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Serological identification and molecular characterization of B (A) 02 subtype in patients and blood donors from Eastern China

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ABSTRACT

This study was designed to identify rare type ABO blood groups, B(A) 02, from Eastern China. Three samples with discordant serological results during routine blood type identification, and four samples from one sample family were selected. All of them were detected by serological method. The exon 6 and 7 of the ABO genes were amplified by PCR and sequenced. They were typed as AsubB by serology and as BO by genotype. In the AsubB samples, nt 700C>G mutation was detected in the B gene, which was previously defined as B(A)02 alleles. In these seven samples, six showed B(A)02/O01 and one showed B(A)02/O02. B(A)02 allele was found to be more common in this study than B(A)04, which is considered to be more frequent than B(A)02. The careful identification of rare blood types is important for the safety of clinical blood transfusion.

Keywords: B(A)02, serological identification, molecular characterization, gene mutation, rare blood type

INTRODUCTION

The human blood grouping system is complicated by the presence or lack of antigens expressed on the red blood cell membrane. The ABO blood group is a major blood classification system, which is very important for transfusion safety and as the foundation of crossmatching blood between donors and recipients[1,2]. The clinical significance of the ABO blood group system extends to a broader area beyond transfusion medicine and several reports have suggested an important involvement in the development of cardiovascular[3,4], oncological[5] and other diseases[6]. In addition to 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), which makes blood typing difficult. The human ABO blood groups are controlled by alleles at a single gene locus on chromosome 9. The elucidation of gene sequence, common polymorphisms[7,8], and genomic organization[9,10] of the ABO gene has opened another dimension that complements serological analysis by allowing determination of DNA sequence of alleles responsible for phenotypes observed[11,12].

The phenotype B(A) was firstly discovered in 1985, just after the anti-A BioClone (Ortho Diagnostic System) monoclonal antibody mixture was marketed, when it was found that anti-A reagent was reacting with RBCs that had previously been typed as B[13]. The B(A) RBCs are genetically categorized as group B and the serum of the individual contains anti-A reagent. The serum from A subgroup B individuals usually contain neither anti-A nor anti-B although they occasionally contain a weak anti-A, this difference allows discrimination of phenotypes of B(A). To date, six

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B(A) alleles have been identified and characterized, and B(A)02 is even more rare in China compared with B(A)04. Individuals with the CisAB phenotype are characterized by a non–classic mode of inheritance of A and B antigens expressed at the membrane of RBCs: A and B are not transmitted as two independent alleles but as a single allele, referred to as "CisAB"[14,15]. As rare types, B(A) and CisAB have similar performance in serological identification. The present study was designed to analyze the serological and molecular characteristics of B(A)02.

MATERIALS AND METHODS

Blood sample collection

During the period from 2014 to 2016, three samples with discordant serological results with AsubB were collected during routine blood type identification from all blood donors and patients in the General Hospital of Jinan Command, and then four samples from one sample´ family were collected. The venous blood was selected from a healthy blood donor ZMM and hospital patients ZXL and WCZ with his family members.

The study was approved by the Institutional Review Board of the General Hospital of Jinan Military Command. Informed consent was voluntarily signed by each one before blood samples collection based on the full understanding of subjects on this study.

Blood group serological typing

ABO typing tests including RBC, serum blood grouping procedures, and the saliva substance test were carried out according to latest edition of the AABB Technical manual. Forward and reverse ABO phenotyping was conducted by agglutination testing at 4 ℃ and Room Temperature[16]. The tests were performed with commercial antisera according to the manufacturer´s instructions by agglutination using murine monoclonal IgM: anti–A and anti–B (Shanghai Hemo–pharmaceutical& Biological CO, Ltd.). The adsorption–elution studies with the patients´ RBCs were carried out using human serum samples containing IgG anti–A and high–titer IgG anti–B, which were donated by Shanghai Red Cross Blood Center.

