Received: 25 January 2024 | Revised: 13 April 2024 | Accepted: 17 May 2024

DOI: 10.1111/trf.17910

ONE-PAGE REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

A novel c.29-3C>G variant on the *B* allele forms the B_{el} phenotype

Yongkui Kong[1] | **Li Wang**[1] | **Cunquan Kong**[2] | **Qiankun Yang**[1]

[1]Department of Blood Transfusion, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

[2]Department of Blood Transfusion, Henan Provincial People's Hospital, Zhengzhou, China

Correspondence
Yongkui Kong, Department of Blood Transfusion, The First Affiliated Hospital of Zhengzhou University, No.1 Jianshe East Road, Erqi District, Zhengzhou, Henan, China.
Email: kyk0418@163.com

KEYWORDS: ABO blood group, novel allele, subtype

1 | BACKGROUND

The ABO gene's coding sequence consists of 1065 base pairs arranged in seven exons. Blood group A individuals possess the *A* allele that produces the GTA enzyme, while those with blood group B carry the *B* allele, responsible for generating GTB. Those with AB blood type inherit both alleles. Conversely, type O individuals typically have a deletion at position c.261 based on *A1.01*, which involves the loss of a guanine (G). At the cDNA level, the *A* and *B* alleles diverge by seven nucleotides, changing four amino acids at positions p. 176, p. 235, p. 266, and p. 268. Beyond coding region variants, alterations can also occur in the regions surrounding the *ABO* exons. These changes may induce alternative splicing, giving rise to different ABO protein isoforms.[1] In this report, we identified a novel B_{el} allele marked by the c.29-3C>G gene variant, which is located in the 3′ end of intron 1 and can be retrieved as a single nucleotide polymorphism locus in the dbSNP database (rs587631486).

2 | BRIEF METHODS

A 47-year-old female was hospitalized for excessive menstrual bleeding and extended cycle duration. After obtaining her informed consent, 3–5 mL of whole blood was collected in EDTA anticoagulant for study purposes. The First Affiliated Hospital Ethics Committee of Zhengzhou University approved the research (2023-KY-0870-003). For serologic analysis, adsorption-elution and saliva assays were conducted using the tube method, adhering to the procedures outlined in the 18th edition of the AABB Technical Manual.[2] The reagents employed included monoclonal anti-A and anti-B from Changchun Brother Biotech, polyclonal anti-A and anti-B sourced from the plasma of healthy donors with a titer of 128 or higher, anti-H from Sanquin, anti-D from Zhuhai BASO, and A cells, B cells, and O cells (Ac, Bc, and Oc) from Changchun Bioxun. The proband's genomic DNA was isolated using the Blood Genome Kit from Beijing TIANGEN. The DNA sample was then forwarded to Xi'an Haorui Gene Technologies Ltd. for third-generation long-read sequencing. The sequencing protocol was based on the technique developed by Wang et al.[3] The ISBT-recommended NG_006669 sequence served as the reference genome for analysis.

3 | RESULTS AND DISCUSSION

Serologic testing (Table 1) indicated that the proband's forward typing showed no agglutination with anti-A and anti-B but a strong 4+ agglutination with anti-H. Reverse typing revealed 4+ agglutination with Ac. The adsorption-elution assay confirmed the presence of B antigen, while the saliva assay detected only substance H. These serologic findings clearly define the B_{el}

List of Abbreviations: GTA, α-(1→3)-N-acetylgalactosaminyltransferase; GTB, α-(1→3)-galactosyltransferase; ISBT, International Society of Blood Transfusion.

TABLE 1 Results of serologic grouping and *ABO* gene analysis.

ABO blood group typing										*ABO* genotype and variant	
				Adsorption-elution							
Anti-A	Anti-B	Anti-H	Anti-D	Ac	Bc	Oc	Ac	Bc	Oc	Saliva test	Haplotype 1	Haplotype 2
0	0	4+	4+	4+	0	0	0	2+	0	Substance H	*ABO*O.01.01* (c.261delG)	*ABO*BEL.new* c.29-3C>G;c.297A>G; c.526C>G;c.657C>T;c.703G>A; c.796C>A;c.803G>C;c.930G>A

phenotype. Advanced third-generation long-read sequencing revealed a novel allele on one haplotype which consists of variant c.29-3C>G on a *B.01* background (c.297A>G, c.526C>G, c.657C>T, c.703G>A, c.796C>A, c.803G>C, and c.930G>A). This new B_{el} allele has been documented in GenBank under the accession number OR538571. The other haplotype is *O.01.01* due to c.261delG. The c.29-3C>G variant, located at the end of intron 1, is inferred to trigger alternative splicing due to its proximity to the conserved AG dinucleotide of the intronic GU–AG splicing sequence. Research by Hong et al. has indicated that variants near exon-intron junctions can disrupt normal splicing and activate cryptic splice sites, resulting in irregular mRNA splicing.[1] Based on this evidence, we propose that the c.29-3C>G variant induces a splicing defect in exon 2 of the *ABO* gene, resulting in an aberrant form of the GTB, thus producing B_{el} phenotype.

4 | BRIEF SUMMARY

Our results suggest that c.29-3C>G in *B.01* forms a novel B_{el} allele. The variant may result in an alternative splicing of the *B* allele, which may be responsible for the reduced activity of GTB.

CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

ORCID

Yongkui Kong https://orcid.org/0000-0003-3583-4606

REFERENCES

1. Hong X, Ying Y, Zhang J, Chen S, Xu X, He J, et al. Six splice site variations, three of them novel, in the ABO gene occurring in nine individuals with ABO subtypes. J Transl Med. 2021; 19:470.
2. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM. AABB technical manual. 18th ed. Bethesda: AABB; 2014.
3. Wang C, Tang Y, Zhang P, Xiong L, Chen W, Lv X. Detection and phenotype analysis of a novel Ael blood group allele. Vox Sang. 2024;119:74–8.

How to cite this article: Kong Y, Wang L, Kong C, Yang Q. A novel c.29-3C>G variant on the *B* allele forms the B_{el} phenotype. Transfusion. 2024;64(7):E28–9. https://doi.org/10.1111/trf.17910