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Case Report

A_2B 표현형을 가진 캄보디아인에서 발견된 이중 특이성을 갖는 당전이효소를 부호화하는 희귀한 *ABO* 대립 유전자

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Rare *ABO* Allele Encoding Glycosyltransferase with Dual Specificity Found in a Cambodian Individual with the A_2B Phenotype

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Cis-AB and *B(A)* alleles encode an ABO enzyme with dual A and B glycosyltransferase activity. Although globally rare, the *cis*-AB phenotype is found relatively often in Korean, Japanese, and Chinese populations. Cases of the *B(A)* allele have been reported mostly in the Chinese population. Forward typing performed in a Cambodian woman with an ABO discrepancy demonstrated a strong reaction with anti-A and anti-B reagents, while there was no reaction with lectin anti-A_1. The anti-A_1 antibody was detected in reverse typing. Through *ABO* gene sequence analyses of exons 6 and 7, one of the alleles was identified as *ABO*B.01*. In contrast, the other allele harboring a c.803G>C substitution was either *ABO*cisAB.05* or *ABO*BA.06* allele. The *ABO*cisAB.05* and *ABO*BA.06* alleles remain indistinguishable despite routine serological testing and *ABO* genotyping. To the best of the author's knowledge, this is the first case report of these variants discovered in a Cambodian individual residing in Korea. **(Korean J Blood Transfus 2020;31:254-259)**

Key words: ABO, Genotyping, *cis-AB*, *B(A)*

Introduction

The *ABO* gene encodes a glycosyltransferase, which catalyzes the transfer of carbohydrates to the H antigen, thereby converting the H antigen into A or B antigens, depending on the encoding allele. The common ABO phenotypes in Korea are mostly encoded

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by *ABO*A1.02, ABO*B.01, ABO*O.01.01,* and *ABO*O.01.02* alleles, whereas weak ABO phenotypes are encoded by other ABO subgroup alleles, such as *ABO*cisAB.01, ABO*A2.01, ABO*A2.04, ABO*B3.03, ABO*B3.06,* and *Aw10* [1-3]. Among ABO subgroup alleles, the *B(A)* and *cis-AB* alleles can encode an enzyme with both A and B glycosyltransferase activities [4]. This dual enzymatic activity of *cis*-AB results in a typical phenotype of A_2B_3 expressing decreased levels of A and B antigens; B(A), on the other hand, is distinguished by very low A antigen levels and normal B antigen levels.

The *cis*-AB is relatively common in the Korean, Japanese, and Chinese populations. To date, several *cis-AB* alleles such as *ABO*cisAB.01, ABO*cisAB.04,* and *ABO*cisAB.09* have been reported in Koreans [1,5,6]. However, *ABO*cisAB.05* alleles remain unexplored outside the Chinese population. In contrast to *cis-AB* alleles, *B(A)* alleles have not been identified by molecular genetic testing in Koreans and Japanese despite their abundance in Chinese. Finally, no cases of *cis-AB* and *B(A)* alleles have been yet reported in Cambodians.

Here, we report for the first time, a case of a Cambodian individual suspected of having an *ABO*cisAB.05* or *ABO*BA.06* allele.

Case Report

A peripheral blood sample from a 25-year-old Cambodian woman with an ABO discrepancy was sent to Samsung Medical Center. The proband was identified to have an A_2B phenotype by serological method. RBCs of the proband showed strong agglutination reactions with anti-A and anti-B reagents (Ortho Clinical Diagnostics, Raritan, NJ, USA), but no reaction with anti-A_1 lectin (Ortho Clinical Diagnostics). Anti-A_1 antibody was detected in the plasma (Table 1). Sequence analysis of exons 6 and 7 of the *ABO* gene was performed according to the previously described method [1], and revealed a heterozygous sequence (C and G) at nucleotide 803. Allele specific polymerase chain reaction (AS-PCR) for allele separation was subsequently carried out for two sequences. AS-PCR with sequence covering the c.261 to c.803 was performed as previously described [7] and of sequence covering the