Molecular analysis of ABO exons 6 and 7

Genomic DNAs of 7 individuals with the B(A) phenotype were extracted from peripheral EDTA–treated anti–coagulated blood using a whole blood commercial kit (TIANamp Blood DNA Kit, Tiangen Biotech CO., LTD, Beijing, China). Real–Time PCR Assay was performed by ABI 7500 using the fluo–ABO subgroup genotyping kit (ABO subgroup in Chinese, Jiangsu LiBio Pharmaceutical Biotechnology Co., Ltd., China) based on SYBR Green, with the primers to detect the genotypes of B(A)02, B(A)04, cisAB01, and cisAB03. PCR with sequence–specific primers was performed for ABO blood group genotyping using two commercial kits (ABO basic; FluoGene, Inno–Train Diagnostik GmbH, Kronberg, Germany, and ABO genotyping kit; Jiangsu LiBio Pharmaceutical Biotechnology, China). The automatic software (FluoGene, technology transferred from the Germany INNO–Train Company, Jiangsu LiBio Pharmaceutical Biotechnology Co., Ltd.) was used to analyze the genotypes of A1, A2, B, O1, and O2 samples. Sequencing of 7 individuals was performed and sequence data were compared to ABO alleles listed in the RBC database [17].

RESULTS

Serological phenotype analysis

Cases of B (A) phenotype were discovered as a result of discrepancies between forward and reverse typing in routine ABO grouping by the standard hemagglutination test. The RBCs of the samples were agglutinated by monoclonal and human anti–A and anti–B reagents. The serum samples contained middle–strong anti–A activity but no anti–B activity. The ABO phenotypes of W; Family members tested were shown in ***Fig.1***. At the time of study, all the family members were healthy and had no history of hematologic disease. RBCS from the patient WCZ´s mother (GEJ) were agglutinated by anti–A and anti–B reagent, suggesting that her serum samples contained low quantities of irregular anti–A. She was initially typed B(A). WCZ´s father exhibited a classic A1 type. The propositus (WCZ), her mother (GER), her sister (WAZ), her brother (WMQ), her son (MGM), and two individuals without consanguinity were typed as AsubB by serology (***Table 1***). The results from these

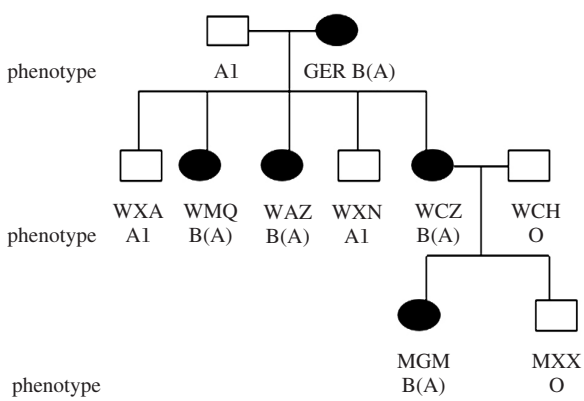

Fig.1 **Schematic diagram of the inheritance of B(A) in W. family.** The phenotypes are indicated below each symbol.

Table 1 **Rare ABO phenotypes for the W. family and the unrelated individuals**

	Forward typing							Reverse typing		
	Anti−human regent			Monoclonal regent						
Member	Anti−A	Anti−B	Anti−A1	Anti−B	Anti−H	Anti−AB	Ac	Bc	Oc	Selfc
GER	1+w	3+	2+s	4+	3+	4+	1+	0	0	0
WCZ	1+w	2+	2+s	3+	3+	4+	1+	0	0	0
WMQ	1+w	2+	2+w	3+	3+	4+	2+	0	0	0
WAZ	1+w	2+	2+w	3+	3+	4+	2+	0	0	0
MGM	1+w	2+	2+s	3+	3+	4+	1+	0	0	0
ZMM	1+w	3+	2+s	4+	3+	4+	2+	0	0	0
ZXL	1+w	3+	3+	4+	3+	4+	1+	0	0	0

w:week; s:strong

seven samples yielded the rare ABO phenotypes for AsubB.

