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SHORT REPORT

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Identification of an $A_{weak}B$ phenotype caused by significant ABO gene deletion in a Chinese woman

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Abstract

Background and Objectives: The $A_{weak}B$ is a rare phenotype in the ABO blood group system. Here, we first report a novel ABO mutation discovered in a Chinese woman with an $A_{weak}B$. Third-generation sequencing was employed to investigate the molecular mechanisms underlying $A_{weak}B$. By correctly identifying the phenotype, it was useful for increasing the safety of blood transfusion.

Materials and Methods: ABO blood group was identified by the standard ABO serological test and polymerase chain reaction with sequence-specific primers (PCR-SSP). To analyse the ABO gene sequence, single-molecule real-time (SMRT) sequencing was performed to obtain full-length sequencing of the ABO gene.

Results: The Chinese individual was serologically identified as $A_{weak}B$ subtype, and SMRT sequencing analysis revealed that there were large fragment deletion mutations in the promoter (c.1-1326_1-1321del, c.1-1010_1-975del, c.1-952_1-1del) and Exon 1 region (c.1_28del).

Conclusion: We report for the first time that large fragment deletions represent a novel molecular basis for the $A_{weak}B$. These deletions may potentially influence the expression of the A antigen.

Keywords

ABO blood group, $A_{weak}B$, gene sequencing, large fragment deletion

Highlights

- In this study, we report a novel large fragment deletion mutation identified in a Chinese woman with an $A_{weak}B$ phenotype.
- Serological methods and single-molecule real-time sequencing technology can assist in identifying this phenotype, thus enhancing the safety of blood transfusions.
- The novel $A_{weak}B$ mutation was not previously included in the International Society of Blood Transfusion database.

INTRODUCTION

The ABO blood group system is the most widely used blood group system in clinical transfusion and transplantation medicine [1]. In addition to the common ABO blood group, there are other subtypes. ABO subgroups are rare, with a detection rate of approximately 0.015% in Shanghai, China [2]. The molecular genetic basis of the ABO blood group system has been known since the cloning in 1990 of complementary DNA (cDNA) corresponding to messenger RNA (mRNA) transcribed at the ABO locus [3]. The ABO gene,

located on human chromosome 9 (9q34.1-9q34.2), consists of seven exons and six introns and spans approximately 25 kilobases (kb) [4]. Numerous variations associated with ABO subgroup have been identified in the coding region; however, the molecular mechanisms underlying some subgroups remain incompletely understood [2].

With the development of technology and science, single-molecule real-time (SMRT) sequencing, a third-generation sequencing technology, has become more widespread for the detection of various ABO subgroups. It yields an average read length of up to 100 kb and highly accurate sequencing results, overcoming challenges in distinguishing haplotypes and multiple recombination events [4, 5]. Here, we report the identification of an $A_{weak}B$ subtype using SMRT sequencing, revealing a novel molecular basis for $A_{weak}B$ individuals.

MATERIALS AND METHODS

Sample collection

The proband was a 56-year-old Chinese woman hospitalized due to a mediastinal mass. Informed consent was obtained from the proband for sample collection and participation in related studies.

Serological analysis

The subject's ABO blood groups were detected using serological methods. Briefly, forward typing was identified using monoclonal anti-A, anti-B and anti-H (Shanghai Blood Biomedical LLC, Shanghai, China), and reverse typing was done with standard A, B and O cells (Shanghai Blood Biomedical LLC, Shanghai, China) using a tube test.

ABO genotyping using the PCR-SSP

Target genomic DNA amplification and ABO blood group genotyping were performed using the Human Erythrocyte ABO Blood Group Genotyping Kit (polymerase chain reaction with sequence-specific primers [PCR-SSP]) (Jiangsu Zhongji Wantai Biological Medicine Co., Ltd). All procedures were carried out strictly in accordance with the manufacturer's instructions.

ABO gene full-length sequencing analysis

Genomic DNA was extracted from the proband's blood sample using a commercial DNA extraction kit (Jiangsu Zhongji Wantai Biological Medicine Co., Ltd). The full-length ABO gene sequence was analysed using long-read sequencing. All amplicons were purified by magnetic beads, followed by repeat sequencing and analysis using a PacBio RS sequencer and Sequel System. ABO genotypes were determined based on the nucleotide sequence of the ABO gene polymorphisms. The obtained nucleotide sequences were compared with known ABO gene polymorphisms, using the International Society for Blood Transfusion (ISBT) as the reference sequence [6].

