

Brief Report

Molecular genetic analysis of weak B blood group allele in Jinan population reveals distribution of ABw phenotype

Wen Wu, Xiaojian Li, Xingying Guo, Peng Wu, Xiangyan Huang*

Department of Transfusion Medicine, the 960th Hospital of the PLA Joint Logistics Support Force, Jinan, Shandong 250031, China.

ABSTRACT

The aim of this study was to investigate the distribution of the ABw phenotype of ABO blood group in the Jinan population. 31 856 samples were tested during the period 2018 to 2019. Thirty-nine samples with discrepant results, as identified by micro-column gel method, were further investigated by serological (tube technique) and molecular (fluorescence PCR, DNA sequencing) methods. Eight samples showed ABw phenotype, which accounted for 0.025% of the population tested. From the sequencing analysis, six samples (6/8) were typed as *ABO*A1.02/ABO*BW.12* and two samples (2/8) as *ABO*A1.02/ABO*BW.03*. The study suggests that ABw12 account for 75% of ABw phenotype and indicate ABw12 is the main ABw phenotype in Jinan population.

Keywords: ABO subtype, Bw alleles, ABw phenotype

INTRODUCTION

The ABO blood group is considered to be the most important group for blood transfusions. The ABO gene that encodes the glycosyltransferases responsible for the conversion of H substance to blood group A and B antigens is located on chromosome 9^[1]. It consists of seven exons ranging in size from 28 to 688 bp. The last two exons (exon 6 and 7), comprising of 823 bp of the transcribed 1 062 bp mRNA, encode for the catalytic domain of ABO glycosyltransferases^[2]. ABO subtype is mainly caused by ABO gene mutation affecting enzyme activity. Blood group subtypes with weak expression of B antigen are usually misjudged as type A or O in clinic. Many ABO subgroups have been found to have a weak expression of A or B antigen on red blood cells

(RBCs), which makes the detection of variant alleles one of the most important criteria for a clinically useful genotyping strategy^[3]. Furthermore, the clinical significance of the ABO blood group system extends to a broader area beyond transfusion medicine and several reports have suggested an important involvement in the development of cardiovascular disease^[4–5], tumor^[6] and other diseases^[7].

ABw phenotype is associated with normal A and weak B antigen expression, but anti-B is also found in serum. This study carried out a molecular genetic analysis of eight samples from an unrelated Chinese population in Jinan city with the ABw phenotype, revealing their main genotypes to be *ABO*A1.02/ABO*BW.12*.

*Corresponding to: Xiangyan Huang, Ph.D, Department of Blood Transfusion, the 960th Hospital of the PLA Joint Logistics Support Force, Jinan, Shandong 250031, China. E-mail: xiangyan73@aliyun.com.

Conflict of interests: The authors declared no conflict of interests.

MATERIALS AND METHODS

Blood sample collection

A total of 31 856 samples were tested in the 960th Hospital of the PLA Joint Logistics Support Force during the period from 2018 to 2019. Seventy-two showed discrepant results by micro-column gel card (Bio-Rad Co., Ltd. California, USA). The phenotype changes in 33 patient samples may have been due to allogeneic hematopoietic cell transplantation. Another 39 samples were considered to have special molecular bases. Eight samples with normal A and weak B during routine blood typing from the 39 samples were collected. The study was approved by the 960th Hospital of the PLA Joint Logistics Support Force's Ethics Committee.

Blood group serological typing

The ABw phenotype cases were discovered as a result of discrepancies between forward and reverse typing in routine ABO grouping according to the AABB Technical manual^[8]. RBCs were tested with polyclonal anti-A, anti-A1, anti-B, anti-H, anti-AB reagents (Shanghai Hemo-pharmaceutical & Biological Co., Ltd.) by tube method. Forward and reverse ABO typing was conducted by agglutination testing at 4 °C and 37 °C.

Molecular analysis of ABO exons 6 and 7

Genomic DNA was extracted from EDTA anti-coagulant peripheral blood by commercial kit (TIANamp HemDNA Kit, Tiangen Biotech). SYBR green fluorescence PCR assay was performed by ABI 7500 using a fluo-ABO subgroup genotyping kit (Jiangsu LiBio Medicine Biotechnology Co., Ltd. China) to detect the genotypes. PCR product sequencing of ABO exons 6 and 7 was performed to further explore the genotypes (Seq ABO exon kit, Jiangsu ZoJiWat Bio-pharmaceutical Co., Ltd. China).

