




# Cis-AB, the Blood Group of Many Faces, Is a Conundrum to the Novice Eye

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*Cis-AB*, a rare ABO variant, is caused by a gene mutation that results in a single glycosyltransferase enzyme with dual A and B glycosyltransferase activities. It is the most frequent ABO subgroup in Korea, and it occurs more frequently in the East Asian region than in the rest of the world. The typical phenotype of *cis-AB* is A<sub>2</sub>B<sub>3</sub>, but it can express various phenotypes when paired with an A or B allele, which can lead to misclassification in the ABO grouping and consequently to adverse hemolytic transfusion reactions. While *cis-AB* was first discovered as having an unusual inheritance pattern, it was later found that both A and B antigens are expressed from the same allele inherited from a single parent; hence, the name *cis-AB*. Earlier studies relied on serological and familial investigation of *cis-AB* subjects, but its detection has become much easier with the introduction of molecular methods. This review will summarize the serological variety, genetic basis and inheritance pattern, laboratory methods of investigation, clinical significance, and the blood type of choice for transfusion for the *cis-AB* blood group.

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## INTRODUCTION

Numerous examples of weak ABO subgroup phenotypes, such as A<sub>2</sub>, A<sub>3</sub>, A<sub>m</sub>, A<sub>x</sub>, A<sub>y</sub>, A<sub>el</sub>, B<sub>3</sub>, B<sub>x</sub>, B<sub>m</sub>, B<sub>el</sub>, B(A), and *cis-AB*, have been reported to date [1-4]. These phenotypes and their allelic frequencies vary according to geographic and ethnic background [1-4]. Among them, the *cis-AB* blood group is rare globally, yet it is relatively common in the Korean, Japanese, and Chinese populations [3, 5, 6]. Cho *et al.* [5] reported that the overall frequency of the *cis-AB* blood group in Koreans is 0.0354% (60/169,605), while in Japanese and Chinese blood donors, frequencies of 0.0012% [6] and 0.00066%, respectively, have been reported [7]. Interestingly, 26.4% (60/227) of

ABO weak subgroups in Korea arise from the *cis-ABO1* allele [5]. Although new alleles are continuously being reported, *cis-ABO1* is the most prevalent allele in Korea [5].

The *cis-AB* blood group has attracted attention in transfusion medicine because of the interesting phenomenon that a single allele encodes both A and B antigens, as opposed to the regular *trans-AB* genotype [6, 8]. Therefore, it can be difficult to correctly match ABO group for transfusion for the *cis-AB* subgroup, and paternity disputes can arise because of the unusual inheritance pattern, which can result in, for example, the birth of an O child from an AB mother.

The *cis-AB* subgroup still stands as a challenge to the novice eye in the clinical blood bank. This review provides an overview

**Table 1.** Classification, *cis*-AB alleles, nucleotide and amino acid changes, and phenotypes of *cis*-AB blood groups reported in the literature

Backbone	Allele	Nucleotide* changes	Amino acid* changes	Phenotypes	GenBank accession No. (when available)	Reference
A backbone	<i>cis</i> -AB01	467C>T; 803G>C	P156L; G268A	A <sub>2</sub> B <sub>3</sub>	<a href="#">AF134427-4428</a>	Cho <i>et al.</i> [5]; Cho <i>et al.</i> [12]
	<i>cis</i> -AB01var	803G>C; 1,009A>G	G268A; R337G	AxBx	<a href="#">JQ824867</a>	Cai <i>et al.</i> [3]
	<i>cis</i> -AB04	467C>T; 796C>A	P156L; L266M	A <sub>2</sub> B	Not submitted	Yoon <i>et al.</i> [37]
	<i>cis</i> -AB08	467C>T; 724G>T; 803G>C	P156L; E242X; G268A	NA	<a href="#">JF304777</a>	Liu <i>et al.</i> [38]
	<i>cis</i> -AB new	467C>T; 803G>C; 930G>A; 1,096G>A	P156L; G268A	A <sub>2</sub> B <sub>3</sub>	<a href="#">KR870035</a>	Not published
B backbone	<i>cis</i> -AB02	297A>G; 526C>G; 657C>T; 703G>A; 803G>C	R176G; G235S; G268A	A <sub>2</sub> B, A <sub>int</sub> B <sub>x</sub>	<a href="#">AF062487</a>	Mifsud <i>et al.</i> [39]
	<i>cis</i> -AB03	297A>G; 526C>G; 657C>T; 700C>T; 703G>A; 796C>A; 803G>C; 930G>A	R176G; P234S; G235S; L266M; G268A;	A <sub>2</sub> B	<a href="#">AF408431</a>	Roubinet <i>et al.</i> [40]
	<i>cis</i> -AB05	297A>G; 526C>G; 657C>T; 703G>A; 796C>A; 930G>A	R176G; G235S; L266M	A <sub>2</sub> B	Not submitted	Deng <i>et al.</i> [41]
	<i>cis</i> -AB06	297A>G; 657C>T; 703G>A; 796C>A; 803G>C; 930G>A	G235S; L266M; G268A	A <sub>2</sub> B	<a href="#">FJ851690</a>	Zhu <i>et al.</i> [42]
	<i>cis</i> -AB07	297A>G; 526C>G; 657C>T; 703G>A; 796C>A; 797T>C; 803G>C; 930G>A	R176G; G235S; L266M; G268A	A <sub>2</sub> B <sub>w</sub>	<a href="#">JX473237</a>	Mifsud <i>et al.</i> [39]
	<i>cis</i> -AB09 <sup>†</sup>	297A>G; 526C>G; 657C>T; 703G>A; 796C>G <sup>‡</sup> ; 803G>C; 930G>A	T99T; R176G; H219H; G235S; L266V; L310L	A <sub>2</sub> B with 1+ agglutination with A <sub>1</sub> cells	<a href="#">KJ766004</a>	Lee <i>et al.</i> [20]

