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SHORT COMMUNICATION

Characterization of a novel *B(A)* allele with BBBA type at the ABO blood group

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Abstract The ABO blood group is one of the main blood group systems, and it plays an important role in transfusion medicine and transplantation. To date, most of the many ABO subgroups with a weak expression of the A or B antigen on red blood cells have been elucidated to have specific molecular genetic background with respect to the *ABO* gene. The *ABO*B(A)* allele or *CisAB* allele is a type of dual *ABO* allele which can encode glycosyltransferases responsible for the conversion of H substance to both A and B antigen. We report here our characterization of a novel *B(A)* allele which differs from those reported previously.

Keywords ABO · Allele · Chinese · Novel *ABO*B(A)* allele

Introduction

The ABO blood group is one of the main blood group systems that plays an important role in transfusion medicine, transplantation and paternity testing. In addition to four common ABO phenotypes, many ABO subgroups have been found to have a weak expression of the A or B antigen on red blood cells (RBCs). To date, more than 100 *ABO* alleles within the coding region of the *ABO* gene have been reported in the literature; these can explain the weak expression (Chester and Olsson 2001; Olsson and Chester 2001; Yip 2002; Seltsam et al. 2003a, b). The mechanisms which lead to numerous polymorphisms found in various *ABO* alleles include substitution, gene conversion and recombination.

Yamamoto et al. (1990a) were the first researchers to analyze the molecular background of the three main alleles: *A101*, *B*, and *O*. The *ABO* gene consists of seven exons, each of which ranges in size from 28 to 688 bp, with most of the coding sequence lying in exons 6 and 7. The DNA of the *A101* allele comprises a 1062-bp nucleotide (nt) sequence that has been calculated to encode a 41-kDa enzyme protein. The A, B, and O cDNA structures are highly homologous (Yamamoto et al. 1990b). Compared with the reference *A101* allelic sequence, the *O01* allele has a single nucleotide deletion, G261, in exon 6. This results in a stop codon after nt 352 and premature termination of translation after amino acid 117, which explains the recessive nature of the *O* allele. The cDNA from the *B101* allele differs consistently from that of *A101* by seven single nucleotide substitutions at nt 297, 526, 657, 703, 796, 803, and 930, which results in four amino acid changes (Arg176-Gly, Gly235Ser, Leu266Met, Gly268Ala) in the expressed protein. Changing these four amino acids alters the specificity of transfer from GTA (A-synthesizing α1–3 N-acetylgalactosaminyltransferase) to GTB (B-synthesizing α1–3 galactosyltransferase).

We report here our molecular analysis of an individual diagnosed with the AwB subgroup by serological testing. The sequencing of the *ABO* alleles allows the molecular basis of the *ABO* variant allele to be elucidated. This knowledge is, in turn, helpful in revealing the effect of certain amino acid variations on

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the serological specificity or the activity of the glycosyltransferase.

Materials and methods

A discrepancy between red blood cell (RBC) and plasma ABO grouping results was observed in a 31-year-old male Chinese donor from HeNan, China. Additional testing was subsequently carried out on the donor's family (father and his brother only as his mother was dead). Informed consent was obtained before collecting the blood samples. Venous blood was collected according to standard blood banking practice: 5 mL of blood (no additives in tube) was collected for ABO blood grouping; 5 mL of EDTA blood was collected for DNA testing.

Serological methods

ABO typing tests, including RBC and serum blood grouping procedures, and the saliva substance test were carried out together with the serological standard as described previously. The antisera used included three monoclonal anti-A and anti-B reagents (Dominion, N.S., Canada; Bioscot, Livingston, UK; Brother, ChangChun, China), polyclonal anti-A (blend of human serum), anti-A1 from *Dolichus biflorus* (Lectin, Dominion), and monoclonal anti-AB (Immucor, Norcross, USA). Lectins were used for anti-H from *Ulex europaeus* (Dominion). All reagents were used according to the manufacturers' instructions.

Direct sequencing of Exon 6 and 7

Because 77% of the protein and 91% of the catalytic active part can be found in exons 6 and 7, PCR-based gene analyses were performed on these two exons. Primer pairs mol-46/mol-57 and mol-71/mol-101 [described in Olsson and Chester (1996)] were used to amplify exons 6 and 7. The PCR fragments for exons 6 and 7 were 252 (251 bp for *O1*) and 843 bp, respectively. PCR amplification was carried out in a 50-μL reaction volume containing PCR buffer, 400 μM of each dNTP, 0.2 μM each primer, 300–500 ng of genomic DNA, and 0.44 U *Taq* DNA polymerase (Promega, Madison Wis.). Amplification was carried out under the following conditions: one cycle of 95°C for 10 min; ten cycles of 94°C for 60 s, 63°C for 90 s, and 72°C for 60 s; 25 cycles of 94°C for 60 s, 61°C for 90 s and 72°C for 60 s; this was followed by a final elongation cycle of 72°C for 10 min. The purified products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif.) on an ABI PRISM 3100 DNA Sequencer.

