A Novel c.796 A>C Mutation in the ABO*B.01 Allele Responsible for CisAB Phenotype

Xiaofei Li^{a, b} Huiwen Bi^c Liping Sun^a Faming Zhu^{d, e} Deqing Wang^a

^aDepartment of Blood Transfusion, Chinese PLA General Hospital, Beijing, China; ^bDepartment of Blood Transfusion, Beijing Friendship Hospital, Capital Medical University, Beijing, China; ^cMaternity and Child Care Hospital of Jinan, Shandong, China; ^dBlood Center of Zhejiang Province, Hangzhou, China; ^eZhejiang Provincial Key Laboratory of Blood Safety Research, Hangzhou, China

Keywords

CisAB phenotype · Gene mutation · Sequencing · ABO blood group

Abstract

Background: Individuals with the CisAB phenotype are rare in the Chinese population. In the present study, we investigated the sequence of the ABO gene and family members of a newborn suspected to have the CisAB phenotype. **Methods:** The ABO phenotype was detected using conventional serological tests. The full coding region of exons 1 to 7 of the ABO gene was amplified by polymerase chain reaction and was sequenced. The ABO haplotype was determined by the allele-specific primer sequencing method. **Results:** The proband and his father and grandfather were assigned the CisAB phenotype according to the results of the serological tests and family investigation. A novel CisAB allele was identified in the proband and his father and grandfather, which has only one nucleotide difference at position 796 from A to C (c.796A>C) compared with the ABO*B.01 allele. **Conclusion:** A novel CisAB (c.796A>C mutation in ABO*B.01) allele is the first identified in the Chinese population.

© 2019 S. Karger AG, Basel

Introduction

The ABO blood group plays an important role in transfusion and transplantation medicine [1, 2]. The ABO gene encodes specific glycosyltransferases to induce the formation of A or B blood antigens. The mutations in the ABO gene may lead to diminished amounts of A or B antigens in red blood cells giving rise to ABO subgroups [3–5]. The CisAB phenotype is a unique ABO subgroup [3, 4, 6]. Currently, only six CisAB alleles are recognized by the Red Cell Immunogenetics and Blood Group Terminology working party of the International Society of Blood Transfusion (ISBT) [7]. Herein, we report a novel c.796A>C mutation in the ABO*B.01 allele responsible for CisAB phenotype in China.

Materials and Methods

Subjects

The proband was a neonate boy who lived in Ji'nan City, Shandong Province, China. He was suspected to have hemolytic disease of the fetus and newborn. In the primary serological tests, the child was assigned the AB type, his mother the O type, and his father the

Table 1. Serological characteristics of the proband and his family members

	anti-A	anti-B	anti-AB	anti-H	anti-A1	Ac	Bc	Oc	Control self	Phenotype
Proband	3+	2+	3+	3+	0	0	0	0	0	CisAB
Father	3+	4+	4+	3+	0	1+	0	0	0	CisAB
Mother	0	0	0	4+	0	4+	4+	0	0	O
Grandfather	2+	4+	4+	2+	0	3+	0	0	0	CisAB
Grandmother	4+	4+	4+	1+	4+	0	0	0	0	AB

1+ to 4+ means different strength agglutination. 0 means no agglutination. Ac is A group cells, Bc is B group cells, Oc is O group cells.

Table 2. Partial polymorphism of the *ABO* gene in the proband and his family members

Position	nt261	nt297	nt467	nt526	nt657	nt703	nt796	nt803	nt903	Genotype or allele
Reference	G	A	C	C	C	G	C	G	G	<i>ABO*A1.01</i>
Reference	del	A	C	C	C	G	C	G	G	<i>ABO*O.01.01</i>
Reference	G	G	C	G	T	A	A	C	A	<i>ABO*B.01</i>
	G	G	C	G	T	A	C	C	A	<i>CisABnew</i>
Proband	del/G	A/G	C/C	C/G	C/T	G/A	C/C	G/C	G/A	<i>CisABnew/ABO*O.01.01</i>
Father	G/G	G/G	C/C	G/G	T/T	A/A	A/C	C/C	A/A	<i>CisABnew/ABO*B.01</i>
Mother	del/del	A/A	C/C	C/C	C/C	G/G	C/C	G/G	G/G	<i>ABO*O.01.01/ABO*O.01.01</i>
Grandfather	G/G	G/G	C/C	G/G	T/T	A/A	A/C	C/C	A/A	<i>CisABnew/ABO*B.01</i>
Grandmother	G/G	A/G	C/T	C/G	C/T	G/A	C/A	G/C	G/A	<i>ABO*A1.02/ABO*B.01</i>

AB type. For his mother, it was the first delivery, and she had no history of blood transfusion or abortion. Family members, including the proband's father, mother, grandfather, and grandmother, were investigated. After the consent of the family members, the specimens were collected for serological testing and *ABO* gene sequencing.

Serological Test

ABO phenotypes of the samples were firstly tested with monoclonal anti-A, anti-B (Shanghai Hemo-pharmaceutical Biological Company, Shanghai, China), anti-AB, and anti-H (Sanquin, Amsterdam, Netherlands), as well as with A1, B, and O cells using the conventional tube method.

DNA Sequencing for Exons of the *ABO* Gene

Genomic DNAs of the samples were extracted from the whole blood using a commercial kit (Tianjin Super Biotechnology Development Company, Tianjin, China). The full coding region of exons 1 to 7 of the *ABO* gene was amplified by polymerase chain reaction and was sequenced as previously reported [8].

ABO Haplotype Analysis in the Proband, His Father, and Grandfather

The sequence of the exons 6 through 7 was amplified using the allele-specific primers [9] and was sequenced as previously reported [8].

