Original Article

Access this article online

Quick Response Code:

Website: www.ajts.org

DOI: 10.4103/ajts.ajts_235_23

A_{weak} phenotype associated with novel *ABO*A* allele variant c.106delinsGG

Sanmukh Ratilal Joshi, Glenda Millard[1], Mayuri Vekariya, Priya Radadiya, Manisha Rajapara, Hiren Dhanani[2], Gaurav Shastri[2], Prabhat Sharma[3], Brett Wilson[1], Yew-Wah Liew[1]

Abstract:

BACKGROUND AND OBJECTIVES: Discrepancy between forward and reverse ABO grouping could be due to several reasons including genetic mutations of the alleles encoding group specific transferase. The healthy donors found with weak A antigen were investigated to ascertain the allele responsible for variation.

MATERIALS AND METHODS: Standard serological methods were employed using commercial antisera. The molecular sequencing was performed on DNA with enrichment library prep kit and a custom designed overlapping probe panel. Binary alignment mapping files, generated on board the Illumina MiSeq instrument and aligned to the GRCh37/Hg19 reference genome, were uploaded to the QIAGEN CLC genomics workbench software (version. 20) where variant call files were generated and analyzed.

RESULTS: Red blood cells (RBCs) of six healthy donors, showing weak mix-field agglutination by anti-A and anti-A, B and plasma with absence or weakly reacting anti-A, were investigated serologically. The RBCs incubated with anti-A yield positive elution and their saliva lacked A but possessed H antigen thereby classifying as a historical known phenotype A_{end}. Family study on 4 probands showed inheritance of the trait. Molecular studies revealed presence of *ABO*A* allele carrying rare novel variant referred to as c.106delinsGG in line with HGVS recommendation that was thought to be responsible for the variant of A.

CONCLUSION: Six cases serologically defined as A_{weak} were found to be associated with novel *allele ABO*A* (c.106delinsGG). The A_{weak} phenotype with the novel allele has not been displayed on International Society of Blood Transfusion database, though c.106delinsGG is listed in the UCSC genome browser under rs782544248.

Keywords:

A_{weak} phenotype, c.106delTinsGG, Indian population, novel allele

Lok Samarpan Raktadan Kendra and Research Center, [2]Sterling Accuris Diagnostics, Surat, [3]Department of Blood Bank, Shalby Hospital, Ahmedabad, Gujarat, India, [1]Red Cell Reference Laboratory, Pathology and Clinical Governance, Australian Red Cross Lifeblood, Brisbane, Australia

Address for correspondence:
Dr. Sanmukh Ratilal Joshi, Lok Samarpan Raktadan Kendra, Miranagar, Minibazar, Varachha Road, Surat - 395 006, Gujarat, India.
E-mail: sanmukhj@yahoo.com

Submitted: 08-12-2023
Revised: 16-12-2023
Accepted: 17-12-2023
Published: 21-06-2024

Introduction

Genetic variants of ABO blood group antigens are rare with heterogeneous serological properties, some of which are detected by discrepant results between the forward grouping, performed on red blood cells (RBCs), and the reverse grouping, carried out on serum or plasma of a person. The absence or weakly presence of expected antibody in reverse grouping can indicate the presence of the corresponding antigen on RBCs. Such weakly expressed phenotypes are further classified by determining the secretor status of the person based on the presence of secretory antigens in saliva in accordance with the ABO blood group.[1] Most of these variants result from mutations of the alleles that encode the group specific glycosyltransferase responsible for biosynthesis of the antigen at phenotypic level. Thus, the weak variants of A and B antigens are classified on the basis of serological reaction patterns (obtained

How to cite this article: Joshi SR, Millard G, Vekariya M, Radadiya P, Rajapara M, Dhanani H, *et al*. A_{weak} phenotype associated with novel *ABO*A* allele variant c.106delinsGG. Asian J Transfus Sci 2024;18:1-6.

through simple blood grouping) and testing for salivary secretion of the antigens. Adsorption-elution study with positive elution results may further help in confirming the presence weak of antigen on RBC surface. Molecular studies can reveal the genetic factors responsible for weaker expression of the antigen on RBC surface if it is due to a variation of the allele causing altered group specific glycosyltransferase. Different weaker variants of the ABO blood groups are listed in the database of the website of the International Society of Blood Transfusion (ISBT).[2] The present study was aimed to investigate the blood samples of healthy blood donors showing discrepant results on the forward and reverse grouping as well as the secretor status to classify them serologically as shown earlier.[3] Further testing was performed to understand the genetic variation responsible for the altered expression of the A antigen on the RBCs.

