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SHORT REPORT

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Novel missense mutation c.797T>C (p.Met266Thr) gives rise to the rare B(A) phenotype in a Chinese family

Lin-Nan Shao | **Yi-Cheng Yang** | **Yue-Xin Xia** | **Chun-Xiang Li** | **Shi-Hang Zhou** | **Xiao-Hua Liang**

Dalian Blood Center, Dalian, Liaoning, China

Correspondence
Shi-Hang Zhou and Xiao-Hua Liang, Dalian Blood Center, Dalian, Liaoning 116001, China.
Email: zshsail@163.com and liangxiaohua1968@126.com

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Abstract

Background and Objectives: B(A) phenotype is usually formed by nucleotide mutations in the *ABO*B.01* allele, with their products exhibiting glycosyltransferases (GTs) A and B overlapping functionality. We herein report a *B(A)* allele found in a Chinese family.

Materials and Methods: The entire ABO genes of the probands, including flanking regulatory regions, were sequenced through PacBio third-generation long-read single-molecule real-time sequencing. 3D molecular models of the wild-type and mutant GTB were generated using the DynaMut web server. The effect of the mutation on the enzyme function was predicted by PROVEAN and PolyPhen2. The predictions of stability changes were performed using DynaMut and SNPeffect.

Results: Based on serological and sequencing features, we concluded the two probands as possible cases of the B(A) phenotype. Crystallization analysis showed that Thr266 substitution does not disrupt the hydrogen bonds. However, some changes in interatomic contacts, such as loss of ionic interactions and hydrophobic contacts, and addition of weak hydrogen bonds, may have affected protein stability to some extent. This mutation was predicted to have a benign effect on enzyme function and slightly reduce protein stability.

Conclusion: The probands had the same novel *B(A)* allele with a c.797T>C (p.Met266Thr) mutation on the *ABO*B.01* backbone.

Keywords

allele, B(A), mutation, sequencing

Highlights

- The B(A) phenotype is usually caused by nucleotide mutations in the *ABO*B.01* allele, resulting in their products having glycosyltransferase A and B overlapping functionality.
- The probands have the same *B(A)* allele with a mutation of c.797T>C (p.Met266Thr) on the *ABO*B.01* backbone.
- This allele at position 266 did not belong to glycosyltransferase A or B.

INTRODUCTION

The ABO blood group plays a vital role in blood transfusion and organ transplantation. The ABO blood group gene, located on chromosome 9, encodes different glycosyltransferases (GTs). These GTs bind N-acetylgalactosamine or D-galactose to the end of substance H, thereby forming the A or B antigen. The variations between GTA and GTB are Arg176Gly, Gly235Ser, Leu266Met and Gly268Ala. Positions

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TABLE 1 Amino acid substitutions associated with the B(A) phenotype.

	Amino acid position					
Alleles	**176**	**214**	**234**	**235**	**266**	**268**
*ABO*A1.01*	**Arg**	Met	Pro	**Gly**	**Leu**	**Gly**
*ABO*B.01*	**Gly**	Met	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.01/BA.03*	**Gly**	Met	Pro	**Gly**	**Met**	**Ala**
*ABO*BA.02*	**Gly**	Met	Ala	**Ser**	**Met**	**Ala**
*ABO*BA.04*	**Gly**	Val	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.05*	**Gly**	Thr	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.06*	**Gly**	Met	Pro	**Ser**	**Met**	**Gly**
*ABO*BA* allele in this study	**Gly**	Met	Pro	**Ser**	**Thr**	**Ala**

Note: Bold indicates amino acid substitutions at the four Positions (176, 235, 266, 268).

266 and 268 are particularly crucial in differentiating sugar specificity [1].

B(A) is a rare ABO subgroup. *B(A)* alleles are typically formed because of nucleotide mutations in the *ABO*B.01* allele, which results in their products with GTA and GTB overlapping functionality [2]. In serological tests, B(A) is easily confused with cisAB or other AB subgroups. Nevertheless, A antigen is stronger than B antigen in the cisAB phenotype, whereas B(A) is characterized by the expression of weak A antigen on type B red blood cells (RBCs) and detection of anti-A antibodies in the serum [3]. The frequency of B(A) is low among Caucasians in Europe, ranging from approximately 1 in 170,000–580,000, but it is relatively high in China (approximately 1 in 50,000–100,000) [4]. Moreover, the frequency of B(A) gradually increases from north to south China [5]. Nowadays, six *B(A)* alleles, namely *ABO*BA.01–06*, have been documented on the ISBT website (https://www.isbtweb.org/resource/001aboalleles.html, accessed 15 Nov 2023). Most common *B(A)* alleles in China are *ABO*BA.02* and *ABO*BA.04*, the latter predominately reported from north China [4].

