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REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

A new missense variant in exon 7 of the *ABO* gene, c.662G>A, in a family with B_w phenotype

Annika K. Hult[1,2] | **Åsa Hellberg**[1,2] | **Jill R. Storry**[1,2] | **Martin Písacka**[3] | **Martin L. Olsson**[1,2]

[1]Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden

[2]Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Lund, Sweden

[3]Institute of Haematology & Blood Transfusion, Transfusion Departement, Prague 2, Czech Republic

Correspondence
Annika K. Hult, Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Klinikgatan 23, SE-221 85 Lund, Sweden.
Email: annika.hult@med.lu.se

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1 | BACKGROUND

Weak expression of ABO antigens is encountered in the clinical laboratory occasionally, and subgroups of A are more commonly observed in Europeans than subgroups of B. To date, weakly expressing B variant phenotypes have been associated with 38 different alleles according to ISBT (https://www.isbtweb.org/resource/001aboalleles.html). This number is an underrepresentation since there have been several reports of aberrant B expression due to variant alleles since the last update of the ISBT allele table. The current study was initiated by an unusual blood group typing result in a 55-year-old male patient of Czech origin and previously reported as an abstract.[1]

2 | BRIEF METHODS

Blood grouping was performed according to standard blood banking practice, initially using an automatic analyzer (Galileo, Immucor) followed by confirmation with manual gel (BioRad; DG-Gel) and tube agglutination techniques. Initial genotyping analysis was done using a PCR-SSP kit (Innotrain), microarray (BloodChip Reference, Progenika) and subsequently verified by expanded PCR-ASP and PCR-RFLP as described previously.[2,3] *ABO* exons 1–7 and splice sites were amplified and analyzed, together with the product(s) of PCR-ASP for exons 6–7, by Sanger sequencing.[4] A single nucleotide variation (SNV) was detected, and the localization of the affected amino acid is visualized in a 3D-model of ABO glycosyltransferase by Cn3D (v.4.3.1, www.ncbi.nih.gov) and a detailed view obtained by AlphaFold.[5,6] Flow cytometry testing with monoclonal ABO reagents was performed as described previously.[7]

3 | RESULTS

The proband's red blood cells (RBCs) initially typed as group O but the plasma typing gave negative or weak reactions with test RBCs of group B, depending on the method used, Table 1. An *ABO*B.01/O.01.01* genotype was revealed, normally consistent with group B. Screening for selected A and B subgroup allele markers was negative.[2] After informed consent, samples from family members were drawn and further investigation was performed.

In samples from the proband, his sister and niece, sequence analysis revealed heterozygosity for a SNV in *ABO* exon 7, c.662G>A (no rs number available) in an otherwise normal *ABO*B.01* allele. Significantly weakened B antigen expression was observed in all three individuals. An overview of serological testing and genetic results is shown in Table 1.

SNV c.662G>A encodes an amino acid change, p.Gly221Asp. The glycine residue is completely evolutionarily conserved among the members of the GT6 family of glycosyltransferases[8] and centrally located in the enzyme, seven amino acids away from the DVD motif (pp. 211–213) that coordinates the Mn^{2+} ion and the UDP part of the UDP-galactose donor substrate (Figure 1A). However, it is not directly interfering with the catalytic site. Instead, the change of the small neutral glycine to the bulkier and charged aspartic acid is predicted to abolish selected hydrogen bonds and is therefore hypothesized to destabilize the protein conformation (Figure 1B).[5,6]

Other family members were also tested but did not display the phenotype nor the genotype (Figure 2A).

Flow cytometric analysis showed very weak B antigen expression on RBCs from the proband and his sister and notably stronger expression on RBCs from the niece (Figure 2B). While the same mutation was present in all three individuals, the niece had also inherited a normal *ABO*A1.01* allele *in trans*. The increased B antigen expression in this individual is consistent with the phenomenon of allelic enhancement.[7]

TABLE 1 Overview of serological testing and genetic results from affected family members

		Serology					Genetics					
		Forward typing		Reverse typing			Critical change (SNV)	Backbone	Allele *in trans*	Amino acid change	Evolutionary conservation[8]	GenBank acc. no
		Anti-A	Anti-B	A_1	B	O						
Proband	Gel cards	0	0	4+	0	nt	c.662G>A	*ABO*B.01*	*ABO*O.01.01*	p.Gly221Asp	Invariant	OL470661
	Galileo	0	0	4+	2+	0						
Sister	Gelcards	0	0	4+	0	nt	c.662G>A	*ABO*B.01*	*ABO*O.01.01*	p.Gly221Asp	Invariant	OL470661
	Galileo	0	0	4+	0	nt						
Niece	Gel cards	4+	1+	0	0	0	c.662G>A	*ABO*B.01*	*ABO*A.01.01*	p.Gly221Asp	Invariant	OL470661
	Galileo	4+	0	0	3+	0						

Abbreviation: nt = not tested.

