Accurate cisAB typing is essential to ensure the safety of a transfusion: A case of a cisAB01 neurosurgery pediatric patient and family study

Yanan Zhang1 | Nuochuan Wang1 | Yongji Tian2

Abstract
The rare cisAB subgroups inherited from a single parent are characterized by the activities of dual A and B glycosyltransferases encoded by a gene on one chromosome. The serological complexity of cisAB challenges clinical blood transfusion practice because of misclassification in ABO grouping.

KEYWORDS
cisAB, genotype, phenotype, transfusion practice

1 | INTRODUCTION
Preoperative blood preparation is a necessary procedure before elective neurosurgery if the patient requires blood transfusion during the perioperative period. Among pre-transfusion compatibility tests, an accurate interpretation of ABO group discrepancies is critical to ensure patient transfusion safety in good practices.1 The current perspective of blood typing tests is to reduce missed subgroup detection to avoid incorrect transfusion. Here, we present a case of a pediatric patient with a cisAB blood type undergoing elective neurosurgery and the pedigree of the propositus family.

2 | MATERIALS AND METHODS
2.1 | Patient and family study
The propositus, a 6-year-old girl (height: 128 cm; weight: 25 kg) who lived in Hefei City, Anhui Province, China, presented headaches accompanied by nausea and vomiting due to obvious causes and visited the local hospital. She was subjected to brain magnetic resonance imaging and was suspected of having “lesions of the right frontal lobe and basal ganglia”; surgery was recommended by the local doctor. For further treatment, the propositus was admitted to the Department of Pediatric Neurosurgery,
Beijing Tiantan Hospital, with "intracranial space-occupying lesions."

During pretransfusion compatibility testing for elective neurosurgery, we observed a blood type discrepancy between the forward and reverse results. Because no blood transfusion history existed in the patient's medical history, further molecular tests to resolve the ABO discrepancy were required. Family members, including the propositus's father, mother, grandfather, grandmother, aunt, and brother, were investigated. Written informed consent for ABO genotyping was obtained from the propositus and her family.

2.2 Routine serologic tests

Pretransfusion compatibility testing was performed using the gel card and manual tube methods according to standard methods and procedures described in the National Guide to Clinical Laboratory Procedures and reagent instructions. Briefly, routine ABO serological tests were performed using the gel card method. Discrepancies between forward and reverse grouping required further investigation using the manual tube method. If accurate discrimination was not possible, the sample was sent to the Beijing Red Cross Blood Centre for identification.

The following commercially available reagents were used: RBC antigen typing test using the monoclonal anti-A and -B antisera (Changchun Bode Biotechnology Company), and serum typing test using A1 and B RBCs (Shanghai Hemo-pharmaceutical Biological Company; Jiangsu Libo Pharmaceutical Biotechnology Company). Gel card using DiaClon ABO/D + reverse grouping for patients (DiaMed GmbH).

2.3 ABO genotyping

Samples from the propositus and her family were posted to an immunohematology and genetics reference laboratory (Jiangsu Zhongji Wantai Biomedical Company) to further detect ABO genotyping using the commercially available human red blood cell ABO blood group genotyping kit (fluorescence polymerase chain reaction [PCR] method). Before genotyping, serological tests were performed using anti-A1, anti-A, anti-B (Merck; Jiangsu Zhongji Wantai Biomedical Company), anti-H (Sanquin), anti-A, and anti-B (Immucor) antibodies.

Fluorescence PCR method detection sites included specific primers for the A, A205, B, O (261G deletion), O1, and O2 alleles of the ABO blood group system. The detection principle of this method is that PCR amplifies only alleles complementary to the first base at the 3' end of the primers and does not amplify non-complementary alleles. Melting curve analysis was used to confirm whether the target product has been specifically amplified to determine the allele of ABO.

2.4 Sequencing for exons 6 and 7 of the ABO gene

To identify the ABO gene haplotypes, exons 6 and 7 were analyzed using an ABO gene sequencing ABO exon sequencing kit according to the manufacturer's instructions (Jiangsu Zhongji Wantai Biomedical Company). The nature of the reference bases present at nucleotide positions (nps) 261, 297, 467, 526, 646, 657, 681, 703, 771, 796, 803, 829, and 930 was determined for the specificity of alleles at the ABO locus.

3 RESULTS

3.1 Patient blood management

Preoperative autologous blood donation (PAD) was limited because of the patient's low body weight and worsened state. We prepared two units (140 ml/unit) of type O washed RBCs and 200 ml of type AB fresh frozen plasma (FFP) before surgery. After microscopic total resection of the tumor during neurosurgery, the size was ~8.0 × 7.0 × 7.0 cm, of which the solid part was ~5.0 × 4.0 × 4.0 cm. The operation was uneventful. Intraoperative bleeding was ~100 ml without using blood transfusion. The propositus recovered and was discharged with an ependymoma (right frontal and basal ganglia, WHO grade II–III, focal anaplastic) on postoperative day 19.

