**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\textsubscript{1}B\textsubscript{1}, 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.

**Key Words:** ABO, cis-AB, Genotyping, Serology

**INTRODUCTION**

Numerous examples of weak ABO subgroup phenotypes, such as A\textsubscript{x}, A\textsubscript{y}, A\textsubscript{m}, A\textsubscript{x}, A\textsubscript{y}, A\textsubscript{m}, B\textsubscript{x}, B\textsubscript{m}, B\textsubscript{y}, B\textsubscript{x}, B\textsubscript{y}, B\textsubscript{m}, B(A), and cis-AB, have been reported to date [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-AB01 allele [5]. Although new alleles are continuously being reported, cis-AB01 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 | cis-AB01 | 467C>T; 803G>C | P156L; G268A | A_B2 | AF134427-4428 | Cho et al. [5]; Cho et al. [12] |
|     | cis-AB01var | 803G>C; 1,009A>G | G268A, R337G | A_Bx | JQ824867 | Cai et al. [3] |
|     | cis-AB04 | 467C>T; 796C>A | P156L; L266M | A_B | Not submitted | Yoon et al. [37] |
|     | cis-AB08 | 467C>T; 724G>T; 803G>C | P156L; E242K; G268A | NA | JF304777 | Liu et al. [38] |
|     | cis-AB new | 467C>T; 803G>C; 930G>A; 1,096G>A | P156L; G268A | A_B2 | KR870035 | Not published |
| B backbone | cis-AB02 | 297A>G; 526C>G; 657C>T; 703G>A; 803G>C | R176G; G235S; G268A | A_B, A1B1 | AF062487 | Mitsud et al. [39] |
|     | cis-AB03 | 297A>G; 526C>G; 657C>T; 700C>T; 703G>A; 796C>A; 803G>C; 930G>A | R176G; P234S; G235S; L266M; G268A | A_B | AF408431 | Roubinet et al. [40] |
|     | cis-AB05 | 297A>G; 526C>G; 657C>T; 703G>A; 796C>A; 930G>A | R176G; G235S; L266M | A_B | Not submitted | Deng et al. [41] |
|     | cis-AB06 | 297A>G; 657C>T; 703G>A; 796C>A; 803G>C; 930G>A | G235S; L266M; G268A | A_B | FJ851690 | Zhu et al. [42] |
|     | cis-AB07 | 297A>G; 526C>G; 657C>T; 703G>A; 796C>A; 797T>C; 803G>C; 930G>A | R176G; G235S; L266M; G268A | A_B | JX473237 | Mitsud et al. [39] |
|     | cis-AB09 | 297A>G; 526C>G; 657C>T; 703G>A; 796C>A; 803G>C; 930G>A | T99T; R176G; H219H; G235S; L266V; L310L | A_B with 1+ agglutination with A1 cells | KJ766004 | Lee et al. [20] |

*Changes in nucleotides and amino acids in the cis-AB01 allele are described according to the A101 allele; †The cis-AB09 allele arises from a de novo nucleotide substitution c.796A>G (p.M266V) in the B glycosyltransferase gene; ‡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 A1B2 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_B2, A1_B2, and A11_B3, 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 A1_B2, A11_B2, A111_B3, and A1B 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. 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
Blood Group Antigen Gene Mutation Database [1, 18]. *cis*-AB01, 04, and 08 have an A allele background, whereas *cis*-AB02, *cis*-AB03, *cis*-AB05 to *cis*-AB07, and *cis*-AB09 have a B allele background. Yamamoto et al. [19] first identified structural changes in the *cis*-AB01 allele, using the A102 allele as a reference. The coding sequence of the *cis*-AB01 allele is identical to that of the A102 allele except for Gly268Ala (c.803G>C) in exon 7, whereas the *cis*-AB02 allele sequence is identical to that of the B102 allele except for Leu266Met (c.796A>G) (GenBank accession No. AF062487). The most recently discovered *cis*-AB09 arises from a *de novo* c.796A>G nucleotide substitution in the ABO* B101 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:B1 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*-AB09 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-A1B3, the trans-A1B3 blood group originated from heterozygosity of A1 and B3 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 A1B3 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 A1B3 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 A1B1 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 (cis-A1B3)             | - Weak or delayed red cell reactivity to anti-B reagent                 | Chun et al. [25]; Kim et al. [34] |
| Plate (tile)                    | - Weak red cell reactivity to anti-B reagent (mixed field agglutination)| Chun et al. [25]               |
| Tube                            | - Weak serum reactivity to B cells can be enhanced by incubation at room temperature for 15 minutes | Unpublished data              |
| Microcolumn                     | - Medium-sized clumps of agglutinated cells in the upper half of the gel column, can be observed in cell typing (to anti-B) | Chun et al. [25]               |
| Automated microplate            | - Misidentification of cis-A1B3 samples as typical AB can be possible  | Unpublished data              |
| Molecular                       | - Can be only used for known cis-AB alleles                             | Fukumori et al. [29]           |
| AS-PCR/PCR-RFLP                 | - For clinical purpose, sequencing of ABO gene exons (6, 7) are commonly used | Won et al. [28]; Won et al. [30] |
| Sequencing                      | - For research purpose, sequencing of the all of ABO gene coding region (exons 1–7) and regulatory regions is used | Unpublished data              |
| Cloning/allele-separation and sequencing | - Required for novel cis-AB allele study                          | Lee et al. [20]                |
| Next-generation sequencing      | - Required for novel cis-AB allele study                               | Moller et al. [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-A₂B₂, interpreted as typical A₁, 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-A₂B₂ 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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