



## Case Report

# Serological and Molecular Identification of Rare Type B (A): A Case Report

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## Abstract

**Objective:** To investigate the characteristics of one case of rare B (A) blood group was identified by serology test and DNA sequencing. **Methods:** ABO blood phenotype were determined by routine serological methods and ABO genotype was determined by direct sequencing. **Results:** ABO group identification is inconsistent with the forward and reverse typing, an antigen is relatively weak, the forward typing is A2B or AwB, and the reverse typing shows the existence of weak anti-A1, H antigen and O phenotype of reaction agglutination intensity is consistent. Forward and reverse direct gene sequencing on exons 6 and 7 of the ABO gene with the O 02 allele in exon 6 and a unique single base 700C/G mutation in exon 7 confirmed that the patient's genotype was ABO\*BA.02/O02. The serological phenotype of the patient was determined to be B (A). **Conclusion:** Blood group serology identified A2B or AWB, which should be further characterized by B(A) typing and molecular analysis, the case was identified as A rare ABO\*BA.02/O 02.

**Keywords:** B (A) blood type; ABO genotype; Gene sequencing; Molecular

## Introduction

ABO group system is of great significance in clinical blood transfusion, and the accuracy of group identification is an important guarantee for transfusion safety. However, some cases of forward and reserve typing inconsistency of ABO group are often encountered in clinic, which brings a lot of trouble to timely complete the cross match. In our laboratory, 1 rare case of B (A) was found in the samples, which is often mistaken for A2B subtype or AwB subtype. The group is extremely rare, being approximately 1/170000-580000 in Caucasian populations [1], while accurate frequency data are not available in China. B (A) 02 type in this paper has not been reported in the world except in China [2,3]. We performed blood group serology and gene sequencing respectively, and reported below.

## Subjects and Methods

Subjects the patient, female, 45 years old, originated from Fusun County of Sichuan Province. On 21 August 2021, she was

admitted to the Department of surgery of Nanchang hospital in our city for cholecystolithiasis preservation. The Department of hospital examination was admitted because ABO type identification was inconsistent and ABO type could not be determined.

Reagents and instruments Mab-A1, Mab-H, RBC irregular antibody screening cells, RBC irregular antibody identification spectrum cells, Polybrene kits were all from Shanghai blood biomedical Co., Ltd., Mab-A, anti-B, anti-AB were from Changchun boschen Biotechnology Co., Ltd., and microcolumn gel card was from Daina(Spain); Human ABO blood group reverse stereotyping reagent RBCs are self-made by our laboratory. The main instruments include: KA-2200 serology centrifuge (Kubota, Japan), Microcolumn gel card centrifuge and incubator (Daina), 3730 gene sequencer (ABI, USA).

Blood group serological methods: ABO group identification were performed by immediate centrifugation, RBCs irregular antibody screening, identification, and IgG type antibody was performed by polybrene method and microcolumn gel method. The methods were in accordance with the national operation protocol for clinical testing (4<sup>th</sup> Edition). The microcolumn gel method is

performed according to the product instruction manual, and the other tests are performed according to the standard operating protocol of our laboratory.

**Genomic DNA extraction** Genomic DNA was extracted using the magcore automated nucleic acid extractor, which was operated strictly according to the reagent instructions, with sample DNA concentrations of 30-60 ng /  $\mu$ L and A260 / 280 ratios of 1.70-1.90.

**ABO blood group genotyping** ABO whole exon coding region sequence analysis was performed as described [4], Briefly, as follows: three specific pairs of primers were used to amplify the sequences of exons 6-7 of the ABO alleles, respectively. After digestion and purification of the amplified products, sequencing was performed with Big Dye Terminator v3.1 cycle sequencing reagents and electrophoresis was performed on ABI3730 DNA sequencer, and the results were analyzed with Seqscape V2.5 software (the reference sequence was the ABO gene sequence in GenBank, accession number NG\_006669.1), and the genotypes of the specimens were finally adjudicated based on the base case. The ABO allele nomenclature principles were in accordance with the standards of the international society for blood transfusion (ISBT) Red cell immunogenetics and blood group Terminology Committee.

