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Identification and analysis of a rare CisAB group

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INTRODUCTION

Incompatible ABO transfusion can lead to immune hemolytic transfusion reaction. Accordingly, a correct ABO subgroup identification is vital for blood transfusion safety[1]. ABO subgroups are often characterized by their abnormal appearances and a weakening or disappearance of antigens or antibodies, resulting in mismatched forward and reverse typing. This is mainly caused by ABO gene mutations, leading to a decrease in A/B transferase activity. This makes ABO subgroups prone to misidentification, which has an important impact on the safety and efficacy of transfusion. The CisAB group is one of such ABO subgroups with a complex phenotype, which due to various factors is difficult to determine using routine serological testing. Here, one CisAB group case in the Dongguan Blood Center was identified and reported as follows.

MATERIALS AND METHODS

Subjects

A blood donor, male, 22 years old, Han nationality, from Anhui province, working in Dongguan was involved in ABO group identification at his third blood donation. After forward and reverse ABO group identification were found to be inconsistent, serological analysis and subgroup identification were performed. Nucleotide sequencing analysis and sequence alignment were performed after DNA extraction. This study was approved by the Ethics Committee of the Dongguan Blood Center and all aspects of the study complied with the Declaration of Helsinki.

Reagents and instruments

Monoclonal antibodies anti-A, B (Lot: 20181210), anti-A1 (Lot: 20190220), anti-H (Lot: 2018120), anti-AB (Lot: 0ABM087), were all provided by Shanghai Hemo Pharmaceutical & Biological Co., Ltd (China). Reverse typing reagent were prepared in our laboratory. Nucleic acid detector (Biotek, USA), 9700 PCR amplifier (ABI, USA), and 3130xl sequencing machine (ABI, USA) were used.

Serological detection

The blood sample (EDTA anticoagulant) was detected for routine ABO group by the microplate method. If weakened and inconsistent agglutination intensities were detected in forward and reverse typing, an absorb-elusion technology was used to verify the existence of weak A or B antigens.

ABO DNA sequence analysis

DNA was extracted from 0.5 mL EDTA anticoagulant whole blood with the TIANamp Blood DNA Kit (Qiangen, Germany). The design of primers, amplification of DNA and analysis of bidirectional sequencing were performed by Zhejiang Blood Center´s Transfusion Department[2]. Sequence analysis was carried out by Chromas software, and the A101 sequence was used as the reference for nucleotide inser–

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tion, deletion and mutation. The genotype was determined by referring to the International Blood Group Antigen Gene Mutation Database (BGMUT).

RESULTS

Blood group serological results

Serological results showed that A and B antigens were present, but the expressions in forward and reverse typing were inconsistent. There was a weak anti-B in the serum (***Table 1***), and further absorbelusion tests confirmed the existence of weak B antigen on erythrocytes. The agglutination reaction of erythrocytes to anti-H in this blood donor was higher than that in normal AB blood donors. The preliminary serological results were A_2B_3 or CisAB.

Table 1 **Blood group serological test results**

Forward typing					Reverse typing			Control	Condition
anti-A	anti-A1	anti-B	anti-AB	anti-H	Ac	Bc	Oc		
4+	–	1+	4+	4+	–	1+	–	–	nt
4+	–	2+	4+	4+	–	$2+^{w}$	–	–	4℃

Note: nt, normal temperature, referring to the room temperature; Ac/Bc/Oc, A/B/O group red blood cell; w, weak.

Sequence analysis of PCR products

The sample´s mutation sites were confirmed by multiple PCR amplification and sequencing, and errors in PCR amplification were excluded. The genotype was identified by direct comparison with the A101 reference sequence. The sample´s result was CisAB01/O04. Exon 7 with two nucleotides different from A101, namely: nt467 (C>T) and nt803 (G>C) (***Fig. 1***).