SMRT sequencing

Three primer pairs were designed to amplify the ABO gene sequence, generating three overlapping amplicons of 9, 9.5 and 11.5 kb (Figure 1, Table 1). The long-range polymerase chain reaction (LR-PCR) technique was performed by KOD FX Neo (TOYOBO). The PCR cycling conditions were set according to the manufacturer's protocol, and a two-step cycle of 10 min each was used for a total of 30 cycles to achieve high accuracy of sequencing data and determine the haplotype of the sample.

The PCR products were confirmed by agarose gel electrophoresis, and the library was constructed. A reaction master mixture was prepared before use, which contained 10 μL of reaction mix that included 4 μL of PCR product, 5 μmol/L barcoded adaptor (Integrated DNA Technologies), 1× T4 DNA ligase buffer (Enzymatics), 1 mmol/L adenosine triphosphate (New England Biolabs), 200 μmol/L deoxynucleotide triphosphate (New England Biolabs), 2.5 units of T4 polynucleotide kinase (Enzymatics), 0.75 units of T4 DNA polymerase (Enzymatics) and 180 units of T4 DNA ligase high concentration (Enzymatics). Then, 120–250 ng of PCR product was mixed with the enzyme mixture. Reaction mixes were then incubated at 37°C for 20 min, 25°C for 15 min and 65°C for 10 min. After that, exonuclease I (Enzymatics) and exonuclease III (Enzymatics) were added to remove the failed ligation products, and the final pre-library was purified with 0.6× Ampure PB beads. For multiple samples sequencing, pre-libraries were pooled together according to equal masses. After pooling, the pre-libraries were purified two times with 0.45× Ampure PB beads. The final library was bound with sequencing enzymes and primers through Sequel Binding Kit 2.2 (Pacific Biosciences) and Internal Control Kit 1.0 (Pacific Biosciences); 150 pM DNA polymerase complexes

FIGURE 1 Primer sequence diagram of ABO gene haplotypes. Three primer pairs were designed to amplify the full-length sequence of the ABO gene.

were finally loaded and sequenced with the Sequel II platform (Pacific Biosciences) with a 20-h movie time. All procedures were performed in strict accordance with the manufacturer's instructions. SnapGene software was used for sequence alignment and analysis using ISBT Names for ABO (ISBT 001) blood group alleles v1.1 171023 as the reference data source [4].

RESULTS

Serological characteristics of the ABO phenotype, PCR-SSP

Forward typing revealed that the red blood cells (RBCs) had no agglutination reaction with anti-A and 4+ agglutination with anti-B. Reverse typing demonstrated no agglutination reaction with A1 cells, B cells and O cells; however, a mild response was observed with A1 cells at 4°C. Based on the serologic characteristics (Table 2), the proband was indicated to have a weak A phenotype. PCR-SSP analysis (Table 2) confirmed the ABO blood group as A_{weak}B, consistent with the serologic results.

TABLE 1 Primer design for ABO gene single-molecule real-time sequencing.

	Forward primer	Reverse primer
Primer 1	5′-catccctttcaccttggcattt-3′	5′-agctacattgaccagagagaga-3′
Primer 2	5′-gcccaccaaaactccctggaa-3′	5′-ccagttcctgccagggagagga-3′
Primer 3	5′-gtgtgaaactcatcaaaacc-3′	5′-cgcagggattgcagtgagg-3′

TABLE 2 Results of serological grouping and ABO gene analysis.

	Forward typing			Reverse typing			
	Anti-A	Anti-B	Anti-H	Ac	Bc	Oc	PCR-SSP
25°C	–	4+	3+	–	–	–	A_{weak}B
4°C	–	4+	3+	1+	–	–	

Note: '–' denotes the serology did not agglutinate. '+' denotes the agglutination strength of serology.
Abbreviation: PCR-SSP, polymerase chain reaction with sequence-specific primers.