Materials and Methods

The monoclonal antisera used were from commercial sources (Tulip Diagnostics, India or Seqirus, Australia; and the reagent red cells group A_1, and B used in reverse grouping were *in house* preparation or commercial (Seqirus, Australia). ABO blood grouping was carried out by the forward the reverse grouping by tube method and results were read macroscopically. Any weak or negative reactions were confirmed microscopically. Elution of antibodies from the sensitized RBCs was carried out by heat elution method and were tested with appropriate group of red cells in parallel to the last wash. Secretor status was carried out by hemagglutination inhibition technique using saliva from the subjects and appropriate reagent antisera.

Molecular testing

DNA was extracted from the ethylenediaminetetraacetic acid blood specimen using the QIAGEN EZ1 DSP DNA Blood kit and the QIAGEN EZ1 advanced XL instrument (QIAGEN Sciences 19300 Germantown Rd Germantown MD 20874 USA). For molecular typing (or Next Generation Sequencing) DNA Library preparation from the extracted DNA was then carried out using the Illumina DNA prep with enrichment kit and a custom Targeted Sequencing Panel (Illumina Inc. Worldwide Headquarters 5200 Illumina Way, San Diego, CA 92122 USA). This *in house* designed panel enables comprehensive genotyping for all 43 blood group systems and transcription factors KLF1 and GATA1. The prepared library was then loaded onto the Illumina Miseq platform which performs DNA paired end sequence generation. Binary alignment mapping files generated on board the Illumina MiSeq instrument from the FASTQ files and aligned to the GRCh37/Hg19 reference genome were uploaded to the QIAGEN CLC genomics workbench software (version.20, QIAGEN, Aarhus, Denmark) where variant call files (VCFs) were generated and analyzed. Incidence of the A_{weak} phenotype among the studied was derived by counting the number of donors with A and AB group and by eliminating the repeat donors over a 7 year-period between 2016 and 2022 within which these rare donors under study were encountered. One of the probands and his brothers donated at same time so was counted as one to calculate the incidence.

Results

All 6 cases showed discrepant results for ABO blood group with respect to A antigen on their RBCs and corresponding antibody in their plasma [Table 1 and Figures 1 and 2]. Their RBCs showed weak, mix-field agglutination pattern with anti-A reagent with absence of anti-A. Probands 1, 4 and 5 showed a presence of weakly reacting anti-A_1 in serum/plasma that preferentially reacted at lower temperature. Adsorption-elution experiment yielded active eluate containing anti-A from five of these probands' RBCs sensitized by anti-A and anti-A, B. The secretor status on 5 of the donors showed an absence of A but a presence of H in saliva, thus serologically defining the variant A_{weak} akin to historical description of A_{end} phenotype. The family members of the 4 probands, viz. 1, 3, 5 and 6, were tested for ABO blood groups and the A_{weak} phenotype was found as an inherited character [Figures 3]. Four of the 5 donors with this rare phenotype were found among a total of 43,931 blood donors bearing A antigens, i.e., groups A and AB, at one blood center in Western India during a 7 year-period between 2016 and 2022 giving an incidence of this rare phenotype as 1 in 10,983 donors possessing A antigen.

Molecular typing was carried out on the A allele and analysis of the VCF output showed a

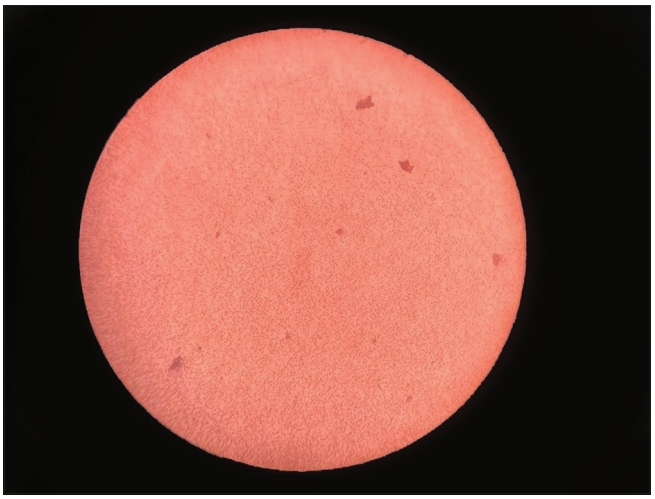

Figure 1: The microscopic view on agglutination pattern obtained on the red blood cells from one of the donors tested with anti-A