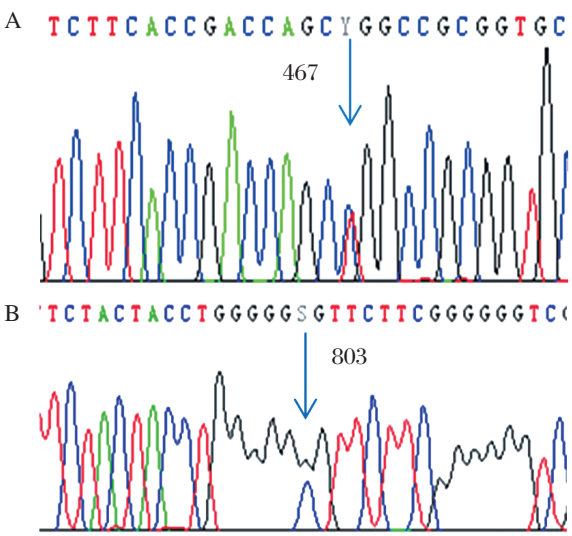

Fig. 1 **The mutation sites of gene sequencing.**A: Arrow points to nt467(C>T); B: Arrow points to nt803(G>C).

DISCUSSION

Yamamoto *et al*. reported the A_2B_3 group as displaying weak A and B antigens, with A and B genes located on the same chromosome[3]. CisAB is a unique ABO phenotype, because its encoded product from the same allele has both A and B specific glycosyltransferase. Meanwhile, CisAB is a relatively rare ABO subgroup with a very low frequency (about 1 in 160,000~580,000)[4]. There are two theories concerning the genetic mechanism of CisAB. One suggests that an unequal exchange of chromosomes causes adjacent A and B genes to produce independent gene products. The other explains that mutations in A or B genes allow the gene product to become a bifunctionally active enzyme, able to transfer both N-acetylgalactose and galactose to H receptors[3,5,6]. From the literature, the latter is more popular.

The CisAB group is under reported in China, due to the lack of further molecular biological analysis. However, a small percentage of samples are confirmed as ABO subgroups when examined by serological analysis. Common characteristics for CisAB group are the presence of both A and B antigens on the membrane of red blood cells, and unexpected antibodies against B antigen in the serum. Additionally, the anti-H reaction is usually positive (4+) and the strength is higher than normal B typing. The antibody titers in serum vary from the different antigenicity in A/B/H antigens. In addition, when detecting irregular antibodies, it is necessary to increase the serum volume or to set the sample at 4℃ to enhance the response[7]. This significantly increases the agglutination strength of reverse typed B cells, better indicating the pre sence of natural anti-B. Compared with the normal AB subgroup, the antigen on CisAB group red blood cells is slightly weaker, while the B antigen is significantly decreased.

Meanwhile, the distinction between CisAB and B(A) blood groups should be clarified. The formation mechanism of B(A) blood group has been reported to be a B allele point mutation on the basis of the normal B gene sequence, with the mutated B gene able to encode bifunctionally active enzymes. The serology shows not only B antigen specificity, but also weak A antigen specificity. Therefore, a high sensitivity monoclonal anti-A reagent should be used for the detection of B(A) blood group[8]. From the results of

this sample, A_2B_X, A_XB, A_2B and CisAB groups demonstrated some serological similarities, but there were also many different manifestations. The sequencing found that the sample contained both A and B genes, which made serological testing more complicated. In short, the CisAB subgroup cannot be correctly identified by serological detection alone. Thus, genotyping is necessary to confirm complicated blood groups[9].

It is generally believed that the co-existence of B antigen and B antibody in the CisAB group seriously affect the safety and efficacy of transfusion. Clinical blood group identification and crossmatching are often very challenging. Consequently, for patients with the CisAB group, the use of O blood group for washed red blood cells and AB blood group plasma is optimal for blood transfusion[10]. Owing to the particularity of blood group inheritance, data regarding rare blood groups´ incidence and molecular mechanisms in Chinese people have important clinical implications and are worthy of further research in the future.

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