Table 1. Comparison of the serological results of *cis-AB05, B(A)06,* and proband

	Forward typing							Reverse typing		Ref
	Anti-A		Anti-A_1	Anti-B		Anti-A,B	Anti-H	A_1 cell	B cell	
	monoclonal	polyclonal		monoclonal	polyclonal					
*ABO*BA.06/ *O.01.01*	4+	−	−	4+	4+	4+	4+	2+	−	[4]
*ABO*cisAB.05 /*O.01.01*	4+	4+	−	4+	4+	4+	4+	−	−	[14]
*ABO*cisAB.05* or *BA.06/*B.01*	4+	NT	−	4+	NT	NT	NT	3+	−	In this case

c.803 to c.1096 was performed using primer pairs 803G-F (GGCGATTTCTACTACCTGGGCGG) and ABO+19915AS (GGCGTATCTGCGATTGCGTGT) [8]. The proband harbored a rare c.803C>G substitution (p.Ala268Gly) in the *ABO*B.01* allele (Fig. 1). This variant is annotated as *ABO*cisAB.05* and *ABO*BA.06* in the International Society of Blood Transfusion (ISBT) database [9].

Discussion

To date, several weak ABO subgroups have been discovered in various populations. Although ABO subgroups are rare, they are a leading cause of ABO discrepancy [10]. In most cases, weak phenotypes result from the expression of a variant ABO allele, which can be revealed by molecular genetic analysis, thereby resolving the ABO discrepancy. Cho et al. reported their results with the resolution of ABO discrepancies by *ABO* genotyping [11]. In this study, serological analysis revealed ABO discrepancy with A_2B phenotype and the presence of anti-A in patient's serum. It was speculated that the underlying genotype of the A_2B phenotype could be *cis-AB/B,*

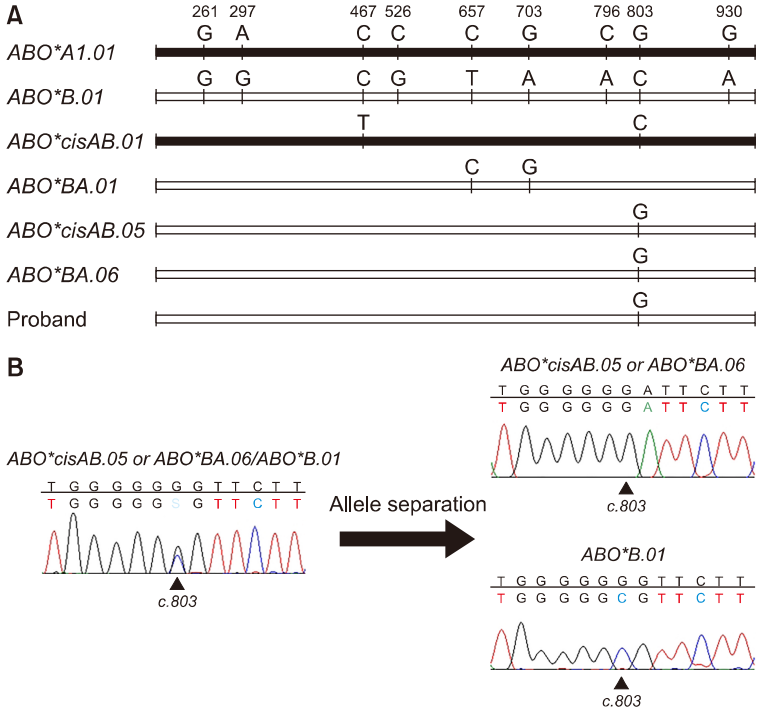

Fig. 1. (A) Genetic characterization of *cis-AB* and *B(A)* alleles (in exons 6 and 7 of the *ABO* gene). The black and white boxes indicate the A and B backbones, respectively. (B) Sequencing chromatograms of a proband with nucleotide substitutions at position 803. The left chromatogram reveals a heterozygous sequence (C and G) at c.803. The two right chromatograms reveal the *ABO*cisAB.05* or *ABO*BA.06* allele and *ABO*B.01* allele.