\*Changes in nucleotides and amino acids in the *cis*-AB01 allele are described according to the A101 allele; <sup>†</sup>The *cis*-AB09 allele arises from a *de novo* nucleotide substitution c.796A>G (p.M266V) in the B glycosyltransferase gene; <sup>‡</sup>The c.796C>G on the A101 allele background is the same as c.796A>G on the B101 allele background.

Abbreviation: NA, not applicable.

of the serological characteristics, genetic basis and inheritance, laboratory investigation, and clinical importance of the *cis*-AB blood group.

## SEROLOGICAL CHARACTERISTICS OF THE CIS-AB BLOOD GROUP

There exist various phenotypes of the *cis*-AB blood group globally, and these phenotypes are associated with various *cis*-AB alleles (Table 1). Among them, *cis*-AB01 is the most common allele. Yoshida *et al.* [9-11] first characterized a transferase enzyme with bifunctional activity (both A and B transferase activities) in the sera of individuals with the *cis*-AB01 allele. The *cis*-AB01 allele causes the A<sub>2</sub>B<sub>3</sub> phenotype when co-inherited with the O allele, and more than seven different phenotypes when paired with A or B alleles have been reported [5].

Yamaguchi [6] reported three phenotypes of the *cis*-AB blood group in the Japanese population: A<sub>2</sub>B<sub>3</sub>, A<sub>2</sub>B, and A<sub>1</sub>B<sub>3</sub>, which are derived from the *cis*-AB01/O, *cis*-AB01/B, and *cis*-AB01/A genotypes, respectively. In large-scale blood donor studies, Cho

*et al.* [5, 12] reported that most Korean *cis*-AB donors exhibit not only the above three typical phenotypes but also a variety of other phenotypes ranging from A<sub>1</sub>B<sub>x</sub>, A<sub>1</sub>B<sub>el</sub>, A<sub>int</sub>B<sub>3</sub>, and A<sub>int</sub>B to typical A. These different phenotypes from a single *cis*-AB01 allele are presumably due to allele competition (i.e., *cis*-AB01/A); the *cis*-AB mutant enzyme might not be able to produce its usual number of antigens due to competition for H-antigen with the co-inherited normal A transferase enzyme [5, 13, 14]. Four Korean cases with typical A phenotype without detectable B antigen expression on red blood cells (RBCs) in individuals with *cis*-AB01/A have been reported [12, 15-17]. Without careful family studies, these cases would have been typed as typical A. Phenotypes, frequencies, serological characteristics, and genotypes of *cis*-AB blood groups reported in Korea are summarized in Table 2.