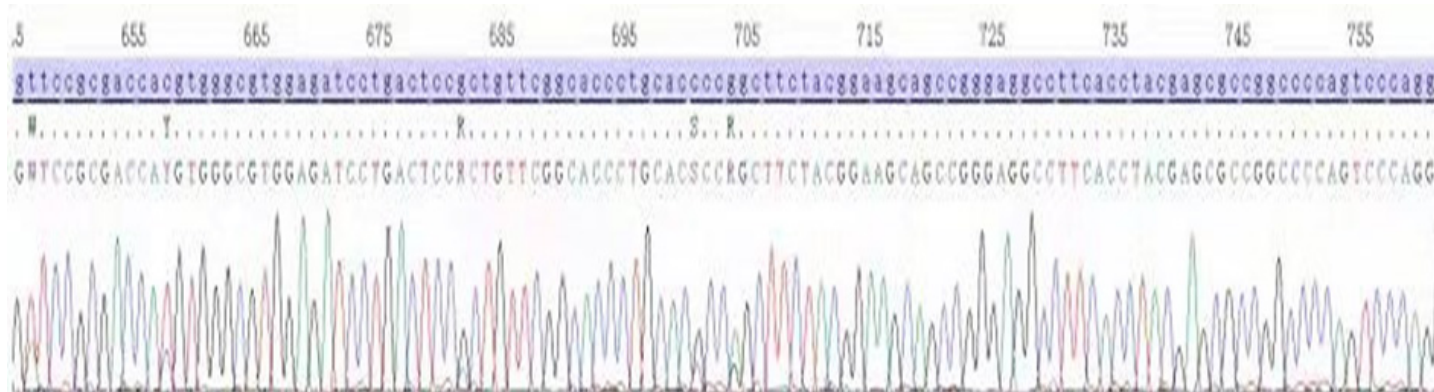
## Results

Blood group serological ABO group identification: the forward type was AB, A antigen agglutination intensity (2+), obviously weaker than normal A antigen, with monoclonal anti-A1 did not agglutination, suggesting may be A2 subtype or other A subtype; The RBC expressed normal B antigen and agglutinated “4 +” with the monoclonal anti-B and “4+” with the monoclonal anti-H reaction, consistent with normal type O RBC, and were significantly stronger than normal type B RBC. There was weak agglutination reaction with Ac and Oc, but no agglutination reaction with Bc. There was no obvious enhancement of each reaction tube at 4°C and room temperature. The patient’s RBCs were positive for irregular antibodies screened by saline medium, and anti-M was further detected by antibody identification with saline medium. No IgG irregular antibodies were found in irregular antibodies screened by condensed amine method and microcolumn gel method. Ac and Oc with negative M antigen were selected for reverse typing test, and the results showed that AcM(-) still had “1+” agglutination, while OcM (-), indicating the presence of anti-A1 in patients’ plasma. The results were shown in Table 1.

Forward typing				Reverse typing					Self-control	Reaction condition
anti-A	anti-A1	anti-B	anti-H	Ac	Bc	Oc	Ac,M(-)	Oc,M(-)		
2+	-	4+	4+	1+	-	±	1+w	-	-	NT
2+s	-	4+	4+	1+s	-	+	1+	-	-	4°C
A2B ? AwB ?				B ? anti-A1						
NT: Normal Temperature										

**Table 1:** Serological identification results of blood group.

ABO gene sequencing Direct sequencing of the 6th and 7th exons of ABO gene revealed that there were heterozygotes of 261G/delG, 297A/G, 646T/A, 657C/T, 681G/A, 700C/G, 703G/A, 771C/T, 796C/A, 803G/C, 829G/A, compared with the A101 standard sequence. The B (A)02 allele 700C/G was found (Figure 1), and the genotype was identified as ABO\*BA.02/O 02.