RESULTS

Serological phenotype analysis

Eight samples showed normal A and weak B serological results during routine blood type identification by micro-column gel card. All samples were further confirmed by tube technique based serological testing. Their RBCs showed strong hemagglutination with monoclonal anti-A reagent (3+ to 4+), but weak hemagglutination with monoclonal anti-B and anti-H (1+ to 2+). Their serum showed weak anti-B activity but no anti-A activity against

standard RBCs.

Genotyping and Sequencing

A commercial fluorescence PCR kit was used to evaluate the 8 samples' ABO genotype. The fluorescence PCR results confirmed all the samples were AB (**Fig. 1**).

The sequencing results showed different mutations in these samples (**Table 1**). On the basis of *ABO*B1.01* allele, c.721C>T mutation in exon 7 had been detected in two samples, which is the characteristic mutation of *ABO*BW.03*. The genotypes of these two samples were all *ABO*A1.02/ABO*BW.03* (**Fig. 2A**). The remaining six samples' genotypes were identified as *ABO*A1.02/ABO*BW.12*, which had the point mutation c.278C>T in exon 6 when compared with *ABO*B1.01* allele (**Fig. 2B**).

DISCUSSION

ABw phenotype shows normal agglutination with anti-A, enhanced agglutination with anti-AB, but weak agglutination with anti-B and anti-H^[9]. 33 types of Bw-allele are known to exist^[10]. In a previous study, the detection rate of weak ABO subgroups in the Shanghai population was approximately 0.0125%–0.025%^[11]. Meanwhile, a Bw allele frequency study in Germany showed Bw25 frequency to be much higher, with Bw14 frequency being the highest (0.060%–0.070%)^[12]. The Bw alleles in our study were Bw12 and Bw03, which differed from those found in the German population. The results derived in our study showed that the frequency of ABw phenotype in the Jinan area was about 0.025% and ABw12 accounted for 75%. Bw12 accounts for a relatively high proportion of Bw alleles, which is similar to that reported in the previous literature^[13]. It is considered that the results are affected by population distribution and sample size. In clinical work, ABw with relatively weak B antigen is easily misjudged as type A, which leads to incorrect blood group determination and clinical blood transfusion complications.

At present, the molecular mechanism of Bw alleles is known as point mutation. Compared with B101 allele, *ABO*BW.03* is characterized as a single nonsynonymous substitution at 721C>T (R241W) in exon 7 (**Fig. 2A**), and *ABO*BW.12* with a single nonsynonymous substitution at 278C>T (P93L) in exon 6 (**Fig. 2B**). The change of amino acid caused a conformational change of galactosyltransferase, which led to decrease of enzyme activity and weakening of

