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CASE REPORT

Identification of a Novel Mutation in B Allele in a Chinese Individual

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SUMMARY

Background: **There are 49 B alleles in the ISBT ABO blood group list. This study will describe a new missense mutation, c.784G>T, in exon 7 of the ABO in a Chinese individual.**
Methods: **A weak B was analyzed by serologic techniques. Exons 6 and 7 were sequenced directly through polymerase chain reaction-based typing (PCR-SBT). Subsequently, the heterozygous mutation sites in exon 7 were determined through cloning and sequencing. The mutated GTB proteins were expressed in HEK293F cells after being subcloned into a pCAG vector with a Strep-tag. The potential impact of the mutations on GTB stability was predicted using mCSM software, while UCSF Chimera X software was utilized for visualization of the mutation.**
Results: **The ABO blood typing of serologic characteristics showed weak B phenotype, and the heterozygous site ABO*B.01 (c.784G>T) in Exon 7 was identified by PCR-SBT analysis after TA cloning, which led to an alteration of Asp to Tyr at residue 262 in B glycosyltransferase. Like the ABO*BW.17 (D262Y), D262N also significantly decreased ABO*B.01 expression and lead to GTB destabilizing.**
Conclusions: **The novel B allele with 784G>T caused an alteration of Asp to Tyr at residue 262 in B glycosyltransferase, affecting the expression of GTB protein and influencing GTB structural instability.**
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KEYWORDS

weak B phenotype, gene mutation, glycosyltransferase

INTRODUCTION

ABO blood group antigens play a critical role in safe and effective clinical transfusion. Since the discovery of the genetic basis of the *ABO* gene in 1990 [1], different phenotypes and genotypes have been identified and reported. Most *ABO* gene mutations that influence the activity of glycosyltransferases lead to weak expression of blood antigens or antibodies or a discrepancy between forward and reverse typing of the ABO blood group [2-4]. Here, the serological test of a 47-year-old Chinese female patient with uterine fibroids who underwent surgery showed a discrepancy between forward and reverse typing results. Thus, molecular analysis of the *ABO* gene was performed, and a missense mutation in exon 7 of ABO*B.01 (c.784G>T)/O01 was found to result in decreased B antigen expression. Here, we report the identification of a novel mutation.

CASE PRESENTATION

Clinical feature and serologic investigation

A 47-year-old female patient with uterine fibroids underwent blood typing prior to surgery. Routine serological tests were performed using the microgel method (ORTHO AUTOVUE Innova, USA and GRIFOLS, Spain), and the manual tube method was also retyped. Monoclonal anti-A, anti-B, anti-A1, anti-AB, and anti-H antibodies (Blood Grouping Reagent, Shanghai Hemo-Pharmaceutical & Biological, Inc.) were used for forward typing, whereas A1, B, and O RBCs were used for reverse typing (ABO RBC reagent kit, Jinhao, Beijing). The patient provided written informed consent before enrollment. This study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (2020051).

ABO genotyping and clone sequences

Genomic DNA (Tiangen blood DNA kit, Tiangen Biotech Co.) extracted from the patient's blood sample was sequenced on exons 6 and 7 of the ABO gene using PCR-SBT after serologic testing. The PCR product was cloned into the pMD19-T vector (pMD19-T vector cloning kit; Transgene) to identify the mutated allele of the *ABO* gene.

ABO*B allele expression

Full-length ABO cDNA was synthesized by Sangon Biotech and subcloned into a pCAG vector with a Strep-tag, to generate ABO*BW.17, and a 784G>A mutation was introduced using a PCR-based strategy. After endotoxin-free plasmid extraction by the Endo-Free Plasmid Mini Kit (Omega) and transfection into HEK-293F cells, briefly, 20 µg of plasmid was diluted into 1 mL of 293F medium and mixed with 60 µg PEI, and the mixture was diluted into 20 mL of a HEK293F cell suspension, at a density of 1.8 x 10^6 cells/mL. The cells were centrifuged at 800 g, 15 minutes after 60 hours of incubation at 37°C. The cells were then lysed in lysis buffer with 1% Triton X100 over 1 hour in a 4°C rotator, and the supernatants were isolated via centrifugation and used for western blot analysis.

GTB protein stability prediction and 3D structural analysis

To determine the effect of the mutation on the conformation of the GTB enzyme produced from the new ABO*Bw allele, 3D molecular models of wild-type GTB, D262Y (ABO*BW.17) [5] and D262N mutant GTB were generated using a template X-ray structure of wild-type GTB (PDB code, 1LZ7) [6]. Three-dimensional molecular models of these mutants were generated using the mCSM [7].
Web site (https://biosig.lab.uq.edu.au/mcsm/stability).

RESULTS

Serologic results

The serologic results are shown in Table 1. The RBCs were 3+ agglutinated by anti-B and 4+ agglutinated by anti-H, but were not agglutinated by anti-AB and anti-A1. However, reverse typing showed 4+ for anti-A1 antibodies, thus suggesting the presence of blood group B.