**Figure 1:** Sequencing part of the 6<sup>th</sup> and 7<sup>th</sup> exon of ABO gene (700C/G).

## Discussion

B(A) type was the first discovered in 1985 [5]. Shanghai Blood Center is the first reported the study of B(A) group by molecular biology in China in 2004 [2]. The B(A) group belongs to type B in serological and genetic classification and is a very rare subtype B. In the European Caucasian, the frequency of B (A) type is about 1/ (17-580,000) [1], and in Chinese blood donor, the frequency of B (A) type is about 1/ (50-100,000) [6], which is much higher than that of the European. At present, there are 6 types of B (A) types, which are B (A) 01~06. The reports of B (A) in China are more for type B (A) 02 (c. 700 C > G) and B (A) 4 (c. 640 A > G) and B (A) 5 (c. 641 t > c) [2,3,7]. B (A) 04 and B (A) 02 are the main types in mainland China. The frequency of B (A) 02 was reported as 0.78/100,000 in China [8].

Because of the limitations of group serologic method, some variants may be misidentified, which cause the inconsistency between serology and molecular biology, and there are differences in the operator's judgment of the degree of weak agglutination in serology. B (A) type are often defined as A<sub>sub</sub>B on the basis of the detection of an A antigen on RBCs. In fact, B (A) group is different from A<sub>sub</sub>B in serological characteristics. The B(A) type RBCs did not agglutinate with monoclonal anti-A1, but weakly agglutinated with some monoclonal anti-A and human anti-A, strongly agglutinated with anti-B (4+), and strongly agglutinated with anti-H in most cases. Anti-A and A1 cells could agglutinate, and A2 cells could agglutinate. However, forward type of A<sub>sub</sub>B had strong agglutination with monoclonal anti-A1 and weak agglutination with anti-H, and irregular anti-A1 might be found in the serum of reverse type. The ABO subtype occurs mainly as a result of mutations at the exon 6 and 7 gene loci that control the expression of glycosyltransferases, reducing or altering the

activity of the enzyme, leading to an altered species or quantity of A or B antigen expressed. The formation mechanism of B (A) blood type may be the single base mutation of B allele on the basis of normal B gene sequence, and the mutated B gene may have the ability to encode double-functional active enzymes, thus showing not only B antigen specificity but also a small amount of A antigen specificity in serological methods [9].

The sample, when tested by group serology, was found to have a weakly reactive A antigen and not reacting with anti-A1, with strong agglutinating B and H antigens, anti-A1 was present, we initially presumed to be A2B or A<sub>sub</sub>B, also likely to be B (A) group due to a strongly agglutinating with H antigen. So it could be B (A). The patient is a migrant worker, the family investigation cannot be carried out, and so the genetic status of the family is unknown. After sequencing, the B allele of the sample was found to have a single base mutation of C>G at the 700th nucleotide, which may lead to a change from (Proline, Pro) to (Alanine, Ala) at the 234th amino acid. The mutant allele belongs to the published B(A)02 type. Sequencing showed that there were two heterozygous mutations in exon 6, namely 261delG and c.297A>G. There were heterozygous mutations in the 7th exon region of: c.526C >G, c.640A>G, c.646T>A, c. 657C >T, c.681G>A, c.700C>G, c.703G>A, c. 771C >T, c. 796C >T, c.803G> C, c. 829G>A, c.930G>A. A101 allele was used as the standard sequence for comparison, and the genotype of the patients was B (A)02/O02. Molecular basis of ABO subtypes is often described in terms of amino acids at 4 key locations (G526C, A703G, A796C and C803G). If glycosyltransferase A is described as AAAA and glycosyltransferase B as BBBB, then glycosyltransferase B (A) may be BABB or BBBB [10-12]. B(A)02, also known as B(A)700 (526C>G,700C>G,703G>A,796C>A,803G>C,BBBB),

expressed B alleles at 4 key locations, but there was A missense mutation at nt700 site, which might change the glycoltransferase characteristics and catalytic activity. The B gene that causes the mutation has the ability to encode bifunctional active enzymes. For patients with B (A) type, O washed RBCs or AB plasma should be selected to prevent hemolytic transfusion. If donors or patients are clinically found to have B (A) type, family study and molecular analysis should be carried out, and identification should be combined with serology and molecular test to further ensure the safety of transfusion.

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