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The use of DNA analyses for subtyping Aend or Bm in ABO blood group system

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Abstract

We sequenced genomic DNA of Aend and Bm subtypes. Our data showed that sequences of the Aend allele from two of Aend and three of AendB samples were identical to those of A_1 allele. And sequences of the Bm allele from two of Bm and one of ABm samples were identical to those of B allele. These sequence data could not be accepted for subtyping A_1 or B blood group.

Keywords: ABO blood group subtypes; Genotype; Sequence; A allele; B allele

1. Introduction

In 1990, Yamamoto et al. [1] elucidated the molecular genetic basis of the three major alleles (A_1, B, and O) at the blood group ABO gene locus by cloning A_1 transferase cDNA, and by cloning B and O alleles followed by nucleotide sequencing. The A_1, B and O alleles are sequenced [1,2]. In addition, another O allele (O^2) without the nucleotide 261 deletion characterized by typing as B allele at position 526 and A allele at positions 703, 796, and 803 has been reported [3,4].

Since then, rare A or B subtypes such as A2, A3, Ax, Aend, Ael, Am, B3, Bx, Bm, Bel and *cis*-AB have been analyzed [5–13]. Some of these rare samples showed differences from the original A_1 and B sequences, while other did not. It is not known at present, however, whether these sequence data can be accepted for subtyping or not.

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The purpose of this study is to achieve an evaluation of the sequence for rare ABO subtyping. In this paper, we sequenced genomic DNA of Aend and Bm subtypes. We found that these subtypes did not possess any specific mutation.

2. Material and method

2.1. DNA

Saliva samples obtained from Japanese ABO subtype individuals were used for DNA preparation. Genomic DNA was extracted by the phenol–chloroform method.

2.2. Primers

Pairs of primers are used for PCR in four regions. Primer 1E and primer 2E [14] were used for the amplification of exon 6 in the ABO genes (size 217 bp, named 6-1 region). Primer 3 [15] and ABOi7con [16] were used for the amplification of exon 7 (size 577 bp, named 7-3 region). Four pairs of primers were also used for sequence-specific PCR of exon 7: GA01N and GA13 (424 bp, named 7-1 region) [17], and ABOx7B [16] and ABOi7con (461 bp, named 7-2 region) [16] were used for group B. GA01N and GA14 (425 bp, named 7-1 region) [17], and ABOx7not B and ABOi7con [16] (461 bp, named 7-2 region) for group not B.

2.3. PCR

Template DNA samples were subjected to amplification in a final volume of 50 μl containing 0.2 pmol/μl each pair of primers, 1 × PCR buffer (GIBCO, BRL), 0.05% W-1 (GIBCO, BRL), 1.5 mM $MgCl_2$ (GIBCO, BRL), 0.2 mM dNTPs (Promega) and 1 unit Taq polymerase (GIBCO, BRL). PCRs were performed under the following conditions: 35 cycles of 94 °C for 2 min, 63 °C 2 min and 72 °C 3 min in 6-1 region, 35 cycles of 94 °C for 1 min, 62 °C 2 min and 72 °C 3 min in 7-1 region, 35 cycles of 95 °C for 1 min, 63 °C 1 min and 72 °C 1 min in 7-2 region, 35 cycles of 94 °C for 1 min, 62 °C 2 min and 72 °C 3 min in 7-3 region.

2.4. Sequencing

PCR products of exon 6 (6-1 region) or exon 7 (7-1, 7-2 and 7-3 regions) regions were sequenced using ABI Prism 377 sequencer and BigDye Terminator cycle sequencing v2.0 Ready Reaction.

3. Results and discussion

Our data showed that sequences of the Aend allele from two of Aend and three of AendB samples were identical to those of A_1 alleles, subdivided A102 allele reported by

Table 1
Variations in nucleotide sequence compared to the A102 and B101 alleles

Allele	Allele[a]	Exon 6		Exon 7											Ref.
		261	297	467	526	657	703	796	802	803	826	930	1059–61	1065	
A_1	A101	G	A	C	C	C	G	C	G	G	G	G	CCC	A	[1,11]
A_1	A102	*	*	T	*	*	*	*	*	*	*	*	*	*	[11]
Aend		*	NT	T	*	NT	*	NT	NT	NT	NT	NT	CC	NT	[6]
Aend	A102	*	*	T	*	*	*	*	*	*	*	*	*	*	This work
B	B101	*	G	*	G	T	A	A	*	C	*	A	*	*	[1,11]
Bm	B101	*	G	*	G	T	A	A	*	C	*	A	*	*	This work

[a] Named by Ogasawara et al. [11].

Ogasawara et al. [13]. And sequences of the Bm allele from two of Bm and one of ABm samples were identical to those of B alleles, subdivided B101 allele [13]. Incidences of Aend and Bm phenotypes are infrequent, while those of sequenced alleles are frequent. We found that our Aend and Bm subtypes did not possess any specific mutation. Some of A2, A3, Ax, Ael, Am, B3, Bx, Bel and *cis*-AB were reported to show differences from the original A_1 (A101 allele described by Ogasawara et al. [13]) and B sequences (B101 allele). However, their constant mutation for subtyping were not always found. There might be two types among subtype. One shows specific mutation, and the other did not show any mutation (Table 1).

In conclusion, ABO blood group subtyping based on the sequence data is not always accepted, although the possibility remains that some of these subtypes may be determined by the differences from the original A and B sequences.

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