Molecular genetic analysis revealed that at least six amino acid substitution positions are responsible for the B(A) phenotype. In addition to the four key positions (176, 235, 266, 268) corresponding to the characteristic A and B, there are two positions 214 and 234 (Table 1). The purpose of this work was to report a novel *B(A)* allele with c.797T>C (p.Met266Thr) on the *ABO*B.01* backbone in a father and his daughter belonging to a northern Chinese family and assess the possible impact of this novel mutation.

MATERIALS AND METHODS

Samples

A father (age: 57 years) with his daughter (age: 32 years) visited our blood group reference laboratory of Dalian Blood Center and requested for ABO group tests, because they were found to have an inconclusive blood typing results during their previous hospital visits. After they provided informed consent, EDTA anticoagulant blood samples were collected.

Serology for ABO grouping

ABO forward and reverse typing was performed using the saline tube method. In the forward typing test, anti-A, anti-B, anti-A,B and anti-H (monoclonal anti-A and anti-B: Changchun Brother Biotech Co, Ltd.; anti-A,B: DIAGAST; anti-H: Shanghai Hemo-pharmaceutical Biological Company) were used to detect the A, B and H antigens, respectively, on the RBCs. Reverse typing was determined with A1, B and O cells (Shanghai Hemo-pharmaceutical Biological Company) with a tube test by trained staff according to the manufacturer's instructions. Adsorption and elution were performed to confirm the presence of A antigens on RBCs with monoclonal anti-A antibody according to the standard protocol. Elution was performed using heat elution procedure.

PacBio long-read single-molecule real-time sequencing

Genomic DNA was extracted from peripheral whole blood samples using a commercially available HiPure Blood DNA Mini Kit according to the manufacturer's instructions. The entire ABO gene, including flanking regulatory regions in the three overlapping fragments (Figure S1), was amplified. The fragments overlapped more than 1 kb. Long-range PCR reaction mixtures were composed of 5 μL of 5× PrimeSTAR GXL buffer, 2 μL of dNTP mixture (2.5 mM each), 0.5 μL of PrimeSTAR GXL DNA polymerase, 0.26 μL of each PCR primer mix (100 μM), 30 ng DNA templates and DNase/RNase-free deionized water in a final reaction volume of 25 μL. The cycling program was 94°C for 2 min, followed by 26 cycles of 98°C for 12 s, 68°C for 12 min (starting from the 11th cycle, increase each cycle by 30 s) and a final extension step at 68°C for 10 min. The PCR products were used for preparing the library for PacBio sequencing.

In silico analysis

3D molecular models of the wild-type (PDB ID, 1LZ7) and mutant GTB were generated using the DynaMut web server (https://biosig.lab.uq.edu.au/dynamut/). The effect of the mutation on the enzyme function was predicted by PROVEAN v1.1.3. (http://provean.jcvi.org/index.php) and PolyPhen2 v2.2.3r406 (http://genetics.bwh.harvard.edu/pph2/). The predictions of stability changes were performed using DynaMut and SNPeffect (https://snpeffect.switchlab.org/). These tools provided the difference in the Gibbs free energy between the ΔG mutant and the ΔG wild-type protein, $\Delta\Delta G = \Delta Gm - \Delta Gw$, implying the impact of substituted mutation on the stability of a protein.

RESULTS

Serological results

Two probands were identified to have an ABO phenotype with similar unusual features. Although both A and B antigens were present, A antigen exhibited weak reactivity during agglutination tests. B antigen exhibited normal reactivity, and anti-A antibodies were noted in their serum (Table 2). According to serological characteristics, we concluded that the two probands were possible cases of the B(A) phenotype.

PacBio sequencing results

Based on the PacBio sequencing results, the ABO allele haplotype sequences of each proband were determined. In addition to the *ABO*O.01.01* and *ABO*B.01* alleles present in the father and daughter, respectively, they also carried an *ABO*B.01* allele with a missense mutation at c.797C>T. The c.797C>T mutation causes replacement of methionine by threonine at key position 266. Interestingly, threonine at this position is not associated with GTA or GTB (Table 1).