Sequencing and gene analysis

To confirm the blood types of these samples, the genotypes of the W. Family, ZMM and ZXL were determined by amplification and direct sequencing of exon 6 and exon 7 of ABO. Commercial real−time PCR kit was used to detect B(A) DNA in sample from ZXL. The real−time PCR results confirmed B(A) 02 DNA in small volumes of blood with high concen−trations in patient ZXL (***Fig.2***). When the consensus sequence of the A101 allele was compared with one haplotype of the sample, the O01 allele was deter−mined in the samples from the W. Family and ZMM; the other haplotype of ZXL was identical to O02 al−lele, which included four mutations (646T>A 681G>A 771C>T 829G>A) in exon 7 at the ABO locus. When these 7 haplotypes were compared to the B101 al−lele, nt700C>G mutations in exon 7 were found in all the haplotypes *(**Fig.3A** and **Fig.3B**)*, while another six mutations (526C>G 657C>T 703G>A 796C>A 803G>C 930G>A) in exon 7 were found in the haplo−types of ZXL *(**Fig.3**)*. Consequently, we could deduce

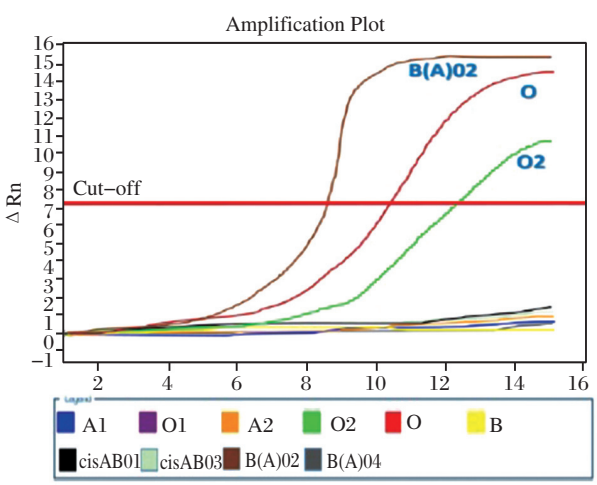

Fig 2 **Results of Real−Time PCR Assay using the fluo−ABO subgroup genotyping kit based on SYBR Green for sample ZXL.**

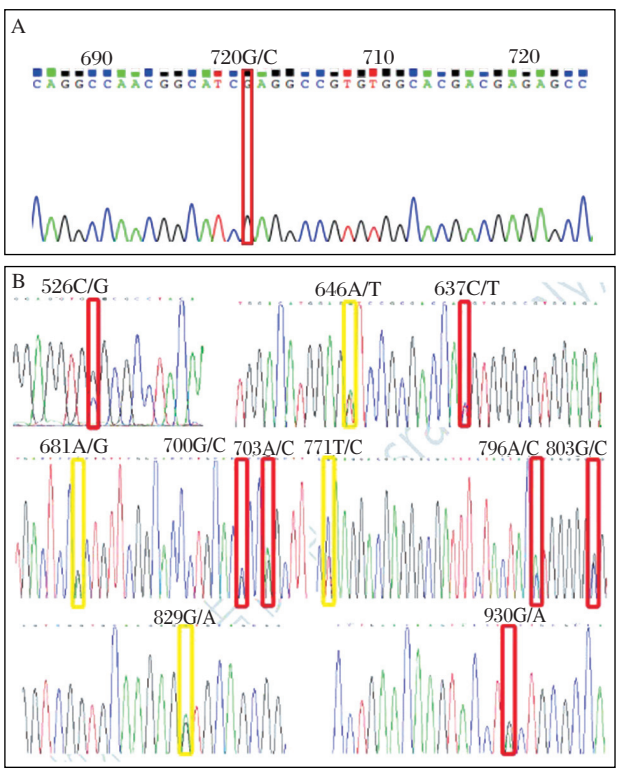

Fig 3 **Mutations in exon 7 in the haplotypes of ZXL.** A: The nt700C>G mutations in exon 7 were found in all the 7 haplotypes compared to the B101 allele. B: While another six mutations (526C>G 657C>T 703G>A 796C>A 803G>C 930G>A) were found in exon 7 in the haplotypes of ZXL.

that the alleles from all samples were B101−like allele with a single nt700C>G or 526C>G 657C>T 700C>G 703G>A 796C>A 803G>C 930G>A mutations.

