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ABO gene in a family with inherited mutation caused rare blood subgroup A

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Abstract
Background and Objectives: *Discrepancies in blood typing are one of the major reasons in causing transfusion reaction. These discrepancies can be avoided through detailed analysis for the blood typing. Although monoclonal antibodies recognizing and agglutinating most weak subgroups exist, the risk for mistyping (eg, Ax donors as blood group O) remains when using commercially available routine reagents. ABO genotyping is a valuable complement to serology for correct determination of donor and patient ABO blood group status*
Materials and methods *In this study, we describe a family with six members carrying a A weak allele through three generations. Blood samples were analyzed by using commercial antisera for blood grouping. The results of forward (known antisera) and reverse (known antigen) reaction were not complimentary. DNA extractions from all six family members were collected after serological ABO blood group typing. On these samples, allele-specific polymerase chain reaction (PCR), direct sequencing of exon 7 and allele separation were performed for ABO gene analysis.*
Results *A single variation segregating with the weak A phenotype has been found. The causative allele is supposed to be Aw40 (c.722C>G, p.Arg241Pro). This novel A allele showed a 722C>G base change in exon 7.*
Conclusions: *Through the molecular analysis in this study, serologically unidentified A subgroups were obviously identified and a new allele for our population was reported.*
Keywords: *blood group, genotype, phenotype, ABO gene*

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I. Introduction

The discovery of the ABO blood group, over 100 years ago, caused great excitement. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. The ABO blood group antigens remain of prime importance in transfusion medicine - they are the most immunogenic of all the blood group antigens. Although monoclonal antibodies recognizing and agglutinating most weak subgroups exist, the risk for mistyping (eg, Ax donors as blood group O) remains when using commercially available routine reagents [(1)]. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused.

The ABO gene indirectly encodes the ABO blood group antigens. The ABO locus has three main allelic forms: A, B, and O. The A and B alleles each encode a glycosyltransferase that catalyzes the final step in the synthesis of the A and B antigen, respectively. The A/B polymorphism arises from several SNPs in the ABO gene, which result in A and B transferases that differ by four amino acids. The O allele encodes an inactive glycosyltransferase that leaves the ABO antigen precursor (the H antigen) unmodified.

There are many other subgroups of blood group A in which RBCs tend to weakly express the A antigen, whereas weak variants of the blood group B phenotype are rare[(2)].

The ABO locus is located on chromosome 9 at 9q34.1-q34.2. It contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most O alleles and results in a loss of enzymatic activity [(3)].

In this study, we describe a family with six members, carried a discrepant A allele and report the identification of the A weak allele in the molecular level.

II. Materials And Methods

Samples

The Preposita, is a sixty year women, known her blood group as O (I), for the first time in her life in this age found that she has A weak blood group. Blood samples of this women and all other family members

(her son and grandson also shown no compatibility blood group with each other) were collected and included for blood group re- typing at the immunohematology laboratory of the National Blood Transfusion Center (NBTC, Tirana, Albania).

Serologic ABO typing

All samples were tested by using DiaClon monoclonal antibodies that detect the presence of the A1 blood group (DiaMed GmbH, Cressier, Switzerland). Blood samples were analyzed by using commercial antisera (Anti-A clones A-11H5; Anti-B clones B-6F9; Anti-AB clones A-5E 10-B-2D7) (CE-Immundiagnostika GmbH, Eschelbronn, Germany) for blood grouping of forward (known antisera) and reverse (known antigen) reaction. Samples showing O blood group in forward reaction but A blood group in the reverse reaction were considered to be A weak.

