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LETTER TO THE EDITOR

PacBio third-generation sequencing detects a new variant, *c.27delC*, in exon 1 of the *ABO* gene resulting in a weak B phenotype

Dear Editor,

The ABO blood group system is complex genetic traits in the human body that has significant applications in transfusion, organ transplantation, and forensic medicine. The *ABO* gene is responsible for encoding glycosyltransferases that synthesise antigens A and B, which are mainly expressed on the surface of red blood cells.[1] Variations within exons 6 and 7 of the *ABO* gene typically result in ABO subtypes, such as single-nucleotide polymorphisms, base insertions, or deletions, which lead to changes in the activity of glycosyltransferases that affect antigen expression. Variations in the splice site and non-coding or regulatory regions of *ABO* can impact its function. These include sequence variations from hybridization between coding exons, flanking introns, and common alleles, such as *c.28G>A*, which downregulates *ABO* mRNA levels through abnormal splicing, resulting in the B_3 subtype.[2] Also, variations in intron 1, like *c.29-10T>A* near the exonic splicing region, may affect gene product splicing, leading to the Ael[3] phenotype.[3] Additionally, variations in regulatory areas such as the CCAAT-binding factor/nuclear factor Y (CBF/NF-Y) binding site, proximal promoter region, and the +5.8 kb site impact transcription factor binding to promoters.[4,5] These variations, alone or in combination, contribute to the diversity of ABO blood group subtypes. The presence of these subtypes increases the complexity of blood type identification in clinical practice. Traditional serological testing methods may not accurately identify certain subtypes, whereas molecular biology methods based on PCR sequence analysis and high-throughput sequencing can provide more accurate genotypic information.[6] Herein we present a new variant in exon 1 of the *ABO* gene associated with a weak B phenotype.

The classic tube test method was used for ABO blood group testing. Forward typing was performed using monoclonal anti-A and anti-B antibodies (clones No. SRBC-B3 + 9113D10 and SRBC-C1 + 9621A8) and anti-A,B (clone No. ES4 + ES15; Lorne Laboratories, Berkshire, UK) to detect antigens A and B on the surface of red blood cells. Reverse typing was performed using red blood cells of types A1, B, and O (Shanghai Hemo-Pharmaceutical Biological Company, Shanghai, China) to detect anti-A and anti-B antibodies in plasma. The H antigen was identified using anti-H lectin (clone No. H5B12) with B and O cells serving as positive controls. The absorption and elution test involved the use of monoclonal anti-B for cold absorption at 4°C for 1 h, followed by six washes and hot elution at 56°C for 10 min to detect the anti-B antibodies in the eluate. Genomic DNA was extracted from the donors' whole blood using a commercial kit (Takara, Dalian, China) in accordance with the manufacturer's instructions. *ABO* exons 6 and 7 were sequenced by Sangon Biotech (Shanghai, China). The full-length haplotype sequence of *ABO* gene was analyzed using PacBio long-read single-molecule real-time sequencing technology (HaoRui Genomics, Xi'an, China). The reference allele *ABO*A1.01* (GenBank accession number: NG_006669.1) was used for sequence comparisons.

During routine blood group testing, we discovered an incongruity in the blood type of a 20-year-old adult female Han Chinese donor. In forward typing, the donor's red blood cells did not agglutinate with the anti-A antibody and showed weak agglutination (±) with the anti-B antibody. The red blood cells exhibited strong 4+ agglutination with anti-H, and positive control cells exhibited normal agglutination intensity with anti-H. In reverse typing, the plasma exhibited strong agglutination (4+) with A1 cells, but no agglutination with B, O, or autologous cells. The absorption elution test detected the B antigen with an agglutination intensity of 3+ (Table 1).

Sequencing of exons 6 and 7 disclosed genotype *ABO*O.01.02/ABO*B.01*. PacBio long-read single-molecule real-time sequencing indicated that on one haplotype the donor harboured the *ABO*O.01.02* allele, whereas on the other haplotype there was a new variant in the *B* allele. This variant involved the deletion of the cytosine base at position 27 in exon 1 of *ABO*B.01*, leading to a synonymous variant at the ninth amino acid translation site (Figure 1). The nucleotide sequence of this new allele has been submitted to GenBank (accession number: PP500623).

