Letter to the Editor
Transfusion and Cell Therapy

Ann Lab Med 2023;43:124-126
https://doi.org/10.3343/alm.2023.43.1.124
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Allelic Enhancement of *BEL.02* With the Single Nucleotide Variant, c.669G>T

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Dear Editor,

The ABO phenotype is determined by the expression of *ABO*—a single glycosyltransferase gene on chromosome 9q34. Genetic variants of ABO subgroup can lead to conformational changes and enzyme dysfunction, impairing the catalytic ability of glycosyltransferase A (GTA) or glycosyltransferase B (GTB). Mutated enzymes can be affected by co-inherited normal alleles, resulting in phenotypic heterogeneity—termed allelic competition or enhancement [1]. While allelic competition has been reported in both the A and B subgroups [1-3], allele enhancement is reported mostly in subgroup A [4-6] (Table 1). One report of allelic enhancement in subgroup B exists; however, the allele could not be identified through genetic testing [7]. We report the first case of genetically identified allelic enhancement in subgroup B in a family carrying the *BEL.02* allele. This study was approved by the Institutional Review Board of Samsung Medical Center (SMC), Seoul, Korea (2022-02-029-001); the participants gave written informed consent for *ABO* genotyping.

A peripheral blood sample from a 30-year-old woman with an ABO discrepancy was sent to SMC. The phenotype of the proband was identified as A_1B_{weak} using routine serological testing (Fig. 1B). We conducted *ABO* genotyping by sequence analysis of exons 6 and 7 of *ABO* [1]. The *ABO*A1.02/ABO*BEL.02* genotype with a single nucleotide variant, c.669G>T, was identified. Routine serological testing revealed that her mother's and father's blood types were O and A, respectively. To uncover the cause of the unusual blood group inheritance pattern in the family and the phenotype–genotype discrepancy, across-family serological testing, adsorption-elution testing, and *ABO* genotyping were performed (Fig. 1B). The *BEL.02* allele was identified in the mother (I-2), the mother's sister (I-3), and a maternal cousin (II-3); however, the phenotypes differed according to the co-inherited allele (Fig. 1A). Routine serological testing revealed that the mother and her sister had the O phenotype; however, adsorption-elution testing revealed the presence of a B antigen showing the B_{el} phenotype. In contrast, the proband (II-2) and maternal cousin showed an A_1B_{weak} phenotype with weak agglutination (+) with anti-B, suggesting allelic enhancement, where the *ABO* allele in *trans* can enhance the expression of its counterpart allele.

Received: April 14, 2022
Revision received: June 15, 2022
Accepted: August 14, 2022

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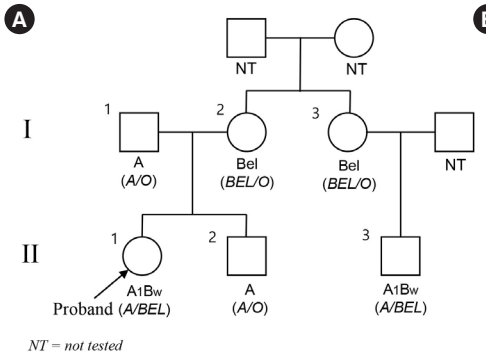

B

Subject No.	Forward typing†				Reverse typing†		Adsorption and elution†	Genotype	
	Anti-A	Anti-B	Anti-A,B	Anti-H	A1 cell	B cell	B cell	Allele 1	Allele 2
II-1*	4+	2+	4+	+-	-	+	NT	*ABO*BEL.02*	*ABO*A1.02*
II-2	4+	-	4+	-	-	3+	-	*ABO*A1.02*	*ABO*O.01.02*
II-3	4+	2+	4+	-	-	-	NT	*ABO*BEL.02*	*ABO.A1.02*
I-1	4+	-	4+	-	-	3+	-	*ABO*A1.02*	*ABO*O.01.02*
I-2	-	-	-	4+	4+	-	+	*ABO*BEL.02*	*ABO*O.01.02*
I-3	-	-	-	4+	4+	-	+	*ABO*BEL.02*	*ABO*O.01.02*