Results

Serological Results

The newborn (proband), his father, grandfather, and grandmother were assigned the AB phenotype, his moth-

er was the O type (Table 1) according to the results of the serological tests. Some members were suspected to have the CisAB phenotype due to obey common *ABO* blood group genetic rule by family investigation (Table 1).

The Sequence of *ABO* Gene in the Proband and His Family Members

According to the results of the nucleotide sequence, his mother was assigned the *ABO*O.01.01/ABO*O.01.01* genotype, and his grandmother *ABO*A1.02/ABO*B.01* (Table 2). However, the results of the 261del/G, 297A/G, 526C/G, 657C/T, 703G/A, 796C/C, 803G/C, 930G/A were found in the proband, which did not completely match any *ABO* allele combination. 261G/G, 297G/G, 526G/G, 657T/T, 703A/A, 796C/A, 803C/C, 930A/A were found in his father and grandfather (Table 2) and cannot be assigned to known *ABO* allele combinations either.

Further, a novel *CisAB* allele was identified in the proband, his father, and grandfather using the *ABO* haplotype analysis and family investigation. Compared with the *ABO*B.01* allele, the novel allele has only one nucleotide difference at position 796 from A to C (c.796A>C). The *ABO* genotypes of the proband and family members are listed in Table 2. The accession number MH137796 in the GenBank nucleotide sequence database was assigned for the nucleotide sequence of the novel *CisAB* *ABO* allele.

Discussion

It is known that there are seven nucleotide differences between the *ABO**A1.01 and *ABO**B.01 alleles. Among them, c.526C>G, c.703G>A, c.796C>A, and c.803G>C can lead to four amino acid changes (p.Arg176Gly, p.Gly235Ser, p.Leu266Met, and p.Gly268Ala). The *ABO* alleles with nucleotide change at position 526, 703, 796, or 803 in the *ABO**A1.01 or *ABO**B.01 alleles were found to have both A and B glycosyltransferase activity and were labelled *CisAB* alleles [3, 4, 6, 7]. At present, six types of *CisAB* alleles responsible for the *CisAB* phenotype are recognized by the ISBT [7]. In our proband, we found a new *CisAB* allele with a c.796A>C mutation in *ABO**B.01. The protein sequence of this novel allele is identical to that of *ABO**cisAB.02. However, this novel case has BBAB characteristics according to the nucleotides at positions 526, 703, 796 and 803.

The *CisAB* phenotype is a relatively rare ABO blood group, and the frequency of occurrence is very low with 1:170,000–1:580,000 [3, 4]. The novel allele was not found in the 417 Chinese Han individuals in our laboratory. In general, the *CisAB* phenotype has normal expression of the antigen A, similarly to the phenotype A2, and weak expression of antigen B. The red blood cells of the *CisAB* phenotype had agglutinated with anti-A and anti-B, while sometimes the serum may be agglutinated with A or B cells. There were various serological reaction patterns of

the three *CisAB* individuals in this study, and this may be due to the difference in *ABO* genotypes of these individuals. However, the novel allele was responsible for both A and B antigen expression and participated in A and B expression in the proband, his father, and grandfather.

In conclusion, A novel *CisAB* allele was identified in this study. When routine work using the serological method leads to the suspicion that an individual has the *CisAB* phenotype, it is necessary to conduct family member investigations or to use the molecular DNA method to identify this phenotype.

Acknowledgements

This work was supported by the National Natural Science Foundation of China General Program (Grant No. 81770194).

Statement of Ethics

The ethical approval for this study was obtained by the Ethical Committee of the Chinese PLA General Hospital, and the family members of the proband gave their informed consent.

Disclosure Statement

The authors declare that they have no conflicts of interest relevant to the publication of this article.

References

- 1 Rummel SK, Ellsworth RE. The role of the histoblood ABO group in cancer. *Future Sci OA*. 2016 Mar;2(2):FSO107.
- 2 Quraishy N, Sapatnekar S. Advances in Blood Typing. *Adv Clin Chem*. 2016;77:221–69.
- 3 Jiao LX, Yu JH, Yu XL, Chen L, Han Y, Yang F. Gene analysis of two cases with *CisAB*/B blood subgroup. *Transfus Apheresis Sci*. 2017 Apr;56(2):223–5.
- 4 Zhao H, Miao T, Yan F, Liu Y. The serological and genetic characterization of *CisAB* blood group in a Chinese family. *Transfus Apheresis Sci*. 2015 Oct;53(2):220–4.
- 5 Huang H, Jin S, Liu X, Wang Z, Lu Q, Fan L, et al. Molecular genetic analysis of weak ABO subgroups in the Chinese population reveals ten novel ABO subgroup alleles. *Blood Transfus*. 2018 Sep:1–6.
- 6 Deng ZH, Seltsam A, Ye YW, Yu Q, Li Q, Su YQ, et al. Haemolytic disease of fetus and newborn caused by ABO antibodies in a *cisAB* offspring. *Transfus Apheresis Sci*. 2008 Oct;39(2):123–8.
- 7 Möller M, Jöud M, Storry JR, Olsson ML. Erythrogene: a database for in-depth analysis of the extensive variation in 36 blood group systems in the 1000 Genomes Project. *Blood Adv*. 2016 Dec;1(3):240–9.
- 8 Zhu F, Tao S, Xu X, Ying Y, Hong X, Zhu H, et al. Distribution of ABO blood group allele and identification of three novel alleles in the Chinese Han population. *Vox Sang*. 2010 May;98(4):554–9.
- 9 Ying Y, Chen S, Ma K, Hong X, Zhu F. Identification of a novel B allele with missense mutation (c.98G>C) in the ABO gene. *Transfusion*. 2017 Jan;57(1):219–20.