***ABO* molecular genotyping**

Genomic DNA from all six related individuals (samples *S 01 – S 06* in the figure 1) including samples showing A weak reactivity and/or discrepant results was extracted from a EDTA blood sample by the Genomic DNA Mini kit (Invitrogen) according to the manufacturer's instructions. ABO gene variant screening was performed at the Laboratoire de Génétique Moléculaire des Groupes Sanguins (Etablissement Français du Sang – Bretagne, Brest, France) with previously published methods by Fichou[(4)]. Briefly, the seven exons of the gene, as well as their respective flanking intronic regions, were individually PCR amplified (Exon 1, TaqPCRx DNA polymerase kit, Life Technologies, Saint Aubin, France; Exons 2 to 7, HotStarTaq Master Mix kit, Qiagen, Courtaboeuf, France) with 0.5 mmol/L forward/reverse primers and 10 to 50 ng genomic DNA as a template. After PCR clean-up with an enzymatic treatment (ExoSAP-IT, USB, purchased from Ozyme, St Quentin en Yvelines, France), sequencing reactions were further carried out with a cycle sequencing kit (BigDye Terminator v1.1, Life Technologies)[(4)].

III. Results

ABO phenotypes and the pedigree

The Preposita confirmed an A weak allele, her son reported an AB blood group and her newborn grandson has O blood group, which after one year we identified the A weak allele. The respective blood groups of the other family members are indicated in the pedigree (see Figure 1).

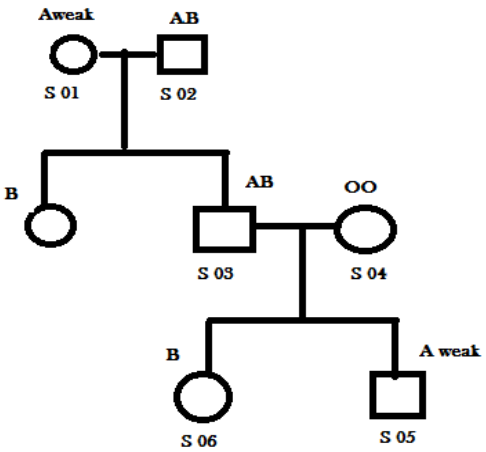

Figure 1 - Pedigree and the ABO blood group for the related individuals. Samples ID are shown below (S 01 – S 06), the preposita is (S 01) A weak women.

ABO genotyping

We identified one single variation related to the A weak alleles variant alleles at the heterozygous state by direct sequencing (Table 1). Examined regions were exons 1 to 7 plus flanking regions in gDNA. Sequencing were required to define the background allele of c.722G>C, (in Exon 7) from S 01, S 03 and S 05, respectively (Table 1). The causative allele is supposed to be *Aw40* (c.722G>C; p.Arg241Pro). The c.722G > C variant, which is carried by an A101 allele (Alias ABO 722C; ABO 10-04) is predicted to generate an endogenous protein product with a weak anti-A reactivity. The individual S 03 was not found to be A weak B with serotyping, but sequencing reaction of exon 7 showed that this individual has inherited the same allele from his mother, Aw40 (c.722G>C; p.Arg241Pro), suggested that his correct blood group is Aw40B (Figure 2).

Table 1. Phenotype and genotype characteristics of the related individuals					
ABO phenotyping				ABO genotyping	
Sample ID	Forward typing	Reverse typing	Blood type	Exon	Nucleotide/amino acid changes
S 01	No anti-A reactivity	Weak reaction with B cells	A weak	7	C.722G > C / p. Arg.241Pro
S 02	anti-B reactivity	Reaction with A cells	B (III)	-	-
S 03	anti-A/anti-B reactivity	No reaction	AB (IV)	7	C.722G > C / p. Arg.241Pro
S 04	No anti-A/anti-B reactivity	Reaction with A and B cells	O (I)	-	-
S 05	No anti-A reactivity	Weak reaction with B cells	A weak	7	C.722G > C / p. Arg.241Pro
S 06	anti-B reactivity	Reaction with A cells	B (III)	-	-