The new deletion variant is predicted to cause all amino acids after the ninth to be affected accordingly, and a premature stop codon (TGA) at positions 31–33 in exon 2, leading to premature termination of translation. Notably however, expression of the corresponding antigen was detected, which may be explained by several mechanisms. (1) The variant *c.27delC* may affect the splicing of *ABO* mRNA, causing the sequence containing the premature stop codon to be excluded from the mature mRNA, thus ensuring that the subsequent exons continue to be translated normally and produce a complete protein.[2] (2) An alternative open reading frame may bypass the premature stop codon with a new start codon.[7] (3) The variant is situated in the CpG

TABLE 1 ABO blood typing results and genotype analysis.

Forward				Reverse			Absorption-elution		
Anti-A	Anti-B	Anti-A,B	Anti-H	A_1C	BC	OC	BC	OC	*ABO* genotype
0	±	0	4+	4+	0	0	3+	0	ABO*O.01.02/ABO*BWnew(c.27delC)

Note: 0 = no agglutination; ± = weak agglutination; 3+ = 2–3 large agglutinates with clear supernatant; 4+ = single large agglutinate.
Abbreviations: A1C, A1 cells; BC, B cells; OC, O cells.

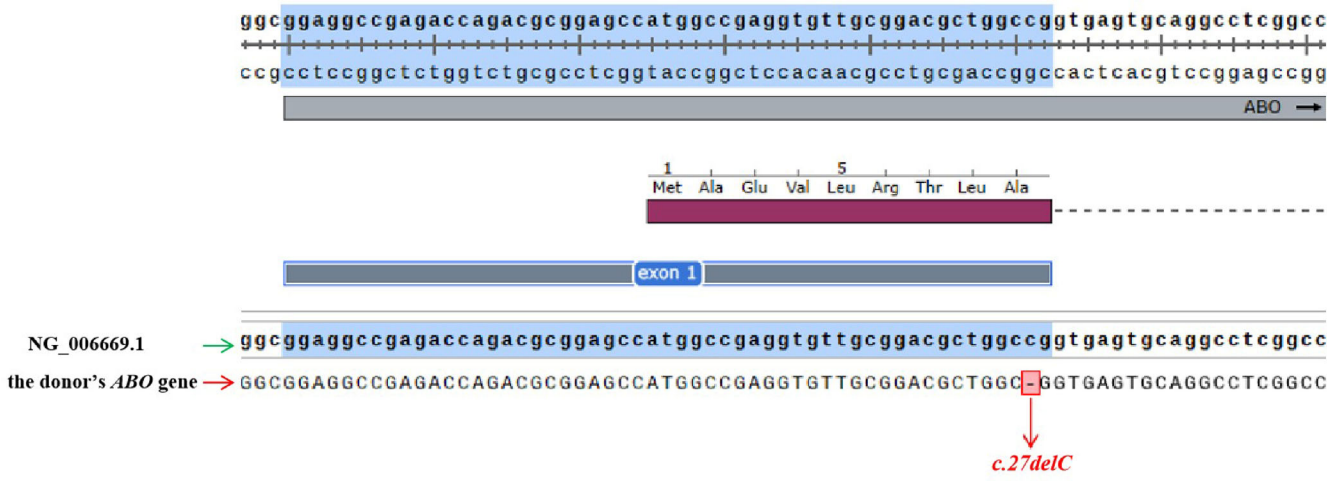

FIGURE 1 The donor's *ABO* gene PacBio third-generation sequencing results.

island region, which is an unmethylated CpG site that is densely distributed in the promoter and exon1 regions of *ABO* (−650 to +50). This variant may influence the methylation status of the CpG island, and such methylation can hinder transcription factors (e.g., SP1) from binding effectively to the promoter, thus diminishing *ABO* expression.[8] (4) Cells may repair the variant via homologous recombination or other DNA repair mechanisms, thereby restoring the normal coding sequence of the protein. The relationship between this variant and weak antigen expression in the current case requires further investigation.

In summary, we discovered a novel deletion variant in the *B* allele of the *ABO* gene, *c.27delC*, located in exon 1 of *ABO*B.01*. This variant results in a weak B phenotype. PacBio third-generation sequencing is effective for identifying alleles resulting in new ABO subtypes.

AUTHOR CONTRIBUTIONS

Shao L. performed the research, analyzed the data, and drafted the manuscript. Ma L. designed the research and reviewed the literature. Xiao J.Y. contributed to DNA sample preparation. Shi L.L. and Liu T.X. analyzed the sequencing results. All authors have contributed significantly to the research and have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

All data included in the study are available upon request by contact with the corresponding author.

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