequences of exons 3–7 in the ABO gene.

More recently, Suzuki and co-workers (1997) determined the nucleotide differences at a total of 20 sites in intron 6 of four major ABO alleles and identified three different chimeric alleles. These three alleles have sequences identical to our *A104, *Q202, and *Q103 at exons 6 and 7 (Ogasawara et al. 1996) and have recombination junction regions in or just downstream of intron 6. Similar chimeric alleles responsible for the A2 phenotype and possessing recombination junction regions in intron 6 have also been reported by Olsson and Chester (1998). These reports indicate that there are at least four different recombination breakpoints in or just downstream of intron 6. Because intron 6 has Chi and Chi-like sequences (Suzuki et al. 1997), this region could be a hotspot for genetic recombination (Smith et al. 1980, 1981).

In this study, we identified new O alleles lacking the guanine deletion at nucleotide position 261 which is shared by a majority of O alleles. These alleles are presumed to be generated from common ABO alleles by substitution, recombination, or gene conversion. Additional analysis of previously and newly identified alleles enabled us to predict hotspots for recombination and gene conversion occurrences in intron 6 and exon 7 of the ABO gene.

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**Materials and methods**

**Blood samples**

All blood samples except for those of four A2-positive Caucasian individuals (genotype *A105/*O) were obtained from Japanese individuals. The blood sample from a serum anti-A antibody-deficient O phenotype individual (SR) was selected by routine serological testing. Eleven blood samples from O phenotype individuals possessing a weak anti-A antibody were selected out of 54,287 blood samples from unrelated donors by the agglutination test using A2-positive red cells. In most cases, the serum anti-A antibodies from group O individuals agglutinated A2-positive red cells as well as A1-positive red cells. However, these 11 samples either did not or only faintly agglutinated A1-positive red cells. These blood red cell (RBC) samples were subjected to an absorption-elution test using polyclonal anti-A antibodies (Dade, Miami, Fla.) to distinguish the O phenotype sharing the weak serum anti-A antibody from the A2 phenotype. Blood samples from 1,302 group O individuals possessing serum anti-A antibody with common agglutinability were selected for comparison. In addition, blood samples from 510 unrelated individuals were also examined for ABO gene polymorphism to estimate allele frequencies. Other blood samples sharing hybrid ABO alleles (*A112, *A113, *B108, and *B109 responsible for phenotypes A1, A2, B, and B3, respectively) were collected from individuals in our laboratory or were kindly provided by the Aichi Red Cross Blood Center. Blood samples of the HM family were kindly provided by the Yamaguchi Red Cross Blood Center.

**ABO gene analysis**

Genomic DNA was prepared from peripheral blood samples from Japanese donors by standard phenol-chloroform extraction (Sambrook et al. 1989) or the Tween 20/protease K method (Mitsunaga et al. 1995). Exons 6 and 7 of the ABO gene (Bennett et al. 1995; Yamamoto et al. 1995) were amplified by polymerase chain reaction (PCR) using four primer pairs under appropriate conditions, and the four fragments (fragments I–IV) obtained were subjected to single-strand conformation polymorphism (SSCP) analysis, as described previously (Bannai et al. 1994; Ogasawara et al. 1996a). The amplified DNA fragments were diluted at 1:7 in denaturing solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF), heated at 95°C for 5 min, and then immediately chilled in ice; 1 µl of the mixture was subjected to polyacrylamide gel electrophoresis. The separated single-strand DNAs in the gel were detected by silver staining (Daichi Pure Chemicals, Tokyo, Japan). The entire intron 6 was analyzed by PCR-SSCP using three primer pairs, as shown in Table 1.

For analysis of genomic DNAs obtained from 1,302 blood samples of group O individuals, exon 6 of the ABO gene was amplified using primer pair ABO-1 and ABO-2 (Ogasawara et al. 1996a) by PCR and digested with Kpn1 (Takara, Tokyo, Japan) to detect deletion of guanine at nucleotide position 261 (Yamamoto et al. 1990b). When the amplified fragment remained undigested, PCR-SSCP analysis and group-specific PCR (Ogasawara et al. 1996a) for the alleles lacking the guanine deletion were performed. The amplified DNA fragments from single alleles were then subjected to PCR-SSCP and/or sequence analyses.

**Provisional ABO allele nomenclature based on intronic sequence**

To understand ABO gene diversity, classifying the ABO alleles according to the variation in their intronic sequences is important, even though their sequences are identical at the exon level. In these cases, a new allele name was created by adding a number to the end of the original allele name after the period.

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**Results and discussion**

**O alleles lacking guanine deletion at nucleotide position 261**

Genomic DNA from the individuals with the serum anti-A-deficient O phenotype (SR) was analyzed by PCR-SSCP. We detected a previously unidentified allele, provisionally called *O301 according to our