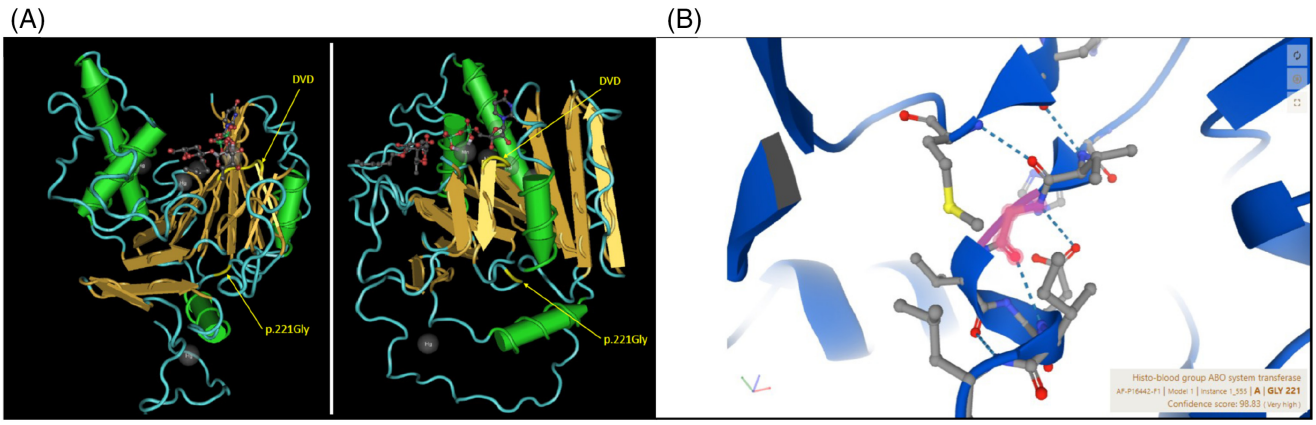

FIGURE 1 Three-dimensional molecular modeling of the ABO glycosyltransferase was performed with two different software packages. (A) A two-angle display of the affected amino acid p.221Gly (yellow and indicated by arrow) shown in relation to the DVD motif (yellow and indicated by arrow) using the Cn3D program (on PDB file 1LZI which includes the UDP and H acceptor molecules). (B) Close-up, as visualized in the AlphaFold protein structure database (https://alphafold.ebi.ac.uk) developed by DeepMind and EMBL-EBI (on Uniprot ID P16442 and protein data Bank [PDB] file AF-P16442-F1), of amino acid p.221Gly and relevant hydrogen bonds (dashed lines) that are predicted to be absent when glycine (highlighted in pink/purple) is substituted by aspartic acid and is likely to result in destabilization of the protein structure.