Haplotype sequence analysis of the ABO gene

The single nucleotide variants (SNVs) of haplotype sequence are shown in Table 3. Haplotype analysis of the ABO gene revealed that the proband's ABO genotype was ABO*B.01/ABO*A.NEW. The novel allele, ABO*A.NEW, is a synonymous variant located in the promoter and Exon 1 region. Notably, SMRT sequencing also captured related sequences upstream and downstream of the coding region, which exhibited distinct sequence patterns.

SMRT sequence analysis of ABO alleles

SMRT sequencing results showed a substantial fragment deletion mutation in the promoter and Exon 1 region, with a total loss of 1022 bp (Figures 2 and 3) when compared with the ABO*A1.02/ABO*B.01 genotype. The locations and nucleotide changes in key variant sites are shown in Table 4. No mutation was found in Exons 2–7 of the ABO gene.

DISCUSSION

Many pioneering studies have revealed weak ABO subgroup phenotypes such as A3, Ax, Am, Ael, B3, Bx, Bm and Bel subgroups, which are predicted to result from extensive polymorphism of the ABO gene [7]. The ABO gene encodes different glycosyltransferases (GTs) that catalyse the attachment of N-acetylgalactosamine or D-galactose to the terminal end of the H substance, thereby forming the A or B antigen. The weak subtype A caused by the mutation mentioned in this case is rare. Besides the coding region, the promoter sequence was also found to be critical for the transcriptional activity of the ABO genes [8]. Kominato et al. discovered that the transcription of the ABO gene was likely regulated by the proximal promoter [9]. Cai et al. provided evidence that promoter abnormality was associated with the formation of weak ABO phenotypes. Dual luciferase assays confirmed that the activity of the mutant promoter was reduced by over 50% compared with that of the wild type [2]. In some human diseases, mutations in promoter regions may disrupt the binding of transcription factors, thus influencing the promoter's transcriptional

TABLE 3 ABO genotyping results.

	Haplotype 1			Haplotype 2		
Sample	Phenotype 1	Allele 1	SNV	Phenotype 2	Allele 2	SNV
Proband	B	ABO*B.01	c.297A>G c.526C>G c.657C>T c.703G>A c.796C>A c.803G>C c.930G>A	A_{weak}	ABO*A.NEW	Exon 1 deletion c.467C>T

Abbreviation: SNV, single nucleotide variant.