Table 1: The ABO grouping and the secretor status on the probands showing A weak phenotype

Probands	Forward grouping				Reverse grouping		Blood group	Antigens in saliva			Secretor status
	Anti-A	Anti-B	Anti-A, B	Anti-H	A_1 cells	B cells		A	B	H	
1	w+, mf	4+	4+	4–3+	w+	0	A_{weak} B	No	Yes	Yes	Nonsecretor for A, secretor for H, B
2	w+, mf	0	w+, mf	4+	0	4+	A_{weak}	No	N/A	Yes	Nonsecretor for A, secretor for H
3	w+, mf	0	w+, mf	4+	0	4+	A_{weak}	No	N/A	Yes	Nonsecretor for A, secretor for H
4	w+, mf	0	w+, mf	NT	w+	4+	A_{weak}	NT	NT	NT	Nonsecretor for A, secretor for H
5	w+, mf	0	w+, mf	4+	1+	4+	A_{weak}	No	N/A	Yes	Nonsecretor for A, secretor for H
6	w+, mf	0	w+, mf	4+	0	4+	A_{weak}	No	N/A	Yes	Nonsecretor for A, secretor for H

mf=Mix-field agglutination pattern, N/A=Not applicable, NT=Not tested

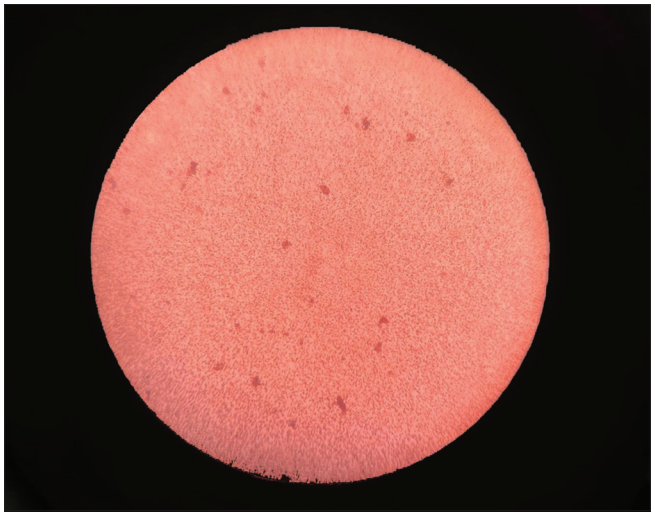

Figure 2: The microscopic view on agglutination pattern obtained on the red blood cells from one of the donors tested with anti-A, B

nucleotide insertion (c.106delins GG) leading to an amino acid frame shift (p.[Phe36fs]) in the AB glycosyltransferase [Tables 2a and b]. This change, not listed in the ISBT database, was present at a heterozygous level in all the samples where serology showed reduced A antigen expression.

Discussion

Weaker form of the A and B antigens of the ABO blood groups are noticed during routine blood grouping as discrepant results on the forward and the reverse grouping. There is a heterogeneity among such cases with respect to a presence or an absence of the antigens on RBCs and that in salivary secretion. The serological patterns shown by weaker forms of A antigen are classified as A_3, A_x, A_m, A_{end} etc.[1] Of these, the variant A_{end} displays an unique serological feature showing a weak, mix-field agglutination pattern, so was initially thought as weak A_3 (A_3^w), with a presence of H but not A antigen in saliva.[3,4] Some of the cases may also show a presence of anti-A_1 in their plasma. Such phenotype with minor variation reported from Finland was termed as A_{finn}[5] and had shown the mix-field agglutination pattern even by flow cytometry.[6] Similar variant, found among the African tribe called Bantu, was named as A_{bantu}.[7,8] The present cases, showing weaker expression of A antigen on RBC with the mixed-field agglutination pattern and a presence of H but not A in saliva may well be considered as A_{end}, though all such cases be referred to as A_{weak} and may be categorized under AW series. In present study, the A_{weak} phenotype showed Mendelian inheritance through 3 generations in one family and 2 generations in other three families.