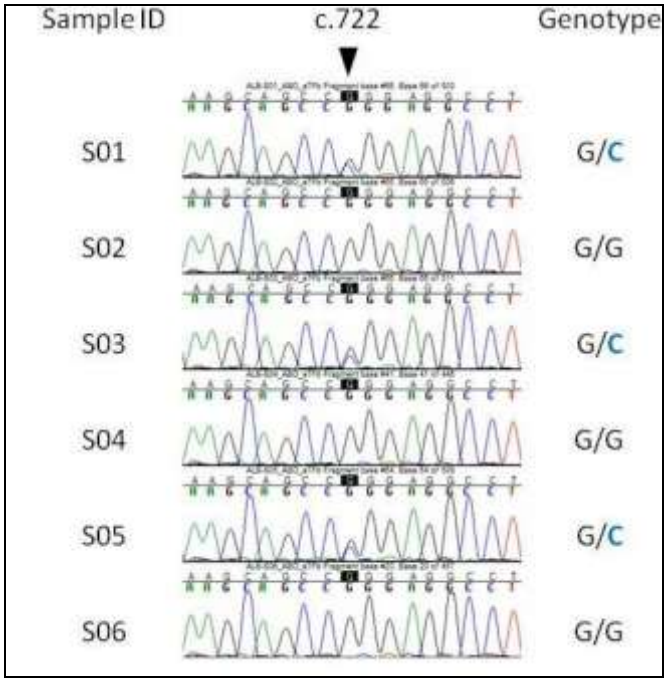

Figure 2: Direct sequencing of ABO exon 7 (electropherograms) for (samples ID in the left line S01-S06). Positions of interest are indicated with black points. Gene-specific nucleotides are mentioned below the electropherograms and in the right line are the replacement founded.

IV. Discussion

Appropriate assignment of the ABO status, has a critical importance in transfusion and obstetrical medicine. We sought to investigate the ABO status, in a family with six members carrying an A weak allele through three generations. We found that an A weak allele blood group not interpreted with serologic methods, carried by three members of this family with molecular genotyping methods were the Aw40; Alias ABO 722C; with nucleotide changes c.722G>C, in exon 7, demonstrated a weak or no anti-A reactivity (Table 1). This is a rare allele, found among 8 unrelated Caucasian donors (GenBank Accession Nrs: KF421135) (Fichou Y et al 2016). This is locating in the vicinity of the catalytic active site and more specifically close to amino acids 235, which is known to be critical for the function of the protein and its specificity [(5)].

Transfusion is now safe, but vigilance is needed to ensure correct identification of blood donor and patient. Staff education should include awareness of ABO incompatibility as causes of life threatening reactions to blood [(6)].

Through the molecular analysis in this study, serologically unidentified A subgroups were obviously identified and a new allele for our population was reported.

References

[1]. Xhetani M, Seferi I, Férec C, Zoraqi G, Fichou Y. Distribution of Rhesus blood group antigens and weak D alleles in the population of Albania. Blood Transfusion. 2014;12(4):565-569.
[2]. Daniels G. Human Blood Groups, Second ed. 2002, Blackwell Science.
[3]. Yamamoto F , Clausen H , White T , Marken J , Hakomori S . Molecular genetic basis of the histo-blood group ABO system. Nature. 1990;345:229–33. [PubMed]

[4]. Fichou, Y., Hennion, M., Dupont, I., Jamet, D., Le Maréchal, C. and Férec, C. Identification of novel variant *A* alleles within the *ABO* gene. Transfusion, 2016; 56: 1244–1246.
[5]. Yamamoto F, Marken J, Tsuji T, et al. Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc alpha 1--2Gal alpha 1----3GalNAc transferase (histo-blood group A transferase) mRNA. J Biol Chem 1990;265:1146-51.
[6]. Williamson LM , Lowe S , Love EM , Cohen H , Soldan K , McClelland DB , Skacel P , Barbara JA . Serious hazards of transfusion (SHOT) initiative: analysis of the first two annual reports. BMJ. 1999;319:16–9.

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