Table 2. Estimated *cis-AB* and *B(A)* frequency in the Asian population

Allele	Frequency(*10-5)			Ref
	China	Japan	Korea	
B(A)02	0.78	N/A	N/A	[13]
B(A)04	1.6	N/A	N/A	[13]
B(A)06	0.3	N/A	N/A	[13]
cisAB01	0.66	1.2	35.4	[12]
cisAB05	N/A	N/A	N/A	

Abbreviation: N/A, not applicable.

B(A)/B, B(A)/O, or A_2/B [12,13]. Interestingly, the *ABO* genotyping demonstrated that the proband harbored the c.803C＞G variant (p.Ala268Gly) in the wild type *B* allele (*ABO*B.01*), which corresponded to *ABO*cisAB.05* or *ABO*BA.06* allele. In this study, we tried to distinguish whether this allele is *ABO* cisAB.05* or *ABO*BA.06*. However, the distinction between the two alleles is inherently infeasible, as the nucleotide sequences of the two alleles registered in the ISBT database (only covering exons 6 and 7) are identical. The regions outside of exons 6 and 7 have not been previously reported and further studies are needed to determine the nucleotide sequences in ABO exons 1～5 and the flanking regions of the two alleles. In fact, the distinction between the two alleles has no clinical implication.

The *ABO*cisAB.05* and *ABO*BA.06* alleles have been reported only in one Chinese subject with an A_2B phenotype [4,14]. However, B(A) blood group types and *ABO*cisAB.05* allele have never been confirmed by molecular analysis, although other alleles of *cis-AB* blood group, such as *ABO*cisAB.01* and *ABO*cisAB.04*, have been reported in Korean and Japanese populations [12]. In Chinese population, B(A) is more abundant than *cis*-AB blood group [13]. Among *B(A)* alleles, *ABO*BA.04* is the most common (Table 2).

The possibility of *ABO*cisAB.05* and *ABO*BA.06* alleles being identical has been noted as the nucleotide changes reported for both alleles are the same based on dbRBC, and the *ABO*cisAB.05* allele has been accepted in dbRBC without publication or GenBank submission [15]. In addition, unlike the common B(A) phenotypes with *ABO*BA.04* allele, *ABO* BA.06* phenotype tends to show strong positive reaction with monoclonal anti-A reagent, which is the same as in *ABO*cisAB.05*. However, in reactions with human polyclonal anti-A reagent (ShuBao, Chengdu, China), RBCs with the *ABO*cisAB.05* phenotype are agglutinated, while RBCs with the *ABO*BA.06* phenotype are not agglutinated [14]. With the advent of potent monoclonal anti-A reagents, human polyclonal anti-A reagents are no longer frequently used as a routine serologic test. Unfortunately, further distinction between *ABO*cisAB.05* and *ABO*BA.06* was not feasible, due to lack of sample.

This is the first case of a Cambodian individual residing in Korea with this ABO subgroup variant. From demographic perspective, foreign residents accounted for 4.57% of total Korean population in 2018 [16]. As the number of foreign residents in Korea continues to grow annually, the need to understand rare blood groups found in other ethnicities as well as in Korean population increases.