Cloning and haplotype sequencing of exon 6, intron 6, and exon 7

A 2170-bp fragment spanning a region from intron 5 to exon 7 was amplified by the following primer pairs: 5′-CTG GAA GGG TGG TCA GAG GA-3′, and 5′-GTT ACT CAC AAC AGG ACG GAC-3′. The amplification was carried out in a 50-μL reaction volumecontaining 2× GC buffer I/II,100 μM each dNTP, 0.1 μM each of two primers, 500 ng of genomic DNA, and 1 U of LA *Taq* polymerase (Takara, Dalian, China). The PCR conditions were: one cycle of 95°C for 10 min; 30 cycles of 94°C for 30 s and 60°C for 30 s; one cycle of 72°C for 150 s; this was followed by a final elongation cycle of 72°C for 10 min. The gel-purified PCR product was cloned into the pCR II vector using the TOPO Cloning kit (Invitrogen, Carlsbad, Calif.). A specific sequencing reaction was performed with the following five forward primers: F1, 5′-GGC GGC CGT GTG CCA GA-3′; F2, 5′-TTG TCC TCC CAG AGG GTA GA-3′; F3, 5′-CAA CCG CAG ACA CAT ACT TGA-3′; F4, 5′-CAG GAC GGG CCT CCT GCA-3′; F5, 5′-CCA GTC CCA GGC CTA CAT-3′.

Results

Serological phenotype

The patient with the AwB phenotype was discovered as a result of discrepancies between forward and reverse typing in routine ABO grouping. The RBCs of the sample were strongly agglutinated by all monoclonal anti-A and anti-B serum but not agglutinated with human anti-A serum and anti-A1 reagent. The serum samples contained middle-strong anti-A activity but no anti-B activity. The saliva sample contained B and H substances, but no A substance. Based only on the serological characteristics and the ABO subgroup definition, the man's phenotype can not be accurately classified and so we defined it as a AwB group. His father and brother were determined to have the common A type. The ABO phenotypes and genotypes of all family members tested are shown in Fig. 1.

Direct sequencing and cloning analysis

According to the sample's direct sequencing results and haplotype sequence, it is possible to determine the

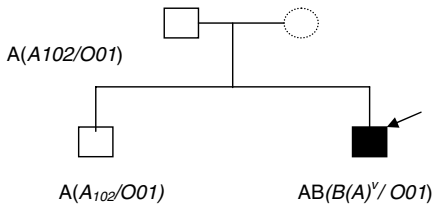

Fig. 1 Schematic diagram of the inheritance of B(A) in one Chinese family. The phenotype and genotype determined are indicated *below* each symbol. *Arrows* indicate propositi. The sample shown in *dashed circle* was not studied

nucleotide sequence of each ABO allele. We defined the ABO allele using the unofficial nomenclature system described in the Blood Group Antigen Gene Mutation Database.

When the consensus sequence of the *A101* allele was compared with one haplotype of the sample, it was determined that the latter was the *O01* allele; another haplotype was identical to it except for six mutations (297A > G,526C > G,657C > T,703G > A,796C > A, 930G > A) in exon 6 and 7 at the ABO locus. When this same haplotype was compared to the *B101* allele, another haplotype was found to be identical to it with the exception of a single nonsynonymous mutation (803C > G) in exon 7. Consequently, we could deduce that the allele under question was a *B101*-like allele with a single nt803C > G mutation. We defined the gene as a novel *ABO*B(A)* allele. The allele nucleotide sequence spanned exon 6 and exon 7 and has been deposited in GenBank with accession number DQ407740.

The sequence of the new *B(A)* allele was compared with consensus *B101* allele at nucleotide 792–809 in exon 7; both are shown in Fig. 2.

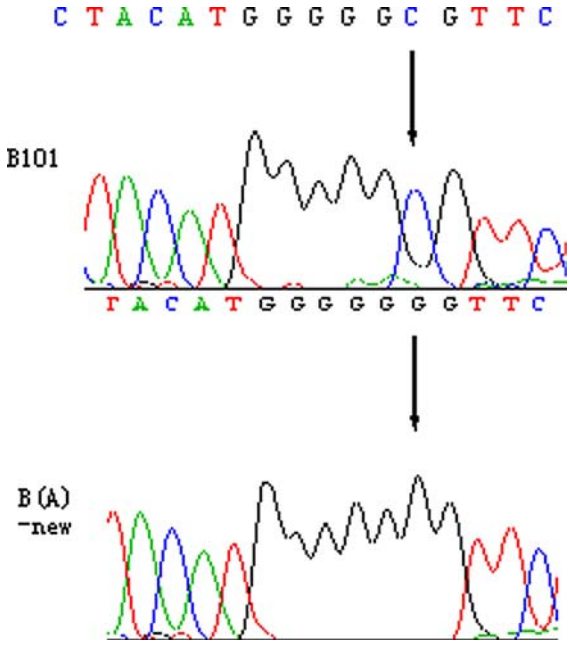

Fig. 2 Sequence of the new *B(A)* allele at nt 792–809. *Arrow* indicates the nt 803 position

Discussion

In our study, the sample phenotyped as AwB actually does not harbor an *A* allele but has instead a novel *B(A)* allele. In a serological reaction, the RBCs were strongly agglutinated by monoclonal serum but they did not agglutinate with human serum. This result provides support that patient had the B(A) phenotype. Unexpectedly, the strong expression of the A antigen on the RBCs differed from previously reports. Classical laws of genetics on inheritance provide the clarification that a person bearing the AB phenotype can be the child of a person with the O phenotype; this occurrence is especially useful in forensic medicine.