DISCUSSION

The frequency of B(A) allele in the Chinese popu−lation is very rare, especially the B(A)02 in Eastern China. The frequency of B(A)04 is usually signifi−cantly higher than that of B(A)02 in China. According to the literature report, six rare alleles were researched in China from 1,716,442 non−repeat donors, which included cisAB alleles, cis−AB01, cis−AB02, cis−

AB02, B(A)02, B(A)04, and B(A)06. The frequency of B(A)04 was higher (1.6/100,000) than B(A)02 (0.78/100,000) and cis–AB01 (0.66/100,000)[18]. Interestingly, the results in the present study showed that the frequency of B(A)02 in Eastern China was significantly higher than B(A)04, which differed from previous reports. A total of 54,305 samples were typed during the study period, 3 cases of B(A)02 allele were identified with high frequency(5.52/100,000). Another four cases of B(A) 02 allele belonged to one patient´s family. The phenotype in the family compatible with B(A) was transmitted from the mother to five of her children and grandson. To confirm this hypothesis, we insisted that a final analysis conclusion was needed to explore the influence of geographical differences. The high frequency of B(A)02 may be related to gene diversity in the Chinese population in different areas, and the limited the number of samples in this study. A more accurate conclusion can be reached when more population groups are screened.

The data regarding the prevalence of different blood group antigens in any given population is always helpful in management of transfusion[19,20]. B(A) and CisAB showed AB blood type in routine serological forward group test, and similar results like "anti–H 3+––4+, anti–A 1+––2+, anti–B–" in reverse group tests. The auto antibodies were negative for samples with B(A) and CisAB. It is difficult to identify B(A) and CisAB by serological methods. The sequencing of the ABO alleles provides the molecular basis of distinguishing subtype. Such knowledge is, in turn, helpful in revealing the effect of certain amino acid variations on the serological specificity or the activity of glycosyltransferase. Sequencing analysis found that seven samples were typed as BO by genotype even though they were typed as AsubB by serology methods. Point mutation was detected in 7 samples of the B gene with 700C>G mutation, which was previously defined as B(A)02 alleles. Nt700 mutation could result in amino acid change at amino acid 234 position. Obviously, mutations at the amino acid 234 position are most probably capable of altering the specificity of the ABO enzyme. Six B(A)02 have a single nucleotide deletion, G261, in exon 6 typed as B(A) 02/O01, and the other B(A)02 has nucleotide deletion on 681,771,829, and G261 in exon 6 typed as B(A) 02/O02.

The *ABO* gene that encodes the glycosyltransferases and is responsible for the conversion of H substance to blood group A and B antigens is located on chromosome 9[21]. It consists of seven exons ranging in size from 28 to 688 bp[22]. The last two exons (exons 6 and 7), comprising 823 bp of the transcribed 1,062 bp mRNA, encode for the catalytic domain of the ABO glycosyltransferases[23,24]. Theoretically, there are eight enzymes (AAA, AAB, ABA,BAA, ABB, BAB, BBA, BBB types) that have been characterized with interchanges in the last three of the four amino acids, including AAAA (Arg–176, Gly–235, Leu–266, and Gly–268) or BBBB (Gly–176, Ser–235, Met–266, and Ala–268). Until now, nine B(A) or cisAB alleles, including five B(A) alleles and four cisAB alleles[25,26], have been identified and characterized. ABO*B(A)01 was typed as BABB, B(A)03 was typed as BABB with nt 657C > T, and B(A)04 was typed as BBBB with nt 640A > G[27,28]. B(A)02 was typed as BBBB with nt 700C > G[29,30]. B(A)02 defined by Yu *et al* in Taiwan[31], had a single substitution in exon 7 for B101 at codon 234, but the proline was replaced by an alanine. Unlike the hydroxyl group–containing polar serine, the nonpolar alanine results in decreased B transferase activity and very low A transferase activity. These previous studies demonstrated the genetic heterogeneity of these subgroups. In these seven samples, six showed B(A)02/O01 and one showed B(A)02/O02, which were type as BBBB.

Under the consistency of cross matching, it is safe and effective to infuse RBCs from B(A) donor to type B receptor. When B (A) blood of donor was infused to type B receptor, Hb rising conformed to the expected value and no hemolytic reaction occurred in the receptor. What is worth paying attention to is how to select the blood for B (A) receptor for clinical transfusion. The B (A) allele blood receptor should be transfused with blood type B or O RBCs and autologous transfusion is recommended.

In conclusion, this study reveals that B(A)02 was found with relative high prevalence in the donors and patients from Eastern China, although a significantly higher frequency of B(A)04 than B(A)02 were reported before in China. Sequencing and gene analysis are helpful for identifying rare types. The genetic essence of rare blood groups and clinical blood transfusion for rare blood groups are worthy of further study.

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