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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 A1Bweak 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 Bel phenotype. In contrast, the proband (II-2) and maternal cousin showed an A1Bweak phenotype with weak agglutination (+) with anti-B, suggesting allelic enhancement, where the ABO allele in trans can enhance the expression of its counterpart allele.

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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*AW3.07                           | (5)       |
|               |                    | ABO*AW.V0.05                         | (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.

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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.

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