Incidence of phenotype A_{finn} among the donors with A antigen was found in Finland as 1:6000 in one study and 1:1000 in the other,[5,9] while that among the French donors with group A was reported to be 1: 75,000.[10] Likewise, the frequency of A_{bantu} was found as 4%–8% of group A individuals among the African tribe.[7,8] In present study, the incidence of A_{weak} phenotype with novel allele *c.106delTinsGG* was found as 1:11,000 among the Indians.

Molecular techniques help to understand the alleles encoding the group specific glycosyltransferase enzymes responsible for synthesis of the A and B antigens. The ABO genes with 7 exons encode 2 glycosyltransferases viz. (1) the A group specific glycosyltransferase, that adds a donor substrate UDP-N-acetylgalactosamine, to an acceptor substrate L-fucose (i.e., the H antigenic substance) to form A antigen and (2) the B group specific glycosyltransferase that adds a donor substrate UDP-galactose to an acceptor H substance. Allelic mutation brings change in activity of the encoded group specific glycosyltransferase enzyme that may reflect as to weak expression of A or B antigen at phenotypic level. In other words, the genetic mutation decreases the activity of the group specific glycosyltransferase enzyme so is reflected as decrease in conversion of H to A or B, resulting into a weak A or weak B subgroups. Weak variants of A, though rare in occurrence, are numerous and classified serologically[1] as well as molecularly, the current status of which are listed on the ISBT website.[2] However, the A_{end} variant with heterogeneity in its form and presumably, its molecular information for all its variety remained unclear thus far has not been displayed on the website, though the variants such as A_{finn} and A_{bantu} have found under the weak variants of A (AW) probably after their molecular genetics information

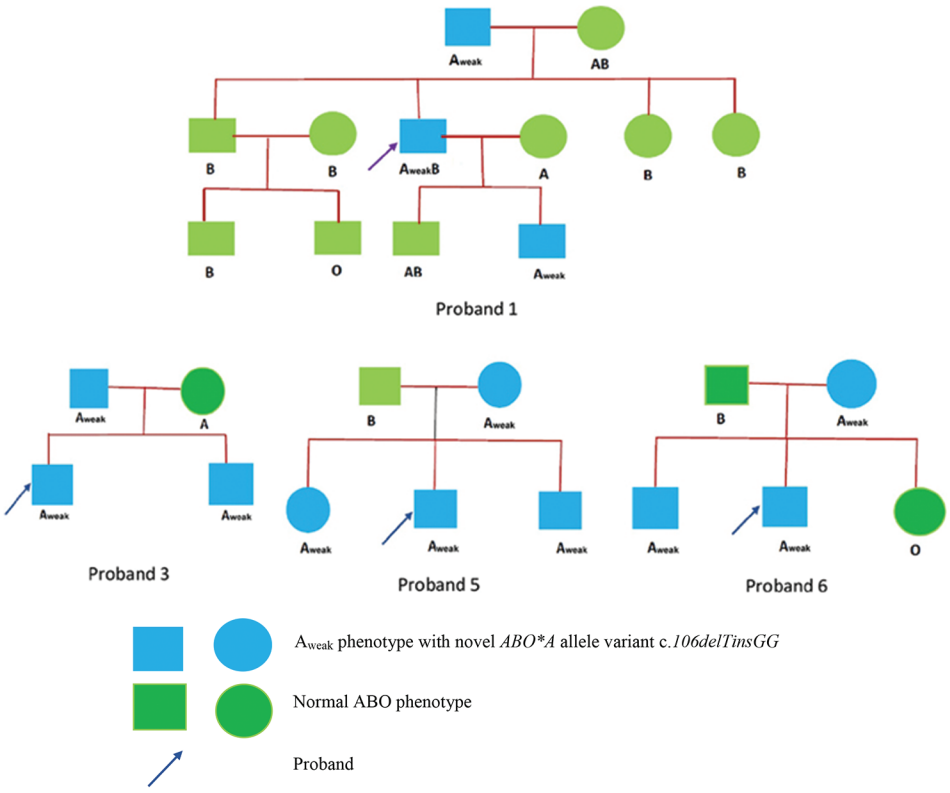

Figure 3: The Pedigrees showing inheritance pattern of the A_{weak} phenotype with novel *ABO*A* allele variant c.106delTinsGG among the four of the probands