In silico analysis

In order to assess the possible impact of this novel mutation, the DynaMut platform was used to generate the mutant's structure using 3D molecular modelling [6]. The crystallization of the wild-type and mutant GTB was analysed. Figure 1b depicts the difference in vibrational entropy and interatomic interactions between the wild type and mutant. As determined by ENCoM, the Δvibrational entropy energy between the mutant and wild type was 0.398 kcal mol^{-1} K^{-1}, which indicated an increase in molecular flexibility. Differences in interatomic interactions such as hydrogen bonds and ionic interactions of the wild type and mutant are depicted in Figure 1c,d. Thr266 substitution does not disrupt the hydrogen bonds (red dotted line). However, some changes in interatomic contacts, such as loss of ionic interactions (yellow dotted line) and hydrophobic contacts (green dotted lines), and addition of weak hydrogen bonds (orange dotted lines), can affect the stability of proteins. The algorithms of PROVEAN and PolyPhen2 predicted the effect of the mutation on the enzyme function. The PROVEAN score of mutation was −0.213, which indicates that the mutation was 'neutral'. Using the PolyPhen-2 analysis, this variant was predicted to be 'benign' based on both HumDiv and HumVar datasets. DynaMut and SNPeffect were employed to calculate the effect of the mutation on protein stability. According to the predicted DynaMut ΔΔG value (−0.454 kcal mol), the mutant destabilized the protein compared with the wild type. Moreover, the mutation at position 266 resulted in a ΔΔG of 0.89 kcal mol, as estimated by the SNP effect. This indicates that the mutation slightly reduced protein stability.

DISCUSSION

GTA and GTB are very similar and have overlapping functions. GTB can synthesize blood group A antigen using the same donor and acceptor substrates as used by GTA [7]. The B(A) blood group is a rare subtype. RBCs of an individual are described as B(A) when, despite them coming from a B individual who lacks the A gene, they are weakly agglutinated by potent anti-A monoclonals. There is also anti-A in their serum that reacts with both A1 and A2 RBCs. Although the B(A) phenotype is inherited in a *cis* manner, it can be classified separately from *cis*AB phenotype due to the presence of anti-A in the serum [8]. The amount of B antigen on B(A) subtype RBCs is lower than that on normal B RBCs [9]. This may cause the H antigen intensity of subtype B(A) to be higher than that of the normal B type.

In the present study, the full-length haplotype sequences of the ABO gene in the probands with the B(A) phenotype were obtained through third-generation long-read single-molecule real-time sequencing. Two probands harboured the novel *B(A)* allele, which was different from the normal *ABO*B.01* allele only by one nucleotide substitution: c.797T>C (p.Met266Thr). This allele was neither recorded on the ISBT website nor found in the dbSNP. The daughter carried the *B* allele in *trans*, whereas the father carried *O* allele in *trans*, explaining the observed increased agglutination reactivity with anti-H (Table 2).

Different amino acids may play varied roles in the protein structure. Modelling 3D molecules and analysing variant changes in the protein structure are crucial for gaining a deeper understanding of the potential mechanisms underlying protein changes. Our 3D structural analysis revealed that the threonine-introducing mutation does not disrupt hydrogen bonds and some changes in interatomic contacts may reduce protein stability. The residue 266 occupies a position in the GTB's active site that can interact with donor-sugar residues. Met266 and Ala268 residues in GTB are both bulkier with respect to GTA, leaving GTB with a smaller and more conformationally restricted active site favouring UDP-Gal over UDP-GalNAc [10]. In this study, a hydrophobic and non-polar molecule methionine in residue 266 in the wild-type GTB was replaced by a hydrophilic and polar molecule

TABLE 2 Results of serological grouping and *ABO* gene analysis.

	RBC grouping					Serum grouping			Genotype	
Proband	-A	-B	-A_1	-AB	-H	A_1 cells	B cells	O cells	Allele 1	Allele 2
Father	1+	4+	-	4+	4+	1+	-	-	*ABO*O.01.01*	*ABO*B.01* with c.797C>T
Daughter	1+	4+	-	4+	w+	1+	-	-	*ABO*B.01*	*ABO*B.01* with c.797C>T