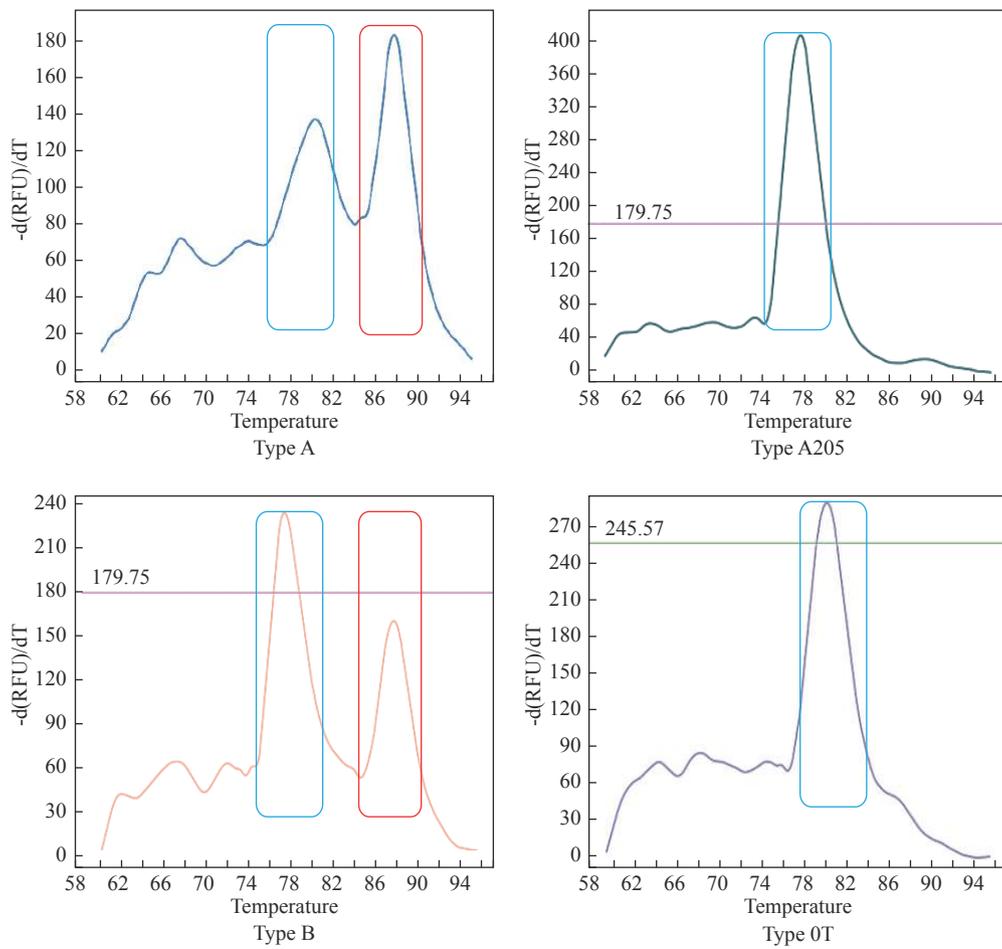


Fig. 1 Fluorescence PCR assay of the ABw samples. The blue box represents the internal reference and the red box shows the positive results. A1 and B genes were positive in all eight ABw samples, while A2 and O genes were not detected.

Table 1 The mutations of the eight samples

Phenotypes	Critical nt change (s)†	Corresponding aa change (s)	Individuals	Genotype
ABw	721C>T	R241W	2	<i>ABO*A1.02/ABO*BW.03</i>
ABw	278C>T	P93L	6	<i>ABO*A1.02/ABO*BW.12</i>

† Characteristic mutations of different Bw genotypes.

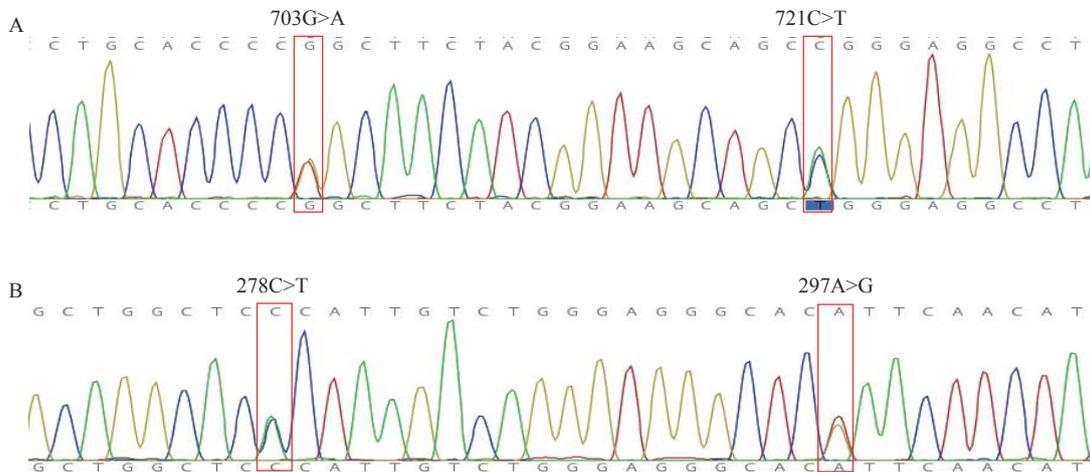


Fig. 2 The gene sequencing results in this study. A: Characteristic mutations in exon 7 of *ABO*A1.02/ABO*BW.03* genotype. B: Characteristic mutations in exon 6 of *ABO*A1.02/ABO*BW.12* genotype.