Table 2a: Genotyping results for the ABO alleles when compared to the GRCh37 reference sequence

Prsobans	Allele	c.106T>G	c.106delTinsGG	c.188A>G	c.189T>C	c.220T>C	c.260_261insG	c.297A>G	c.483C>T
1	1	G	No insert	G	C	C	No insert	G	
	2	G	Insert G^	G	C	C	Insert G	A	
2	1	G	No insert	G	C	C	No insert	A	
	2	G	Insert G^	G	C	C	Insert G	A	
3	1	G	No insert	G	C	C	No insert	A	
	2	G	Insert G^	G	C	C	Insert G	A	
4	1	G	No insert	G	C	C	No insert	A	
	2	G	Insert G^	G	C	C	Insert G	A	
5	1	G	No insert	G	C	C	No insert	A	T[!]
	2	G	Insert G^	G	C	C	Insert G	A	C
6	1	G	No insert	G	C	C	No insert	A	
	2	G	Insert G^	G	C	C	Insert G	A	

Prsobans	c.526C>G	c.657C>T	c.703G>A	c.796C>A	c.803G>C	c.930G>A	Allele	Predicted phenotype
1	G	T	A	A	C	A	*ABO*B.01*	A_{weak} B
	C	C	G	C	G	G	*ABO*A* novel allele	
2	C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	C	C	G	C	G	G	*ABO*A* novel allele	
3	C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	C	C	G	C	G	G	*ABO*A* novel allele	
4	C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	C	C	G	C	G	G	*ABO*A* novel allele	
5	C	C	G	C	G	G	*ABO*O* novel allele	A_{weak}
	C	C	G	C	G	G	*ABO*A* novel allele	
6	C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	C	C	G	C	G	G	*ABO*A* novel allele	

^Change responsible for A_{weak}, [!]Additional rare variant found only in this proband and presumed to be on the *ABO*O* allele

was available. A_{finn} individuals have an A^1allele with a > g in the 5′ donor splice site of intron 6.[6,11] Although skipping of exon 6 would introduce a reading frameshift and no active enzyme product, the mutation is not in the

Table 2b: Genotyping results for the ABO alleles when compared to the International Society of Blood Transfusion reference allele *ABO*A1.01*

Probands	Alleles	c.106delinsGG	c.261delG	c.297A>G	c.483C>T	c.526C>G	c.657C>T	c.703G>A	c.796C>A	c.803G>C	c.930G>A	Alleles	Predicted phenotype
1	1	No insert	c.261G	G		G	T	A	A	C	A	*ABO*B.01*	A_{weak} B
	2	Insert G^	c.261G	A		C	C	G	C	G	G	*ABO*A* novel allele	
2	1	No insert	c.261delG	A		C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	2	Insert G^	c.261G	A		C	C	G	C	G	G	*ABO*A* novel allele	
3	1	No insert	c.261delG	A		C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	2	Insert G^	c.261G	A		C	C	G	C	G	G	*ABO*A* novel allele	
4	1	No insert	c.261delG	A		C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}
	2	Insert G^	c.261G	A		C	C	G	C	G	G	*ABO*A* novel allele	
5	1	No insert	c.261delG	A	T[1]	C	C	G	C	G	G	*ABO*O* novel allele	A_{weak}
	2	Insert G^	c.261G	A	C	C	C	G	C	G	G	*ABO*A* novel allele	
6	1	No insert	c.261delG	A		C	C	G	C	G	G	*ABO*O.01.01*	A_{weak}

^Change responsible for Aweak, [1]Additional rare variant found only in this proband and presumed to be on the *ABO*O* allele

invariable splice site sequence, so a minor fraction of the RNA could be spliced normally. A_{bantu}, on the other hand, results from a hybrid of the common A^2 allele and an O^1-like allele (O^1bantu), with a cross-over region near exon 5 (*Abantu01*).[12] This includes a deletion of a nucleotide in the 5′ intron 4 splice site, leading to the loss of 16 amino acids from the stem region of GTA, but does not include 261delG as exon 6 is derived from A^2. The present cases, with typical serological features similar to A_{end} phenotype, with novel allele *c.106delinsGG* to be reported among the Indian population.