## GENETIC BASIS AND INHERITANCE OF THE CIS-AB BLOOD GROUP

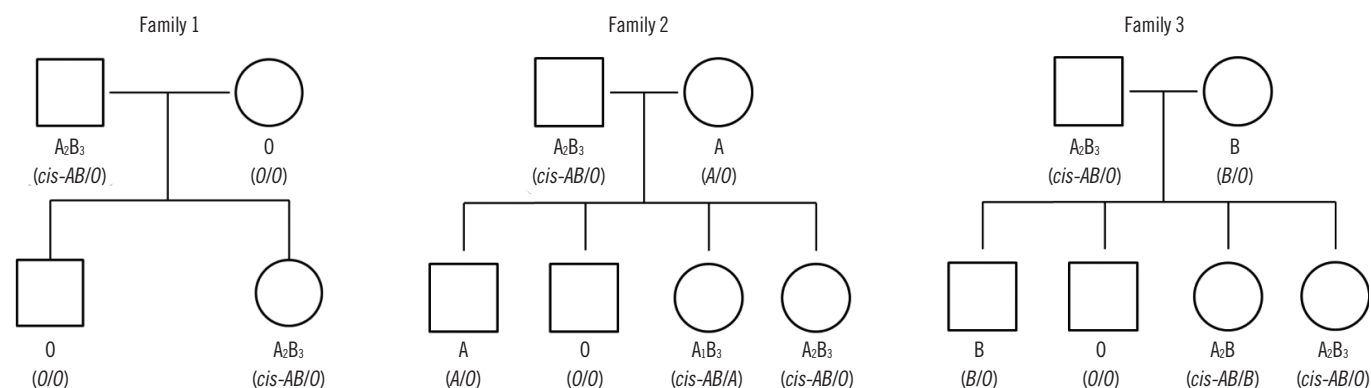
Among reported *cis*-AB alleles, some *cis*-AB alleles (*cis*-AB01, *cis*-AB01var, and *cis*-AB02 to *cis*-AB09) are registered in the

**Table 2.** Phenotypes, frequencies, serological characteristics, and genotypes of the *cis*-ABO1 blood group reported in Korea

Phenotype	Frequency (%)	Cell type					Serum type		Genotypes	Reference
		anti-A	Anti-B	Anti-A1	anti-A, B	Anti-H	A1	B		
A <sub>2</sub> B <sub>3</sub>	60.0	4+	1+ ~ 3+	–	4+	3+ ~ 4+	– ~ 2+	1+ ~ 2+	<i>cis</i> -ABO1/OO1 or OO2	Cho <i>et al.</i> [5]; Cho <i>et al.</i> [12]; Whang <i>et al.</i> [15]; Chun <i>et al.</i> [25]
A <sub>1</sub> B <sub>3</sub>	15.0	4+	1+ ~ 3+	3+ ~ 4+	NA	NA	–	1+ ~ 2+	<i>cis</i> -ABO1/A1O2	Cho <i>et al.</i> [5]; Chun <i>et al.</i> [25]
A <sub>2</sub> B	15.0	4+	4+	–	4+	–	– ~ 2+	–	<i>cis</i> -ABO1/B1O1	Cho <i>et al.</i> [5]; Cho <i>et al.</i> [12]; Cho <i>et al.</i> [22]
A <sub>1</sub> B <sub>w</sub> *	6.7	4+	trace	4+	NA	NA	–	1+ ~ 2+	<i>cis</i> -ABO1/A1O2	Cho <i>et al.</i> [5]; Chun <i>et al.</i> [25]
A <sub>int</sub> B <sub>3</sub>	3.3	4+	1+ ~ 3+	1+	NA	NA	–	1+ ~ 2+	<i>cis</i> -ABO1/A1O2	Cho <i>et al.</i> [5];
A <sub>int</sub> B	Rare	4+	4+	1+	4+	NA	trace	–	<i>cis</i> -ABO1/B1O1	Chun <i>et al.</i> [25]; Park <i>et al.</i> [26]
A <sub>1</sub>	Rare	4+	–	4+	4+	–	–	3+	<i>cis</i> -ABO1/A1O2	Cho <i>et al.</i> [12]; Whang <i>et al.</i> [15]; Kang <i>et al.</i> [16]; Song <i>et al.</i> [17]

\*A<sub>1</sub>B<sub>el</sub>, A<sub>1</sub>B<sub>x</sub>, A<sub>1</sub>B<sub>m</sub>.

Abbreviation: NA, not applicable.



**Fig. 1.** Three representative Korean *cis*-AB family cases illustrating *cis*-AB inheritance of the ABO blood group depending on the mother's genotype (O/O; Family 1, A/O; Family 2, and B/O; Family 3). The ABO phenotype and genotype of each person are shown.

Blood Group Antigen Gene Mutation Database [1, 18]. *cis*-ABO1, O4, and O8 have an A allele background, whereas *cis*-ABO2, *cis*-ABO3, *cis*-ABO5 to *cis*-ABO7, and *cis*-ABO9 have a B allele background. Yamamoto *et al.* [19] first identified structural changes in the *cis*-ABO1 allele, using the A1O2 allele as a reference. The coding sequence of the *cis*-ABO1 allele is identical to that of the A1O2 allele except for Gly268Ala (c.803G>C) in exon 7, whereas the *cis*-ABO2 allele sequence is identical to that of the B1O2 allele except for Leu266Met (c.796A>C) (GenBank accession No. AF062487). The most recently discovered *cis*-ABO9 arises from a *de novo* c.796A>G nucleotide substitution in the ABO\*B1O1 allele [20]. The classification of *cis*-AB alleles according to the allele backbone, along with nucleotide and amino acid changes and reported phenotypes, is presented in Table 1.