요 약

Cis-AB와 B(A)는 A와 B의 당전이효소 활성을 동시에 함께 갖는 효소를 부호화하는 특징이 있

다. cis-AB 표현형은 드물지만 한국인, 일본인 및 중국인 인구에서 일반적으로 발견된다. B(A) 혈액형의 사례는 이전에 보고되었지만 대부분 중국인 인구에 국한되어 있다. ABO 불일치를 가진 캄보디아 여성의 혈구형 검사에서 항-A, 항-B시약에 강한 응집을 보였고 항-A_1 렉틴에서는 응집을 보이지 않았다. 혈청형 검사에서 A_1 적혈구에 응집을 보였다. ABO 유전자의 엑손 6과 7 분석에서 *ABO*B.01* 대립유전자를 동반하는 c.803C>G 변이를 가진 *ABO*cis-AB.05* 혹은 *ABO*BA.06*를 보유하는 것으로 밝혀졌다. 일반 혈청학적 검사와 ABO 유전자 검사를 했음에도 *ABO*cis-AB.05*와 *ABO*BA.06*를 정확히 규명할 수는 없었다. 그럼에도 이 돌연변이들은 국내에서 처음 발견되어 보고하는 바이다.

References

1. Cho D, Kim SH, Jeon MJ, Choi KL, Kee SJ, Shin MG, et al. The serological and genetic basis of the cis-AB blood group in Korea. Vox Sang 2004;87:41-3
2. Cho D, Kim SH, Ki CS, Choi KL, Cho YG, Song JW, et al. A novel B(var) allele (547 G>A) demonstrates differential expression depending on the co-inherited ABO allele. Vox Sang 2004; 87:187-9
3. Cho D, Shin MG, Yazer MH, Kee SJ, Shin JH, Suh SP, et al. The genetic and phenotypic basis of blood group A subtypes in Koreans. Transfus Med 2005;15:329-34
4. Deng ZH, Yu Q, Liang YL, Wang DM, Su YQ, Wu GG. Characterization of a novel B(A) allele with BBBA type at the ABO blood group. J Hum Genet 2006;51:732-6
5. Lee SY, Phan MT, Shin DJ, Shin MG, Park JT, Shin JW, et al. A novel cis-AB variant allele arising from a de novo nucleotide substitution c.796A>G (p.M266V) in the B glycosyltransferase gene. Transfus Med 2015;25:333-6
6. Yoon J, Youk HJ, Chang JH, Jang MA, Choi JH, Nam MH, et al. Identification of the ABO* cis-AB04 allele with a unique substitution C796A: the first case in Korea. Ann Lab Med 2016;36:620-2
7. Cho D, Jeon MJ, Oh BJ, Song JW, Shin MG, Shin JH, et al. A simplified ABO genotyping by allele-specific polymerase chain reaction. Korean J Lab Med 2005;25:123-8
8. Sano R, Nakajima T, Takahashi K, Kubo R, Kominato Y, Tsukada J, et al. Expression of ABO blood-group genes is dependent upon an erythroid cell-specific regulatory element that is deleted in persons with the B(m) phenotype. Blood 2012;119:5301-10
9. International Society of Blood Transfusion (ISBT). Names for ABO (ISBT 001) blood group alleles. https://www.isbtweb.org/fileadmin/user_upload/Working_parties/WP_on_Red_Cell_Immunogenetics_and/001_ABO_Alleles_v1.2.pdf [Online] (last visited on 19 April 2020)
10. 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 2019;17:217-22
11. Cho D, Lee JS, Park JY, Jeon MJ, Song JW, Kim SH, et al. Resolution of ABO discrepancies by ABO genotyping. Korean J Lab Med 2006; 26:107-13
12. Chun S, Choi S, Yu H, Cho D. Cis-AB, the blood group of many faces, is a conundrum to the novice eye. Ann Lab Med 2019;39:115-20
13. Zhu Z, Ye L, Li Q, Gao H, Tan Y, Cai W. Red cell immunohematology research conducted in China. Transfus Med Rev 2017;31:102-6
14. 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 Apher Sci 2008;39:123-8
15. Reid MR, Lomas-Francis C, Olsson ML. The Blood Group Antigen FactsBook. 3th ed. Amsterdam: Elsevier Academic Press, 2012
16. Korea immigration service (Korea). 2018 Korea immigration service statistics annual report. http://www.moj.go.kr/moj/2412/subview.do [Online] (last visited on 19 April 2020)