Sequencing results

The sequencing results showed a heterozygous mutation site c.784G>T in exon 7 in the background of the B.01 allele (shown in Table 1 and Figure 1). After cloning, the novel B.01 allele contained a missense mutation G>T at Position 784, which caused an alteration of Asp (D) to Tyr (Y) at residue 262 of B-glycosyltransferase. It has been suggested that an amino acid change at position 262 decreases the expression of B antigens. The nucleotide sequence of this new allele has been submitted to GenBank with Accession Number MW250917.

Novel B allele expression of the *ABO* mutation

We used the pCAG-Strep vector as a negative control and transfected the vectors into HEK293F cells. After 60 hours cells were collected for western blot analysis. We found that ABO*BW.17 significantly decreased GTB expression, and 784G>T also decreased GTB expression (Figure 2a, 2b).

GTB protein stability of the novel B allele

Using the X-ray structure of wild-type GTB (1LZ7) as a template [6], we found that 262D was relatively close to the active site of the GTB enzyme and only four and six amino acids from critical active site residues, Met266 and Ala268, respectively. This would likely lead the mutation to impact catalytic activity. After predicting protein stability with mCSM software [7], we found that the 262D mutation significantly impacted the stability of GTB protein ($\Delta\Delta G$ = -1.91 kcal/mol), which may be the reason for the difference in GTB enzyme function. Notably, the mutation resulted in reduced expression of the blood group, and the results are altogether shown in Table 1 and Figure 2c.

DISCUSSION

ABO blood group polymorphisms have resulted over the course of human evolution, and accurate identification closely relates to safe blood transfusion. In addition to blood transfusion, the blood group also affects the occurrence and development of many diseases [8,9]. Compared with type A and type O, the variation in the B gene occurs at a slightly lower frequency, with differences between B.01 and A1.01 in c.297A>G, c.526-C>G, c.657C>T, c.703G>A, c.796C>A, c.803G>C, and C.930G>A in exons 6 and 7, and the differences in the amino groups were p.Arg176gly, p.Gly235Ser, p.Leu-266Met, and p.Gly268Ala in exon 7 [10].

Table 1. Results of ABO serologic grouping and genotyping.

Forward typing test					Reverse typing test			Phenotype	Genotype
Anti-A	Anti-B	Anti-AB	Anti-A1	Anti-H	Ac	Bc	Oc		
0	3+	0	0	4+	4+	0	0	B	ABO*B new allele (c.784G>T) ABO*B.01

Table 2. GTB stability of new B allele.

New B alleles	Mutations	Predicted affinity change (ΔΔG)	Effect
ABO*BW.17	D262Y	-1.83 kcal/mol	destabilizing
784 G>T	D262N	-1.91 kcal/mol	destabilizing

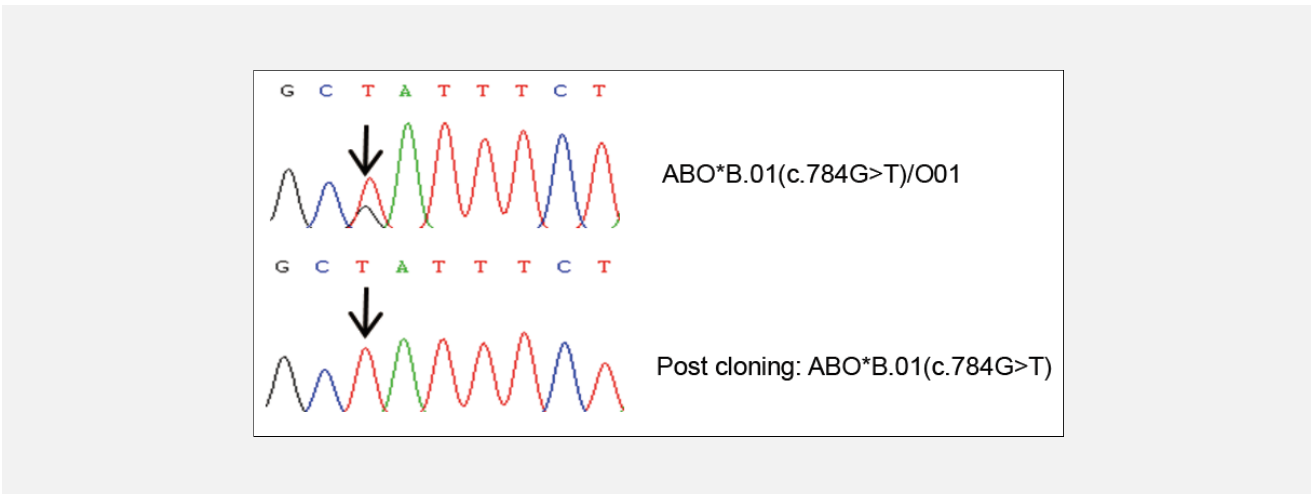

Figure 1. DNA sequences of the new allele.