B antigen. Molecular genetic analysis helps us to understand the polymorphisms of blood group system in a much clearer and broader way. The present cases highlight the importance and necessity of molecular genetic studies in the research of molecular genetic heterogeneity in determining ABO subgroups.

Determining blood group accurately is critical for ABw individuals. Being a patient who requires red blood cell transfusion, it is important to avoid choosing blood products that contain B antigen, since weak anti-B activity in their serum can destroy those transfused red blood cells. On the other hand, when acting as a blood donor, ABw individuals' red blood cells should be classified as blood group AB, and their plasma should be classified as blood group A, since residual B antigens on the red blood cells and weak anti-B activity in the serum could lead to immunological reaction in the recipients.

In summary, ABw accounted for 0.025% of the population tested in Jinan and ABw12 accounted for 75% of ABw, indicating ABw12 is the main ABw phenotype in Jinan population. The identification of ABO gene variants associated with ABO subgroups is an important contribution in each individual case, which adds knowledge to the current understanding of genetic factors that influence blood groups and enriches the genetic data of blood groups in this region.

Acknowledgments

This work was supported by the grant from the 960th Hospital of the PLA Joint Logistics Support Force (No. 2018ZD08).

References

- [1] Yamamoto FI, Clausen H, White T, et al. Molecular genetic basis of the histo-blood group ABO system[J]. *Nature*, 1990, 345(6272): 229–233.
- [2] Yamamoto F, Marken J, Tsuji T, et al. Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc α 1→2Gal α 1→3GalNAc transferase (histo-blood group A transferase) mRNA[J]. *J Biol Chem*, 1990, 265(2): 1146–1151.
- [3] Huang Y, Lin JJ, Zhu SY. Genetic sequencing analysis of A307 subgroup of ABO blood group[J]. *Med Sci Monit*, 2015, 21: 2781–2785.
- [4] Franchini M, Lippi G. The intriguing relationship between the ABO blood group, cardiovascular disease, and cancer[J]. *BMC Med*, 2015, 13: 7.
- [5] Schussler O, Lila N, Perneger T, et al. Recipients with blood group A associated with longer survival rates in cardiac valvular bioprostheses[J]. *EBioMedicine*, 2019, 42: 54–63.
- [6] Stakišaitis D, Juknevičienė M, Ulys A, et al. ABO blood group polymorphism has an impact on prostate, kidney and bladder cancer in association with longevity[J]. *Oncol Lett*, 2018, 16(1): 1321–1331.
- [7] Yang SY, Zeng LY, Li C, et al. correlation between an ABO blood group and primary femoral head necrosis: a case-control study[J]. *Orthop Surg*, 2020, 12(2): 450–456.
- [8] Roback JD, Combs MR, Grossman BJ, et al. AABB technical manual[M]. 16th ed. Bethesda: AABB, 2008.
- [9] Ogasawara K, Yabe R, Uchikawa M, et al. Molecular genetic analysis of variant phenotypes of the ABO blood group system[J]. *Blood*, 1996, 88(7): 2732–2737.
- [10] International Society of Blood Transfusion (ISBT) [EB/OL].http://www.isbtweb.org/fileadmin/user_upload/Working_parties/WP_onRed_Cell_Immunogenetics_and/001_ABO_Alleles_v1.2.pdf.
- [11] Xiang D, Liu X, Guo ZH, et al. Studies of ABO subgroups in the Chinese Han population in Shanghai area[J]. *Chin J Blood Transfus (in Chinese)*, 2006, 19(1): 25–26.
- [12] Lang K, Wagner I, Schöne B, et al. ABO allele-level frequency estimation based on population-scale genotyping by next generation sequencing[J]. *BMC Genomics*, 2016, 17: 374.
- [13] Huang HB, Jin S, Liu X, et al. Molecular genetic analysis of weak ABO subgroups in the Chinese population reveals ten novel ABO subgroup alleles[J]. *Blood Transf*, 2019, 17(3): 217–222.

Received 13 December 2020, Revised 6 March 2021,
Accepted 31 May 2021