3.2 Serologic results

Using the gel agglutination assay, the propositus typed as ABweak for RBCs, while a 2+ anti-B was detected in the serum. The propositus's father typed as A for RBCs and 3+ anti-B for serum. The grandmother typed as A for RBCs and 3+ strong anti-B for serum; the brother typed as ABweak for RBCs and non-anti A and B for serum. The other three members of the propositus's family typed as groups A, O, and A. Thus, the ABweak phenotype of the propositus arose from her father, who carries a cisAB allele from the grandmother. All four cisAB phenotypes showed normal expression of antigen A and weak expression of antigen B on RBCs. The B antigen might even be sufficiently weak to show a negative reaction using the gel card method, such as B antigens of the grandmother and father (Table 1 and Figure 1).
3.3 Genotyping and sequencing results

To confirm the patient’s blood type, genotyping and exons 6 and 7 were analyzed. The genotype of the propositus was A/O1 (Figure 2A), and those of the others were A/A, A/A, and A/O1 (Table 1).

Furthermore, we performed gene sequencing to identify structural changes in the cisAB01 allele using the A101 allele as a reference nucleotide sequence against all other alleles (A102, B101, cisAB01, O01, and O02).3,4 The analyses of exon 6 sequences at nps 261 and 297, and exon 7 at nps 467, 526, 657, 681, 703, 796, 803, 829, and 930 revealed that four members had chimeric structures of the A and B alleles because of B allele specificity at nps 803 (Figure 2B). The thymine (T) residue at nps 467 and cytosine (C) at nps 803 suggested that the cisAB allele arose from both point mutations at nps 467 and nps 803. The final genotypes identified were the propositus and brother with cisAB01/O1 and grandmother and father with cisAB01/A102. Interestingly, the gene sequence at nps 467 was TT homozygous in the father, and that in the others was heterozygous regardless at nps 467 and 803 (Figure 2B).

3.4 Family pedigree

The family described in this study comprises seven individuals, of whom four have the cisAB trait (Figure 3). We confirmed that the cisAB allele was inherited on the same chromosome from the grandmother in this family.

4 DISCUSSION

We identified a case of a cisAB pediatric patient during routine preoperative blood preparation for elective surgery. Because the patient’s individual physical condition limited autologous blood donation management before neurosurgery, we prepared type O washed RBCs and type AB plasma for safe neurosurgery. The operation went well without blood transfusion. Furthermore, family studies revealed three generations in whom the pattern of inheritance could be explained by the inheritance of the cisAB01 genotype.
Figure 2  A, A/O1 genotype of the propositus. The blue box is the internal control; the red box is the positive band. B, Gene sequencing analysis of the propositus and her three family members at nps 467 and 803 of exon 7 of the cisAB allele.
cisAB was first discovered by Seyfried et al.\textsuperscript{5} in 1964 as having an unusual inheritance pattern and was named by Yamaguchi et al.\textsuperscript{6,7} to discriminate this rare phenotype from the transAB phenotype. The cisAB allele, a chimera of normal A and B alleles caused by a gene mutation, encodes a single glycosyltransferase enzyme with dual A and B glycosyltransferase activities. This allele occurs more frequently in the East Asian region than in the rest of the world. The frequency of cisAB was reported to be 0.0354\% in Korean donors,\textsuperscript{8} 0.0012\% in Japanese donors,\textsuperscript{9} and 0.0017\% in Chinese donors.\textsuperscript{10}

The cisAB phenotype is diverse with respect to both antigen and glycosyltransferase expression on RBCs. In previous studies, based on the strength and characteristics of the serologic reactions of RBCs, cisAB was split into three groups: cisA\(_1\)B\(_3\), cisA\(_2\)B\(_3\), and cisA\(_3\)B\(_1\).\textsuperscript{11,12} To date, eleven classification of cisAB alleles presenting various phenotypes of the cisAB blood group have been reported globally in the literature, and seven genotypes of cisAB01 have been reported in Korea\textsuperscript{13} (newly adding the four phenotypes A\(_1\)B\(_{iv}\), A\(_{iv}\)B\(_3\), A\(_{iv}\)B\(_{iv}\), and A\(_1\)). According to the rules of competition between A and B transferases, a strong A transferase may inhibit a weak B transferase so that the expression of B antigen on erythrocytes is not detectable by serological methods in a rare case with the cisAB/A genotype.\textsuperscript{14} For example, A\(_2\)B\(_3\) phenotype RBCs do not type as strongly as transAB RBCs in forward typing using monoclonal reagents but show anti-B agglutination present on reverse typing. In this family, the four cisAB phenotypes had normal expression of antigen A and weak expression of antigen B, in which the expression strength of B antigens in RBCs was proband>brother>grandmother=father. Although both the propositus and younger brother were cisAB/O1, serological manifestations were different. The propositus was anti-B 4+ and B-cell 2+, while the younger brother was anti-B 2+ and B-cell negative. The cisAB serology of the grandmother and father typed cisAB/A102 showed the “A” type (Figure 1). The cisAB/O1 phenotype can be detected by skilled laboratory personnel using several serological methods, but cisAB/A102 is easily misjudged as A type and can miss detection. After introducing an automated ABO grouping technique in a clinical blood bank, manual operations have been substantially reduced. Cases of cisAB samples are mistyped as typical AB or A type during routine serological testing.\textsuperscript{15}

The serologically determined phenotypes have been refined over the years but do not correlate completely with the presence of a single defined allele. By contrast, an increasing number of novel alleles are found within most of the serological groups. Accordingly, various additional ABO genotyping methods may be required to confirm cases of ABO discrepancy to overcome some limitations of the current serological techniques. The serologic and genotypic features of the cisAB subgroup should be highlighted among transfusion service personnel.

