


Identification of a novel O allele with c.777C>G on an *ABO**O.01.02 background

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ABO is considered one of the most clinically relevant blood group systems in the field of immunohematology.¹ Since 1990, the discovery of the genetic diversity at the ABO locus, many different phenotypes have been identified and reported due to *ABO* gene mutations that influence the activity of glycosyltransferase.² The presence of ABO subgroup alleles often is associated with the weakening of A and/or B antigens or inconsistency in the results of forward and reverse typing. It was reported that over 300 *ABO* alleles have been identified worldwide.³ Single-molecule real-time sequencing by Pacific BioSciences (PacBio) has great potential in the quest for comprehensive haplotype sequence collections of blood group gene alleles.⁴ Here, we present one novel O allele of ABO blood group system using PacBio sequencing, which was found in a Chinese individual.

1 | BRIEF METHODS

The proband was a 72-year-old woman with severe anemia. ABO blood typing was performed using standard serologic methods, which included forward typing and reverse typing tests. In the forward typing test, anti-A, anti-B, anti-A,B, and anti-H (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. Genomic DNA was extracted using a commercially available DNA isolation kit on the MagCore Automated Nucleic Acid Extractor (RBCBioscience, Taipei, Taiwan, China). To identify the *ABO* gene haplotypes, the entire *ABO* gene including flanking regulatory regions (more than 23 kb) in the three overlapping fragments (Figure S1) was sequenced by PacBio sequencing (Pacific BioSciences) on a Sequel II/IIe system. The fragments overlapped more than 1 kb.

2 | RESULTS AND DISCUSSION

Table 1 summarizes the results of serological testing and *ABO* genotyping conducted in this study. The RBCs of the proband showed w + agglutination with anti-A, 4+ agglutination with anti-B, anti-A,B, and anti-H reagents. The plasma of the proband exhibited 2+ agglutination with A₁ cells. The eluate displayed 2+ agglutination with A₁ cells in the adsorption and elution test. According to the serologic characteristics and the molecular results (see below), the proband was assigned a B(A) phenotype.

PacBio sequencing showed that the proband was heterozygous for the *ABO**BA.04 allele and an *ABO**O.01.02-like allele containing an additional mutation c.777C>G in Exon 7. The nucleotide sequence of the novel O allele has been lodged in GenBank under accession number OR612064. The c.777C>G of *ABO* gene mutation was not found, but a c.777C>T (rs782713806, with frequency of

TABLE 1 Results of serologic grouping and ABO gene analysis.

Forward typing					Reverse typing			Adsorption and elution	Genotype	
Anti-A	Anti-B	Anti-A ₁	Anti-A,B	Anti-H	A ₁ cell	B cell	O cell	A ₁ cell	Allele 1	Allele 2
w+	4+	0	4+	4+	2+	0	0	2+	ABO*BA.04	ABO*O.01.02 (777G)

0.000011) mutation of the *ABO* gene was listed in the NCBI dbSNP database. Because c.261delG causes a termination codon and premature termination of translation, this novel allele encodes a truncated, inactive protein. B(A) was observed in 1/(50,000-100,000) northern Chinese people, with ABO*BA.04 being the most common allele.⁵ When the patient's blood type is suspected or confirmed to be the B(A) subtype, B or O washed RBCs and AB plasma can be transfused.

In conclusion, a novel O allele was identified in a Chinese female with a B(A) phenotype. This novel allele has the c.777C>G variant on the ABO*O.01.02 allele background.

CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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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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