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SHORT COMMUNICATION

Identify a Novel ABO Allele Similar to *ABO*AW.41* Allele with Additional c. 467C>T

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SUMMARY

Background: **Accurate identification of the ABO blood group is of great significance in ensuring the safety of clinical blood transfusions. Using molecular technologies combined with serological testing helps to identify the new *ABO* variants.**
Methods: **The traditional serological blood type test was performed for a 4-year-old Chinese boy, and the result showed a weaker agglutination with anti-A reagent (3+ strength by microcolumn gel card method; 2+ strength by saline tube method). A molecular genotyping assay was performed to get more information.**
Results: **The ABO gene sequence study indicated two heterozygous nucleotide sites: c.370A>G (p.Lys124Glu) in exon 6 and c.467C>T (p.Pro156Leu) in exon 7, and ABO*B.01 mutations. Further single-strand sequencing analysis showed that the mutation sites were located in gene A. The individual c.467T variant was unique to the ABO*A1.02 allele, while the c.370A>G was not found in the known ABO*A1.02 allele. The individual c.370A>G mutation was characteristic of ABO*AW.41 (previously also known as Ax17), but the ABO*AW.41 allele did not contain the c. 467C>T mutation.**
Conclusions: **A novel A allele with c.370A>G in the *ABO*A102* allele or *ABO*AW.41* with c.467C>T was identified, leading to a weak A phenotype.**
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KEYWORDS

ABO allele, serological test, molecular technology, single-strand sequence

INTRODUCTION

Accurate identification of the ABO blood group is of great significance in ensuring the safety of clinical blood transfusions. ABO variants are usually recognized by apparent discrepancies between the forward and reverse results. With the development of new molecular technologies, ABO genotyping has become widely used to identify ABO variants. Several alleles have been identified in individuals belonging to ABO subgroups worldwide. Here, we describe a novel weak A gene, which has the *ABO*AW.41* allele with variant c.467 C>T in exon 7 or the *ABO*A102* allele with variant c.370A>G in exon 6.

Table 1. Results of serological grouping and ABO gene analysis for the proband.

Method	Forward typing test					Reverse typing test			Phenotype	Genotype
	anti-A	anti-B	anti-AB	anti-A1	anti-H	A1c	Bc	Oc		
Gel card	3+	4+	4+	0	1+	1+	0	0	AwB	AW.41/B.01 with c.467 C>T in gene A; or A1.02/B.01, with c.370A>G in gene A
Tube	2+	4+	4+	0	1+	1+	0	0		