Abbreviation: NT, not tested
* Proband
† The reagents used for serological testing were as follows: anti-A, anti-B, anti-A,B, A1 cell, and B cell (Ortho Clinical Diagnostics, Raritan, NJ, USA); anti-H (Lorne Laboratories, Reading, UK)

Fig. 1. (A) Inheritance of the *BEL.02* allele in the proband's family. The arrow indicates the proband. (B) Serologic testing and ABO genotyping results of the family members.

Table 1. Summary of studies of allelic competition and enhancement

ABO subgroups	Antigen expression	Allele name (according to the ISBT)	Reference
A subgroups	Allelic competition	*ABO*AW.14*	[2]
	Allelic enhancement	*ABO*AW31.02-05*	[4]
		*ABO*AW.35*	[5]
		*ABO*A3.07*	[5]
		*ABO*AW.05*	[5]
		*ABO*AW.10*	[6]
B subgroups	Allelic competition	*ABO*B3.06*	[1]
		*ABO*BW.11*	[3]
	Allelic enhancement	NA	[7]
		*ABO*BEL.02*	This study
Cis-AB	Allelic competition	*ABO*cis-AB.01*	[10]

Abbreviations: ISBT, International Society of Blood Transfusion; NA, not available.

The *BEL.02* allele is characterized by a single nucleotide variant, c.669G>T, compared with the *B.01* allele and was first identified in 1996 by Ogasawara, *et al.* [8], who reported that the *BEL.02* allele exhibited the B_{el} phenotype with minimal amounts of B antigen. We confirmed that the *BEL.02* allele is associated with the B_{el} phenotype when the *O* allele is co-inherited, whereas it showed stronger expression of B antigen with *A* allele.

The c.669G>T variant of the *BEL.02* allele leads to a change in the encoded amino acid (p.Glu223Asp) [8]. However, no studies have evaluated the effect of this variant on GTB's structure and function. We performed *in silico* analysis using PolyPhen-2 and SIFT; the results predicted c.669G>T to cause damage to GTB ("Probably damaging" in PolyPhen-2 and "Damaging" in SIFT). The exact mechanism underlying allelic enhancement remains unknown; however, GTs mixed *in vitro* show enhanced enzymatic activity through heterocomplex formation [9]. We postulate that the amino acid change destabilizes the structure of GTB, and the mutated GTB forms heterocomplexes with GTA to enhance its enzymatic activity. However, we could not perform protein structure modeling to prove our hypothesis. Further studies are warranted to unravel the exact mechanism of allelic enhancement.

The nomenclature of the *ABO* alleles is based on the phenotypes. However, as described above, identical alleles can express various phenotypes according to their counterpart alleles. Several reports [10, 11] claimed that various expressions of *cis*-AB can lead to misclassification in the ABO grouping. Indeed, we were confused by the unexpected phenotype of the proband carrying the *BEL.02* allele because of the stronger expression of the B antigen than expected for the B_{el} phenotype. Clinicians should be aware of the possibility of different phenotypes of the same variant allele, and to avoid misclassification, the phenotype should not be inferred from the gene name.

We report the first case of allelic enhancement in subgroup B in a family carrying the *BEL.02* allele. This case highlights that allelic enhancement can be found in both the A and B subgroups and may cause confusion in ABO genotyping because the phenotype is discordant with the genotype.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Cho D and Chang SH conceived and designed the study. Bae GE, Yu HB, and Seo JY analyzed the data. Bae GE wrote the manuscript. Kim TY and Suh JS reviewed the manuscript. All authors have accepted their responsibilities for the entire con-

tent of this manuscript and approved submission.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

RESEARCH FUNDING

None declared.

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