The inheritance pattern of the *cis*-AB blood group appears to

violate the typical Mendelian inheritance pattern. The *cis*-AB phenotype raises questions about an apparently paradoxical inheritance of the ABO blood group, such as cases of birth of an O or AB child from an AB father and O mother [21, 22]. However, in such cases, the AB type in the family is not a typical AB type, but rather the *cis*-A<sub>2</sub>B<sub>3</sub> blood group in which the A and B characteristics are inherited from one parent. Therefore, the inheritance pattern looks paradoxical, whereas in fact, it exactly follows the general Mendelian inheritance of ABO blood groups. Based on analysis of some unexplained *cis*-AB cases, Yamaguchi *et al.* [6, 23] observed inheritance to follow a *cis*-regulated pattern, in contrast to regular *trans*-AB, and first coined the term *cis*-AB blood group. Representative Korean family trees illustrating the inheritance pattern are shown in Fig. 1. Among the several *cis*-AB alleles, *cis*-ABO9 is of particular interest, as it was reported as a *de novo* mutation (c.796A>G) in a Korean family,

in which both the father and mother had blood group B [20].

It is of sociological interest that, in contrast to Western culture, individuals in Korea and Japan generally know their ABO blood type. In Korea, it is possible to know one's ABO/RhD blood types from routine testing during regular health check-ups at school age (or for men, during military service). In this context, the *cis*-AB type can potentially lead to paternity issues (e.g., when an individual has O or AB [actually *cis*-AB] blood type and the father and mother are known to be O and AB [actually *cis*-AB], respectively). Further, this implies that cases of *cis*-AB can be detected in routine ABO typing of a newborn cord blood samples.

### LABORATORY INVESTIGATION OF THE *CIS*-AB BLOOD GROUP

Owing to its serological characteristic, the *cis*-AB01 blood group is often encountered in pre-transfusion or donor screening. Samples from *cis*-AB01 subjects present forward or reverse ABO blood typing as ABO discrepancy; it may be commonly suspected when there is weak agglutination of RBCs with anti-B reagent in cell typing and weak agglutination with B cells in serum typing. Weak agglutination with B cells can be enhanced when the reaction is incubated at room temperature for 15 minutes. In contrast to *cis*-A<sub>1</sub>B<sub>3</sub>, the *trans*-A<sub>1</sub>B<sub>3</sub> blood group originated from heterozygosity of A<sub>1</sub> and B<sub>3</sub> shows no agglutination with B cells in serum typing, despite prolonged incubation. In addition, a measurable amount of H antigen is suggestive of the *cis*-AB01 blood group [22].

The representative A<sub>2</sub>B<sub>3</sub> phenotype can be detected by skilled laboratory personnel through several serological methods, such as plate and tube methods. Not all medical technologists can be expected to reach this level of expertise, and one study reported serious consequences after *cis*-AB was identified as typical A [24]. After the introduction of automated ABO grouping devices, one research group encountered multiple cases of misidentification of *cis*-AB samples as typical AB when using a device that applies the microplate method [25]. In addition to the confirmation of typical A<sub>2</sub>B<sub>3</sub> phenotypes as *cis*-AB, other phenotypes of *cis*-AB blood are often missed during routine serological testing, and ABO genotyping is the sole method for confirmation. For example, a case of A<sub>int</sub>B phenotype with anti-A antibodies could not be initially suspected of *cis*-AB type, but was confirmed by ABO genotyping [26].

After the introduction of ABO genotyping in clinical blood banks, it has become a valuable tool complementary to serology for correctly determining the ABO blood groups of both patients and donors [2, 27]. Before the broad use of ABO gene testing, the *cis*-AB blood group was confirmed by serological investigations together with family study. However, family study is often impossible, and the introduction of ABO genotyping has thus resolved many issues in this regard [22].