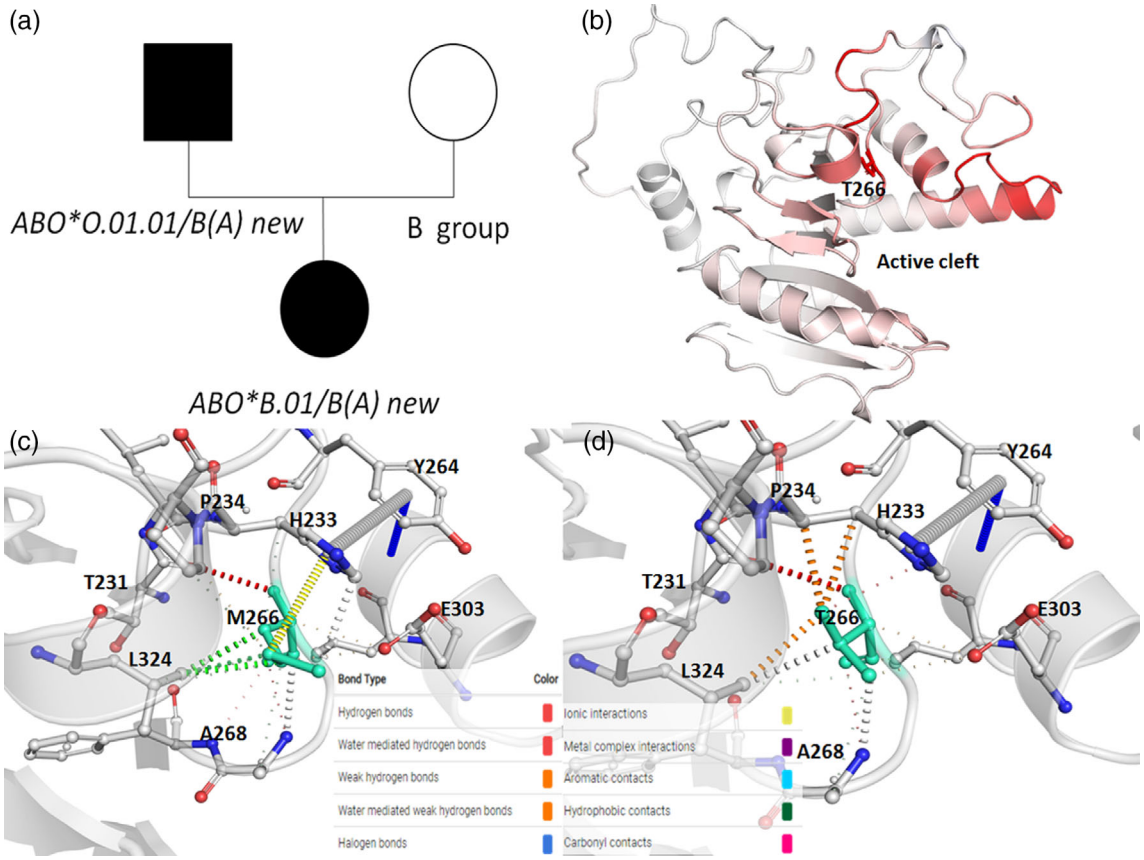

FIGURE 1 (a) Schematic diagram of the inheritance of one Chinese family. Black fill indicates the probands. (b) The structure of 3D molecular mutant and visual representation of protein flexible conformation based on the vibrational entropy difference. Amino acids coloured according to the vibrational entropy change upon mutation. Red represents a gain in flexibility of the structure. The interatomic interaction between wild-type (c) and mutant structures on GTB structure (d). Wild-type and mutant residues are depicted as light green sticks alongside the surrounding residues that are involved in any form of interaction. Thr266 substitution does not disrupt the hydrogen bonds (red dotted line). However, some changes in interatomic contacts.

threonine. Moreover, threonine is slimmer than the corresponding residue methionine in the wild type. We inferred that the substitution possibly alters the spatial structure and causes changes in the sugar donor specificity at that site to GTA, thereby giving rise to an overlapping functional transferase, as suggested by the serological findings of this study.

Another study involving the 3D spatial structure analysis of GTA and GTB confirmed that position 176 had no physical interactions with the substrates, but position 235 was in proximity, and positions 266 and 268 exhibited direct contact [10]. The mutation found in this study was at the key position 266. It causes GTB to synthesize small amounts of A antigen. Interestingly, the *ABO*B.01*, *ABO*cisAB.02* (which was first discovered in a Vietnamese family [11] and rare in the Chinese population [12], is caused by c.796A>C), and the novel *B(A)* allele found in this study (c.797T>C) only differed at position 266 (Met/Leu/Thr). However, they exhibited the phenotypes of normal B, AB_{weak} and $A_{weak}B$, respectively. This further demonstrates that the enzyme's specificity was associated with the crucial position 266. The residue 266 is positioned to contact the characteristic acetamido/hydroxyl groups and so distinguishes between UDP-GalNAc and UDP-Gal [10].

In summary, we here defined a novel *B(A)* allele in a Chinese family. c.797T>C (p.Met266Thr) mutation on the *ABO*B.01* backbone in the subjects exhibited an $A_{weak}B$ phenotype with anti-A antibodies in their serum.

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S.H.Z. and X.H.L. conceptualized the study, L.N.S. conducted experiments, Y.C.Y. and Y.X.X. analysed the data, C.X.L. provided resources and L.N.S. wrote the draft.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests regarding the publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Lin-Nan Shao https://orcid.org/0000-0002-9025-4957
Shi-Hang Zhou https://orcid.org/0000-0001-9295-7747

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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