Genomics is used as an alternative to serological antibody-based methods to determine blood groups because of single-nucleotide polymorphism changes in the respective genes. Importantly, the ability to test for antigens for which serologic reagents are unavailable is a major medical advance to issue compatible donor units for life-saving purposes.\textsuperscript{16} Yamamoto et al.\textsuperscript{17} demonstrated two nucleotide substitutions (C→T at nps 467 and G→C at nps 803) from two cisAB families. Given this perspective, a 467 C>T polymorphism is present, resulting in a Pro156Leu amino acid mutation, as well as an 803 G>C (Gly268Ala). The former mutation may not adversely affect enzyme function, whereas the latter is critically important. A single point mutation reverses the specificity of human blood group B synthesizing galactosyltransferase. Current serological techniques are insufficient to discriminate changes in these levels of GTB activity in cisAB individuals, leading to discrepancies in serology tests.\textsuperscript{18} This event may lead to misclassification in ABO grouping and subsequent adverse transfusion reactions.

Various approaches for transfusion in cisAB patients are available, such as autologous blood transfusion. PAD is suitable to predict intraoperative bleeding and
patients in good preoperative condition. In another approach, blood from a family member with the same blood type can be used for transfusion after irradiation to prevent transfusion-associated graft-versus-host disease. The Korean National Transfusion Guideline suggests that 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 cisAB blood group.\textsuperscript{13} Transfusion therapy is based on cross-match compatibility, and the agglutination effect of the antibody titer in the donor’s serum against the patient’s RBCs is considered to avoid acute or chronic transfusion reactions.

Among the cisAB blood types reported in the literature, cisA\textsubscript{1}B\textsubscript{3} requires increased attention because it is easily misdiagnosed. Woo et al.\textsuperscript{19} reported a case of cisA\textsubscript{1}B\textsubscript{3} interpreted as typical A; the transfusion of four units of type A RBCs and four units of type A FFP caused delayed transfusion adverse effects. However, cisA\textsubscript{2}B\textsubscript{3} may behave differently. Yoo et al.\textsuperscript{20} reported a transfusion case of type A RBCs, FFP, and platelets to a cisA\textsubscript{2}B\textsubscript{3} boy without any clinically significant transfusion reactions. This finding can be explained by the weak B antigen on his RBCs in forward typing but no anti-A antibodies in his serum in reverse typing, against the transfused A RBCs. Theoretically, type O or A donor RBCs can be used for cisAB patients. The underlying premise is that the existence of anti-B in type O or A blood plasma or platelets may not cause a clinical problem when transfusing into a patient with type cisAB because B antigen expression is often low in cisAB patients. Based on our experience with this cisAB pedigree, the various cisAB phenotypes make rapid blood group determination difficult because of the limitations of serological methodology. Therefore, universal blood, type O washed RBCs and type AB FFP/platelets, may be better choice because they can be safely used for cisAB patients.

With the newly discovered cisAB\textsubscript{09} paired with the B allele reported,\textsuperscript{21} various phenotypes of cisAB cases, such as ABO discrepancies, may be determined in pretransfusion compatibility testing. Because of cisAB serological characteristics, establishing own blood group identification procedures is necessary based on local demographics or the feasibility of laboratory operations to avoid ABO mistyping.

5 | CONCLUSION

Our study support that the four cisAB individuals showed various serological reaction patterns. The cisAB\textsubscript{01}/O1 blood type was characterized by weak agglutination strength of anti-B in forward typing and B cells in reverse typing. The cisAB\textsubscript{01}/A102 blood type was easily misjudged as type A. In the case of ABO discrepancies in serological typing, genotype identification is required. For a patient who cannot wait for the result of genotype identification and requires blood transfusion, type O washed RBCs and type AB platelets/plasma are recommended for transfusion.

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CONFLICTS OF INTEREST

The authors have disclosed no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yanan Zhang: conceiving the study design, analysis of data, drafting and revising the manuscript; Nuochuan Wang: posting specimens for tests and communicating with the patient’s family; Yongji Tian: treating patient and communication with patient and their families.

CONSENT

Written informed consents were obtained from guardians of patient and the privacy of patients was effectively protected. Patient consent has been signed and collected in accordance with the journal’s patient consent policy.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Yanan Zhang  https://orcid.org/0000-0001-7345-5629

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