Various genotyping methods can be employed to confirm *cis*-AB in cases of ABO discrepancy. Allele-specific (AS-)PCR, PCR-restriction fragment length polymorphism (RFLP), and/or direct sequencing of exons 6 and 7 of the *ABO* gene have been used for clinical purposes [27-29]. However, sequencing of the full

**Table 3.** Serological and molecular tests for detection of the *cis*-AB blood group

	Method	Remark	Reference
Serology ( <i>cis</i> -A <sub>2</sub> B <sub>3</sub> )	Plate (tile)	- Weak or delayed red cell reactivity to anti-B reagent	Chun <i>et al.</i> [25]; Kim <i>et al.</i> [34]
	Tube	- Weak red cell reactivity to anti-B reagent (mixed field agglutination)	Chun <i>et al.</i> [25]
		- Weak serum reactivity to B cells can be enhanced by incubation at room temperature for 15 minutes	
	Microcolumn	- Medium-sized clumps of agglutinated cells in the upper half of the gel column, can be observed in cell typing (to anti-B)	Unpublished data
	Automated microplate	- Misidentification of <i>cis</i> -A <sub>2</sub> B <sub>3</sub> samples as typical AB can be possible	Chun <i>et al.</i> [25]
Molecular	AS-PCR/ PCR-RFLP	- Can be only used for known <i>cis</i> -AB alleles	Fukumori <i>et al.</i> [29]
	Sequencing	- For clinical purpose, sequencing of <i>ABO</i> gene exons [6, 7] are commonly used	Won <i>et al.</i> [28]; Won <i>et al.</i> [30]
		- For research purpose, sequencing of the all of <i>ABO</i> gene coding region (exons [1-7]) and regulatory regions is used	
	Cloning/allele-separation and sequencing	- Required for novel <i>cis</i> -AB allele study	Lee <i>et al.</i> [20]
	Next-generation sequencing	- Required for novel <i>cis</i> -AB allele study	Moller <i>et al.</i> [31]

Abbreviations: AS-PCR, allele specific-PCR; PCR-RFLP, PCR-restriction fragment length polymorphism.

coding region (exons 1–7), including regulatory regions of the gene, and cloning/allele-separation are necessary for research purposes (i.e., discovery of a novel *cis-AB* allele) [20, 30]. Next-generation sequencing has been applied to blood group genes, and it will also be useful for *ABO* subgroup genes, including *cis-AB* alleles [31]. The *cis-AB* blood group can be detected by several methods (Table 3).

## CLINICAL IMPORTANCE OF THE *CIS-AB* BLOOD GROUP IN TRANSFUSION PRACTICE

Various approaches for transfusion for *cis-AB* patients are available. One is autologous blood transfusion, including preoperative autologous deposit [32], intraoperative salvage, and postoperative salvage [33, 34]. However, preoperative autologous deposit can be applied in few cases, such as when intraoperative bleeding is predicted and the patient's preoperative condition is good [32]. In another approach, blood from a family member having the same blood type can be used for transfusion after irradiation to prevent transfusion-associated graft-versus-host disease. Oh *et al.* [34] reported safe transfusion of type O RBCs to *cis-AB* without adverse transfusion reaction. According to the Blood Transfusion Guideline 4th edition, in Korea, O RBCs (or A RBCs when anti-A is not detectable in the serum) and type AB plasma or platelets are recommended for patients with the *cis-AB* blood group [35].

Although most *cis-AB* subgroups can be accurately typed in the hospital blood bank, some cases may be misinterpreted as AB or A type [25, 26]. In a case of *cis-A<sub>1</sub>B<sub>3</sub>* interpreted as typical A, transfusion of four units of type A RBCs and four units of type A fresh frozen plasma (FFP) caused delayed transfusion adverse effects, because the results of pre-transfusion cross-match had not been properly interpreted [24]. Although reaction between the B antigen of the *cis-AB* patient and anti-B antibodies from the A type FFP is theoretically possible, the authors could not draw a definitive conclusion on the cause of hemolysis [24].

Another group reported a case of transfusion of type A RBCs, FFP, and platelets to a 14-year-old boy with *cis-A<sub>2</sub>B<sub>3</sub>* blood type. Reverse typing showed that his serum contained anti-B but no anti-A antibodies. The patient did not show adverse reactions, which can be explained by the weak B antigen on his RBCs without anti-A antibodies against the transfused A RBCs. The same patient had been transfused with typical AB blood at the age of 13 months without any adverse reaction, which can also be explained by the fact that he may have had no or low anti-A

and anti-B antibodies in his serum against the transfused AB blood during this first transfusion [36].

*Cis-AB* is difficult to determine, as it presents as more than one phenotype. The various phenotypes make quick blood group determination difficult. Therefore, universal blood (O-type RBCs and AB-type FFP/platelets) is recommended, as it can be safely used for patients with the *cis-AB* blood group.

## Authors' Disclosures of Potential Conflicts of Interest

The authors have no conflicts of interest to declare.

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