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The Novel Variant *c.122delG* on the *ABO*B3.0x* Allele Associated with B_3 Phenotype

Yu Zhang[a, b] Yating Ling[a, b] Meng Li[c] Qiang Fu[b] Chengtao He[a, b]

[a]Blood Group Reference Laboratory, Nanjing Red Cross Blood Center, Nanjing, China; [b]Key Laboratory of Transfusion Medicine, Nanjing Red Cross Blood Center, Nanjing, China; [c]Blood Transfusion Department, Children's Hospital of Nanjing Medical University, Nanjing, China

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Abstract
Introduction: B_3 is known to be one of the B subtypes that are characterized by serologic mixed-field agglutination. The proportion of Chinese Han individuals with the B_3 subtype (B type) and AB_3 subtype (AB type) is about 1/900 and 1/1,800, respectively. Here, we identified a novel ABO subgroup allele that caused B_3 phenotype. ***Methods:*** The ABO phenotypes of the proband and his father were typed with the traditional test tube method. The *ABO* genotype was analyzed by SMRT sequencing. ***Results:*** A *c.122delG* variant was identified in both the proband and his father, who exhibited the B_3 phenotype. This variation results in a premature stop codon, leading to mixed-field agglutination of the serological B antigen. ***Conclusion:*** The novel variation of *c.122delG* in the exon 3 of *ABO*B3.0x* allele were identified in Chinese individuals, resulting in mixed-field agglutination of B antigen expression and the formation of ABO subtypes.

Introduction

The ABO blood group system was discovered by Austrian scholar Karl Landsteiner in 1990 [1]. The ABO blood group system is the most critical red blood cell classification in humans, holding significant importance in clinical blood transfusion, organ transplantation, hemolytic disease of the fetus and newborn, and forensic identification [2]. In addition to the common A, B, O, and AB phenotypes in the ABO blood group system, several subtypes of ABO phenotypes have been identified in various populations, which frequently demonstrate discrepancies between forward and reverse ABO typing. This is often attributed to diminished antigen expression and/or reduced antibody activity [3]. The ABO blood group phenotype is affected by genetic changes like nucleotide substitutions, splice site variations, insertions, deletions, and hybrid alleles, which can reduce or eliminate the activity of the ABO glycosyltransferase enzyme, resulting in decreased or absent antigen expression [4]. B_3 is known to be one of the B subtypes that are characterized by serologic mixed-field agglutination with anti-B. The precise identification of the B_3 blood group subtype through serological methods is often complicated by the occurrence of mixed-field agglutination, which may be influenced by factors such as chimerism, recent blood transfusions, or stem cell transplantation. Latest next-generation sequencing can accurately identify ABO subtypes at the genetic level by eliminating interference from other factors. Here, we report a rare B allele associated with a B_3 phenotype in a Chinese Han individual.

karger@karger.com
www.karger.com/tmh

Correspondence to:
Chengtao He, hechengtao413@126.com

Table 1. Serological and genetic results of the proband and his father

Family member	Forward typing			Reverse typing				Phenotype	Genetic results
	anti-A	anti-B	anti-H	A_{1C}	B_C	O_C	self-cell		
Proband	4+	2 + mf	1+	0	0	0	0	AB_3	*ABO*A1.02/ABO*B$_3$*
Father	0	2 + mf	4+	4+	0	0	0	B_3	*ABO*B$_3$/ABO*O.01.01*

mf, mixture of agglutinated and non-agglutinated red blood cells (mixed-field agglutination); 0, non-agglutination; 1–4+, agglutination strength; A_{1C}, 2%–5% suspension of A_1 red blood cells; B_C, 2%–5% suspension of B red blood cells; O_C, 2%–5% suspension of O red blood cells.