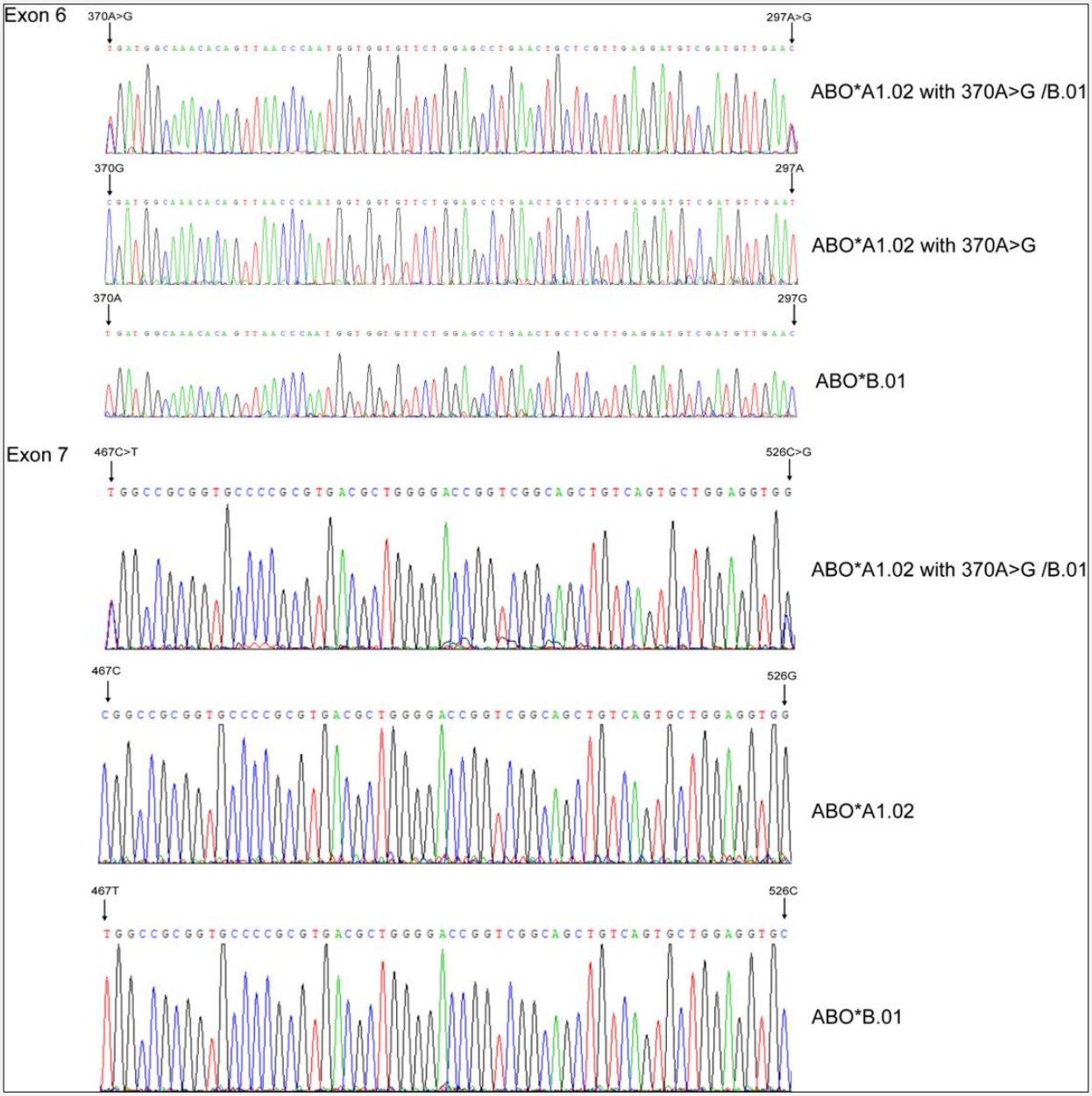

Figure 1. Sequence results of the ABO new allele.

Patient Information

The proband was a 4-year-old Chinese boy, who specifically visited our outpatient clinic for blood type testing, due to difficulty in blood typing during a routine physical examination in an external hospital. The patient has no history of hematologic disorders, his family and psychosocial history is not clear.

Methods

Routine serological tests were conducted by automatic blood group machines (AutoVue; Grifols). The conventional ABO manual tube method was performed if discrepancies were observed. Anti-A (clones; combined 9113D10 and SRBC-B3), anti-B (clones; combined 9621A8 and SRBC-C1), anti-AB (clones; combined 9113D10 and 152D12), and anti-H (clone; H5B12) antibodies (Shanghai Hemo-pharmaceutical Biological Company, Shanghai, China) were used to determine antigens in the assays. Reverse typing was performed using A1, B, and O RBCs (ABO RBC Reagent Kit; Shanghai Hemo-pharmaceutical and Biological Inc.). Genomic DNA was extracted using a blood DNA kit (TIANamp Blood DNA Kit, Tiangen Biotech Co.). Seven ABO exons were amplified and directly sequenced using the method reported by Zhu et al. [1]. A novel ABO allele was confirmed based on cDNA fragments amplified by ABO allele-specific primer sequencing. The patient provided written informed consent before enrollment. This study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (2020051).

RESULTS AND DISCUSSION

During ABO phenotyping, the proband's erythrocytes showed weaker strength agglutination with anti-A reagent (3+ strength by microcolumn gel card method, 2+ strength by saline tube method), 4+ strength with anti-B reagent, and 1+ strength with anti-H reagent and did not react with anti-A1 reagent. The proband's plasma showed 1+ agglutination with standard A1 cells and did not react with standard B and O cells. Therefore, the proband was assigned to the AwB subtype (Table 1).

The proband's ABO sequencing data were compared with those of the ABO reference sequence in the NCBI database. However, the sequencing results did not fully match any known ABO allele combinations by direct sequencing. The proband had two heterozygous nucleotide sites: c.370A>G (p.Lys124Glu) and c.467C>T (p.Pro156Leu), and *ABO*B.01* mutations. Further single-strand sequencing analysis showed that the mutation sites were located in gene A (Figure 1). The individual c.467T variant was unique to the *ABO*A1.02* allele, while the c.370A>G was not found in the known *ABO*A1.02* allele [2]. The individual c.370A>G mutation was the characteristic of *ABO*AW.41* (previously also known as Ax17, its GenBank number is GQ229192) [3], but the *ABO*AW.41* allele did not contain the c.467C>T mutation. Therefore, this genotype could not be defined. The nucleotide sequence of this new ABO allele has been submitted to the GenBank with Accession Number OR823847. Regrettably, we were unable to track down the specimens of the parents for testing. Overall, a novel A allele with c.370A>G in the *ABO*A102* allele or *ABO*AW.41* with c.467C>T was identified, leading to a weak A phenotype. Because the patient has developed anti-A antibodies (3+ strength with A1 reagent cells), it is recommended to use O-type washed red blood cells and AB-type plasma for transfusion. Single-strand sequencing technology plays a significant role in the identification of challenging ABO genes.

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Patient Consent:

Written informed consent was obtained from the patient to publish.

Declaration of Interest:

None of the authors have any conflict of interest in this study.

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