The last three of the four altered four amino acids, R176G, G235S, L266M, and G268A, alter the specificity of transfer from GTA to GTB. Theoretically, there are eight kinds enzymes (AAA, AAB, ABA, BAA, ABB, BAB, BBA, BBB types) that have been characterized with interchanges in last three of the four amino acids; these include AAAA(Arg-176, Gly-235, Leu-266, and Gly-268) or BBBB(Gly-176, Ser-235, Met-266, and Ala-268).

To date, nine *B(A)* or *cisAB* alleles, including five *B(A)* alleles and four *cisAB* alleles, have been identified and characterized. In 1993, a gene that encodes the normal GTA and also possesses trances of GTB activity was first found in Japanese woman (Yamamoto et al. 1993a, b). This allele differs from the *A102* allele in the 803G > C mutation typed as AAAB (amino acid position: 176, 235, 266, 268), which results in an amino acid alteration of Gly268Ala and leads to the A antigen having some B antigen features. Seven known *B(A)* or *cisAB* alleles exhibit mutations affecting these three amino acid residues (Gly235Ser, Leu266Met, Gly268Ala) or its neighbors (nt 700), all of which have been shown to be critical for the specificity of the transferase. *CisAB02* involves different nonsynonymous substitutions of A796C on the *B101* allele background and is typed as BBAB (Mifsud et al. 2000). *cisAB03* was typed as BBBB and is found at the nt 700 position; this mutation C > T) was found in a French family (Roubinet et al. 2002). *cisAB04* was typed as AABA (Tzeng et al. 2005); *ABO*B(A)*01 was typed as BABB; *B(A)02* was typed as BBBB with

Table 1 Mutations in exons 6 and 7 of all *ABO*B(A)* and *cisAB* alleles at the ABO locus analyzed in this study. The *ABO*A101* sequence was used as the reference sequence

	Exon of ABO allele												
	6		7										
Nucleotide position[a]	261	297	467	526	640	641	657	700	703	796	803	930	1096
A101(AAAA)	G	A	C	C	A	T	C	C	G	C	G	G	G
O01	–												
*ABO*B(A) 01*(BABB)		G		G						A	C	A	A
*ABO*B(A) 02*(BBBB)		G		G			T	G	A	A	C	A	A
*ABO*B(A) 03*(BABB)		G		G						A	C	A	A
ABO*B(A)04 *(BBBB)*		G		G	G		T		A	A	C	A	A
*ABO*B(A)05*(BBBB)		G		G		C	T		A	A	C	A	A
CisAB01(AAAB)			T								C		
CisAB02(BBAB)		G		G			T		A		C	A	A
CisAB03(BBBB)		G		G			T	T	A	A	C	A	A
CisAB04(AABA)			T							A			
B(A)-new(BBBA)		G		G			T		A	A		A	A
B101(BBBB)		G		G			T		A	A	C	A	A
Amino acid position	87	99	156	**176**	214	214	219	234	**235**	**266**	**268**	310	
Consensus	Val	Thr	Pro	Arg	Met	Met	His	Pro	Gly	Leu	Gly	Leu	
Change			Leu	Gly	Val	Thr		Ala	Ser	Met	Ala		

[a]The nucleotide positions are numbered starting from the beginning of ABO cDNA

nt 700C > G and nt 657C > T. *B(A)03* was typed as BABB with nt 657C > T (Yamamoto et al. 1993b; Yu et al. 1999; Seltsam et al. 2003a).

In 2005, *ABO*B(A)* alleles with the 640A > G or 641 T > C mutation were reported (Deng et al. 2005) that were significantly different from the *B(A)* alleles described previously. Amino acid changes at amino acid 214 position, which result from nt 640 or nt 641 mutations, are located far outside of those three amino acid residues. Obviously, mutations at the amino acid 214 position are most probably capable of altering the specificity of the ABO enzyme, and the two non-synonymous mutations can lead to B glycosytransferase shifting to B(A) glycosytransferase. Mutations in exon 6 and exon 7 of all *ABO*B(A)* and *cisAB* alleles at the ABO locus presented in study are listed in Table 1.

Six of the eight types of enzymes are characterized in this table. Theoretically speaking, the alleles of the BAA and BBA sequence type should also be found. In our study, we defined a novel *ABO*B(A)* allele with the BBBA type in a Chinese individual. Although this enzyme contains three ''critical'' amino acids associated with the production of blood group B antigen and one critical amino acid, it simultaneously utilizes the blood group A donor UDP-GalNAc and group B donor UDP-Gal.

The individual described here carries a novel *B(A)* allele that differs molecularly from all previously reported *B(A)* or *cisA*B alleles. In conclusion, the data on ABO sequences provided by this study further indicates that amino acid 268 is critical for the specificity of glycosyltransferase.

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