Methods

The proband is an 11-year-old Chinese boy who was admitted to Nanjing Children's Hospital for treatment due to adenoid hypertrophy. Both ABO forward and reverse typing of the proband and his father were performed using the tube method to identify the ABO type, following the standard operating procedure for American Association of Blood Banks (AABB) technical manual. Forward typing was determined with anti-A, anti-B antibodies (Shanghai Hemo-Pharmaceutical Biological Company, Shanghai, China), and anti-H (Sanquin Reagents B.V, The Netherlands). Reverse typing was determined with A_1, B, and O cells (Shanghai Hemo-Pharmaceutical Biological Company, Shanghai, China) using tube tests. Genomic DNAs were extracted from the same blood sample using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). The SMRT sequencing was completed by cooperating with Xian HAORUI Gene Technology Co., Ltd. This study was approved by Children's Hospital of Nanjing Medical University and informed consent was obtained from the proband and his father.

Results

The serological and molecular findings are consolidated in Table 1. The forward typing results showed that the RBCs of the proband had 4+ agglutination with anti-A and mixed-field agglutination reactions with anti-B. The RBCs of the proband's father had no reaction with anti-A and mixed-field agglutination reactions with anti-B. Besides, normal anti-A antibodies were detected in the serum of proband's father using standard cells. The serological findings indicate that the proband exhibits an AB_3 phenotype, while his father exhibits a B_3 phenotype. SMRT sequencing showed that the proband and his father was identified to carry a single nucleotide deletion of G at position 122 in the exon 3 of *ABO*B3.0x* allele. This single nucleotide deletion results in a reading frame shift and a premature stop codon at positions 118–120, and leading to a truncated protein of only 39 amino acids. The sequence of the novel allele has been submitted to GenBank and the accession number PQ278641 was assigned.

Discussion

B_3 is an uncommon blood type characterized by mixed-field agglutination of red blood cells upon interaction with the anti-B antibody. Several gene variants related to the B_3 phenotype have been reported, the most common of which in the Chinese population is *IVS3+5G>A*. The *IVS3+5G>A* variant disrupts the conserved splice donor site sequence, causing exon 3 to be skipped during mRNA processing. This results in a B_3 transcript that encodes a B-transferase protein missing 19 amino acids in the N-terminal, reducing B antigen expression and causing mixed visual field agglutination [5].

In this study, we found that the proband and his father showed a distinct mixed-field agglutination of B antigen in serology. Gene sequencing technology was applied to elucidate the underlying cause of mixed-field agglutination exhibited by B antigens. The results showed that *c.122delG* was present in the *ABO*B3.0x* allele, which was not included in the ISBT database. This variation is similar to *c.261delG* of the O allele. Both have a deletion of nucleotide G, which leads to the premature appearance of the stop codon, but the serological expression is completely different. The deletion of a single nucleotide G in the O allele occurs in exon 6, resulting in a truncated protein and a non-functional catalytic domain. The single nucleotide G deletion of this sample occurred in exon 3, this variation may damage the function of glycosyltransferase B, leading to a decrease in its stability, which serologically manifested as significant mixed-field agglutination of the B antigen on red blood cells. Although both are delet of a single nucleotide of G, the location of the deletion is different, resulting in inconsistent serological results. Further research is required to elucidate the specific molecular mechanisms underlying

these differences. In conclusion, one novel allele with a *c.122delG* variation in the *ABO*B3.0x* background was found, which was predicted to be responsible for B_3 and AB_3 phenotypes in Chinese individuals.

Statement of Ethics

The proband and his father have provided their written informed consent to gain access to and use of confidential and personal information used to write the manuscript and publish the manuscript in a scientific journal. This study protocol was reviewed and approved by the Medical Ethics Review Committee of Nanjing Red Cross Blood Center, Approval No. NJRCBCEC-2024-R01.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Meng Li provides samples of the proband and his father. Yating Ling contributed to the serological testing of the samples. Yu Zhang performed the molecular assays and wrote the manuscript. Chengtao He and Qiang Fu designed the experiments and critically examined the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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