Type B mutations include normal B (ABO* B.01-B.03), B3(B3.01-B3.08), weak B (ABO* Bw.01-Bw.34), and Bel (ABO* Bel.01-Bel.05) [11]. A mutation of base 784, 784G>A, was also noted in the ABO* Bw.17 [1] allele, which results in an amino acid change of Asp-262Asn. This differs from the mutation in the nucleotide base and amino acid (784G>T, Asp262Tyr) evaluated in this study. In addition, ABO*AW.10 also included a change at 784G>A to Asp262Asn, and both alleles lead to weak B or A. Thus, it can be seen that the mutation at this site is a key factor that affects ABO expression. The genetic background of the Bw phenotype is heterogeneous and usually arises through seemingly random missense mutations in exon 7 of the *ABO* gene. We compared these two mutations with the overexpression patterns in HEK293F cells and found that both the 784G>A and 784G>T mutations significantly affected GTB expression.

Based on previous [12,13] analyses of the resulting structural changes in glycosyltransferase, the mutations are likely to disrupt molecular bonds important for enzymatic function. Notably, the 784G>T (D262Y) mutation is located in the C-terminal domain [13], where it forms a salt bridge with Lys314 [6]. The asparagine substitution by tyrosinase disrupts this salt bridge and destabilizes the tertiary structure of the enzyme. Notably, Asp262 is only four and six amino acids away from the critical active residues Met266 and Ala268, respectively.

Using an *in vitro* expression system, we identified differences in GTB expression between the new B variation and the identified genotypes B.01 and Bw17, which

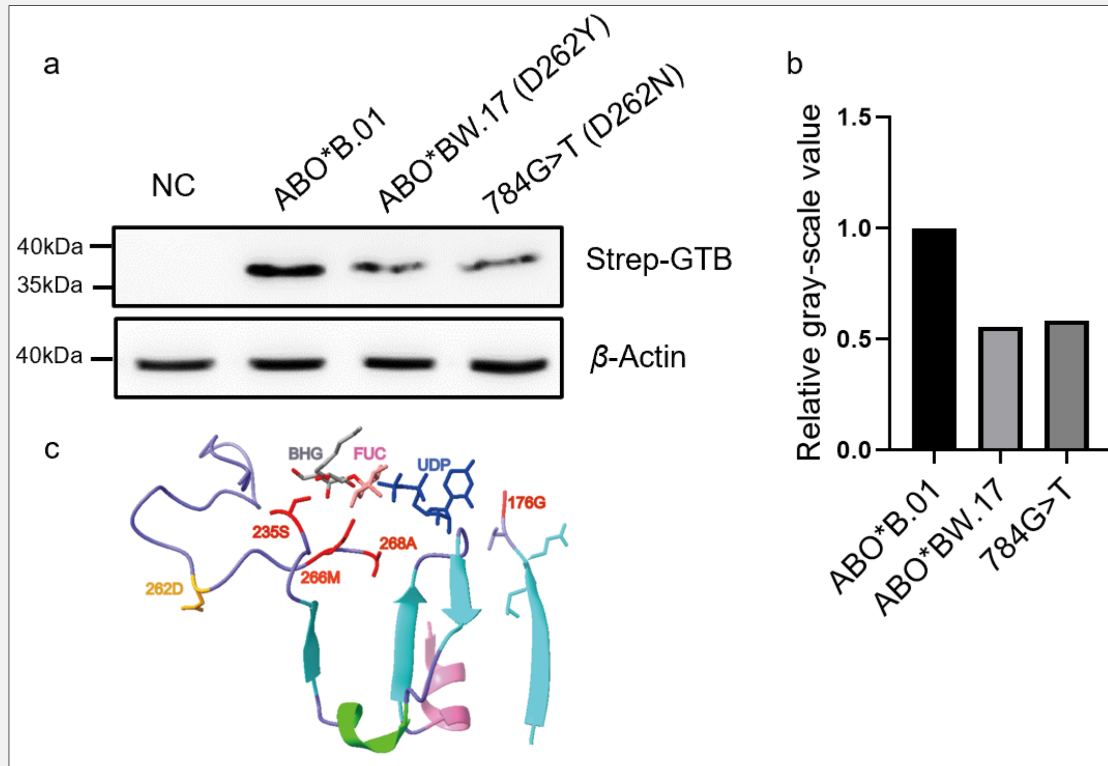

Figure 2. The expression of GTB.

a - The expression of GTB variants, b - Related gray scale level of GTB variants, c - The structure model of GTB mutation.

affect GTB protein expression and its three-dimensional structure, resulting in protein structural instability. We hypothesize that this new B allele may affect the efficiency of GTB enzyme activity through decreasing protein expression and disrupting protein structural stability. Our findings provide a reference for future studies on blood group variations.

CONCLUSION

Here, we identified a novel B allele that contained a missense mutation, 784G>T, which resulted in an Asp to Tyr alteration at residue 262 in the B glycosyltransferase. This change impacts the expression of the GTB protein and results in protein structural instability.

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Ethical Statement:
The study has obtained ethical approval of West China Second University Hospital, Sichuan University (Number: 2020051).

Declaration of Interest:
The authors have disclosed no conflicts of interest.

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