The Hg19 and ISBT *ABO* reference allele differ. For ISBT the reference allele is *ABO*A1.01*,[2] however for GRCh37 (Hg19) a combination of two alleles form the ABO reference: *ABO*O.01.01* (exons 6 and 7) and *ABO*O.01.02* (exons 1–5).[13] This difference has been taken into account with the nucleotide changes reported here based on the GRCh37 (Hg19) genome assembly. In all the six cases a nucleotide insertion (c.106delinsGG, p.[Phe36fs]) was found. This was confirmed as being associated with the A allele following family studies which determined it was travelling with the A and not with the B or O alleles. This therefore defines a novel allele which is comprised of the nucleotide and amino acid changes: c.106delinsGG, p.(Phe36fs), c.188_189delATinsGC, p.(His63Arg), c.220T>C, p.(Ser74Pro) and c.260_261insGp.(Pro88fs) when aligned against the GRCh37 (Hg19) assembly. When aligned against the ISBT reference allele the only change reported is c.106insG p.(Phe36fs). In addition to the probands all samples for which A_{weak} (A_{end}) was predicted by serology were found to have the c.106delinsGG within the A allele [Tables 2a and b]. The variation (c.106delinsGG) is listed in the UCSC genome browser (dbSNP release 153) under rs782544248 with a global frequency in the ExAC study of 0.00003.

Conclusion

The six healthy donors' blood showing A_{weak} akin to historically defined as A_{end} phenotype by serological tests were confirmed by molecular typing as having a *novel ABO*A* variant allele. The new allele identified as *c.106delins GG* is thought to be responsible for reduced/altered expression of the A antigen on RBCs. We propose that this novel *allele* be recognized as being responsible for the A_{weak} phenotype. No doubt, it is possible that other mutations as yet to be described in future course of time may also be responsible for the A_{weak} phenotype and the list would be expanded.

Acknowledgment

SJ and GM both have contributed equally and sequentially place as per alphabetical order of their surname and both be considered as the first author. Dr. D. R. Arya of Govt.

Medical College, Bikaner Vijay Kumar of Immucor, India for collecting and sending the blood samples of the family members of one proband living in distant places.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Harmening DM. Modern Blood Banking and Transfusion Practices. 6th ed. Philadelphia, USA: F.A. Davis Company; 2012. p. 131.
2. Red Cell Immunogenetics and Blood Group Terminology ISBT Working Party. Names for ABO (ISBT 001) Blood Group Alleles v1.1 171023. Available from: https://www.isbtweb.org/resource/001aboalleles.html. [Last accessed on 2023 May 05].
3. Prokop O, Simon A, Rackwitz A. A rare weak A3 receptor "A3w", its analysis and heredity. Dtsch Z Gesgerichtl Med 1960;50:448.
4. Sturgeon P, Moore BP, Weiner W. Notation for two weak A varians: A end and Ael. Vox Sang 1964;9:214.
5. Mohn JF, Cunningham RK, Pirkola A, Furuhjelm U, Nevanlinna HR. An inherited blood group A variant in the Finnish population. I. Basic characteristics. Vox Sang 1973;25:193-211.
6. Hult AK, Olsson ML. Many genetically defined ABO subgroups exhibit characteristic flow cytometric patterns. Transfusion 2010;50:308-23.
7. Brain P. Subgroups of A in the South African Bantu. Vox Sang 1966;11:686-98.
8. Jenkins T. Blood group Abantu population and family studies. Vox Sang 1974;26:537-50.
9. Nevanlinna HR, Pirkola A. An inherited blood group A variant in the Finnish population. II. Population studies. Vox Sang 1973;24:404-16.
10. Garretta M, Muller A, Gener J, Matte C, Moullec J. Reliability in automatic determination of the ABO group by the groupamatic system. Vox Sang 1974;27:141-55.
11. Olsson ML, Irshaid NM, Kuosmanen M, Pirkola A, Chester M. A splice-site mutation defines the Afinn allele at the blood group ABO locus. Transfusion 2000;40:13S. In: Daniels G. Human Blood Groups. 3rd ed. Oxford, UK: Wiley-Blackwell; 2023. p. 36.
12. Hosseini-Maaf B, Smart E, Chester MA, Olsson ML. The Abantu phenotype in the ABO blood group system is due to a splice-site mutation in a hybrid between a new O1-like allelic lineage and the A2 allele. Vox Sang 2005;88:256-64.
13. den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J, *et al*. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat 2016;37:564-9.