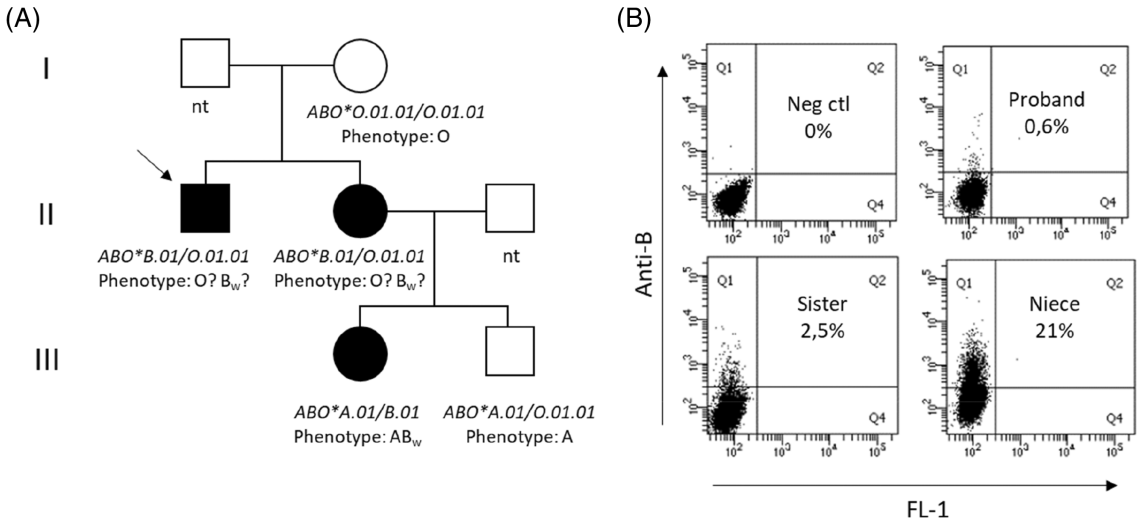

FIGURE 2 (A) Pedigree with phenotype and genotype information. Traditional pedigree symbols are used to depict the family members and their relationships. The proband is indicated by an arrow. (B) Dot plots of the flow cytometry testing of RBCs from the proband, sister and niece tested with anti-B (clone 9621A8), number of positive cells are given. A negative control (group O) is included.

Subsequent to the family study, an unrelated person, also of Czech origin, with the same SNV (genotype *ABO*B.01/O.01.01*) and identical phenotype to the proband, was identified.

4 | SUMMARY

This study revealed an inherited *ABO* variant affecting an invariant residue in the catalytic domain and results in weakened B expression. The activity of the altered B glycosyltransferase clearly demonstrates a paradoxical dependence on the allele *in trans*, that is, allelic enhancement when a full-length, competitive glycosyltransferase is present. In addition to our findings, this SNV has been abstract published[9] and shown to give rise to a similar phenotype in a Caucasian blood donor. A GenBank record (KU206323.1) reports the same SNV but without any information regarding the phenotype and ethnic background.

In clinical routine practice, this SNV gives rise to a phenotype that causes a discrepant typing result or the very weak B antigen expression may even go undetected by routine serological methods and cause erroneous ABO determination.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

ORCID

Annika K. Hult https://orcid.org/0000-0002-2503-7016
Åsa Hellberg https://orcid.org/0000-0002-7000-1899
Jill R. Storry https://orcid.org/0000-0003-2940-2604
Martin L. Olsson https://orcid.org/0000-0003-1647-9610

REFERENCES

1. Pisacka M, Kralova M, Kralova J. B_w phenotype caused by a new missense mutation in exon 7 – a family study in northern Moravia. Vox Sang. 2016;111(Supplement 1):385.
2. Hosseini-Maaf B, Hellberg A, Chester MA, Olsson ML. An extensive PCR-ASP strategy for clinical ABO blood group genotyping that avoids potential errors caused by null, subgroup and hybrid alleles. Transfusion. 2007;47(11):2110–25.
3. Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O 2 versus a/O 1 discriminating nucleotide substitution at the ABO locus. Vox Sang. 1995;69:242–7.
4. Olsson ML, Irshaid NM, Hosseini-Maaf B, Hellberg Å, Moulds MK, Sareneva H, et al. Genomic analysis of clinical samples with serologic ABO blood grouping discrepancies: identification of 15 novel a and B subgroup alleles. Blood. 2001;98(5):1585–93.
5. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596(7873):583–9.

6. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, et al. AlphaFold protein structure database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Res 2022:7; 50(D1):D439–D444.
7. Hult AK, Olsson ML. Many genetically defined ABO subgroups exhibit characteristic flow cytometric patterns. Transfusion. 2010;50(2):308–23.
8. Gastinel LN, Bignon C, Misra AK, Hindsgaul O, Shaper JH, Joziasse DH. N alpha1,3-galactosyltransferase catalytic domain structure and its relationship with ABO histo-blood group and glycosphingolipid glycosyltransferases. EMBO J. 2001;20(4): 638–49.
9. Vege S, Lomas-Francis C, Hue-Roye K, Hu Z, Westhoff CM. ABO donor discrepancy due to a new *B* allele, ABO*B(662A). Transfusion. 2015;55(Supplement 3):145A.

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