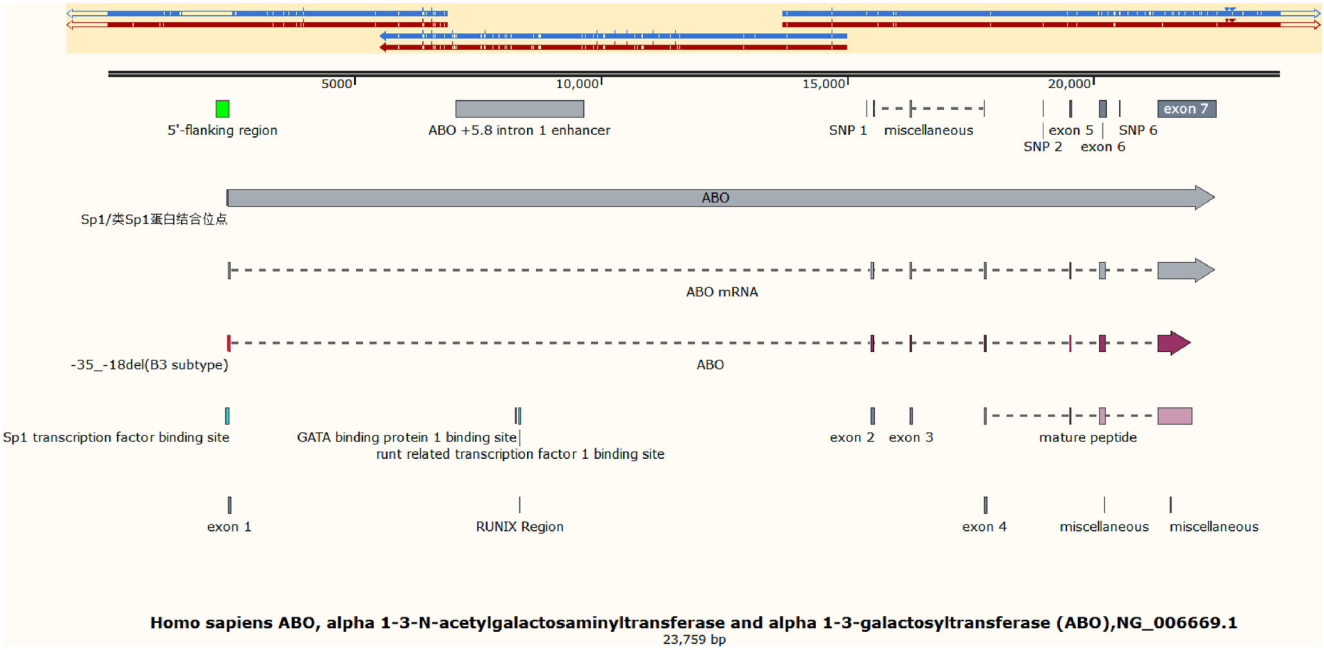

FIGURE 2 Results of single-molecule real-time sequencing. Blue represents haplotype A. Red represents B haplotype. The vertical lines in the blue and red arrows indicate the mutation sites. Abbreviation: SNP, single nucleotide polymorphism.

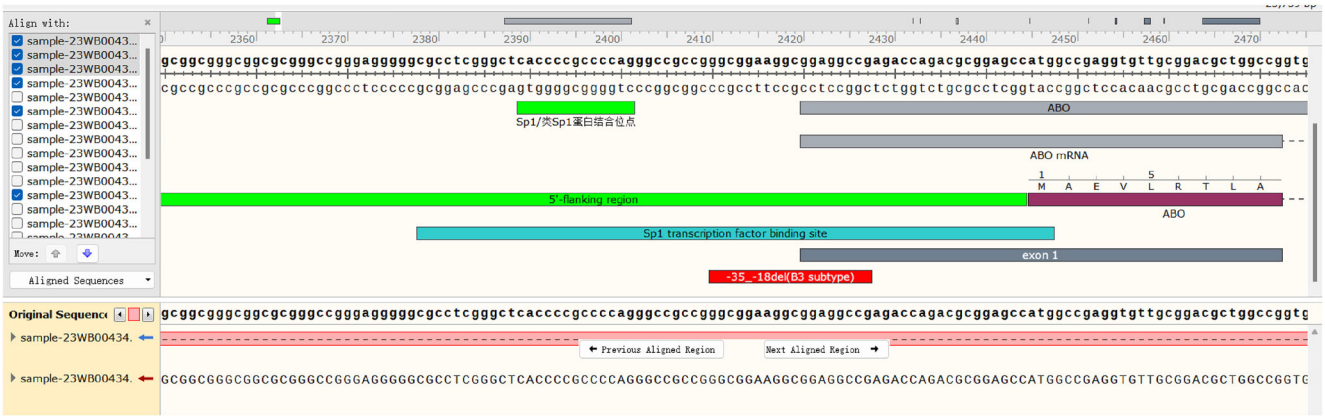

FIGURE 3 The proband's ABO gene sequencing results.

TABLE 4 The nucleotide position in the promoter and Exon 1 region.

Genotype	Location	Nucleotide change
ABO*A1.02	Promoter region	c.1-1326_1-1321del
	Promoter region	c.1-1010_1-975del
	Promoter region	c.1-952_1-1del
	Exon 1	c.1_28del

activity [10–12]. We hypothesize that this mechanism may contribute to the weak A phenotype observed in this case.

Meanwhile, variations in Exon 1 of the ABO gene could affect the expression of the ABO blood gene [13]. Yamamoto et al. clarified the molecular genetic basis of the ABO system; a number of weak phenotypes have been found to be attributable to single nucleotide polymorphisms in the coding exons and splicing sites and hybrid formation between common alleles [9]. Our results demonstrated that the Exon 1 sequence was completely lost. Due to the deletion of Exon 1, Exon 1a was utilized as an alternative starting point for transcription analysis, enabling the production of a functional transferase [14]. Consequently, this deletion could result in the complete failure or significant reduction in the expression level of the entire A haploid protein.

In summary, we identified large fragment deletions in the promoter and Exon 1 region, which were associated with the A_{weak} phenotype. To avoid the adverse transfusion reactions, the proband individuals should be transfused with washed RBCs from blood group B or O, along with AB plasma.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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