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Original Article

Serological and genetic analysis of a rare *CisAB01*/*O01* blood group

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ABSTRACT

The paper aims to analyze a rare blood sample in Ganzhou City Hospital with CisAB subtype and explore a feasible pattern for blood typing of rare blood type patients, so as to ensure clinical transfusion safety. The routine serological methods were used for ABO forward and reverse blood typing and the fluorescence real-time PCR technique was used for sample genotyping. A human ABO blood group 6-7 exon sequencing kit was used for sequence analysis. The nucleic acid sequence of the sample was compared with reference sequences. The forward typing results demonstrated that the sample was ABw, RhD positive. The sample exhibited 4+ agglutination with anti-H and anti-AB antibodies. Reverse typing by microcolumn gel method showed an AB result, but the serum sample demonstrated weak agglutination with B cell under room temperature, 4 °C and 37 °C in saline when tested with tube method respectively. The serological results matched with the A_2B_3 serotype. The fluorescent real-time PCR genotyping results displayed *A*/*O01*. The sequence analysis demonstrated deletion of guanine in 261-position 467C>T (heterozygote) and 803G>T (heterozygote) mutation respectively. The mutation caused the A glycosyltransferase peptide chain to change from proline to leucine (P156L) at 156 and from glutamate to alanine (G268A) at 268. The result demonstrated that the sample's genotype was *CisAB01*/*O01*. The mutation of glycosyltransferase coding gene leads to an abnormal serological reaction pattern. Only by combining the results of genetic analysis can we get the true sample blood type and better ensure the safety of clinical blood transfusion.

Keywords: CisAB, serological blood typing, genetic typing, sequencing

INTRODUCTION

ABO is the most important blood group system of human red blood cells, playing a primary role in ensuring the safety of clinical blood transfusion. According to the types of erythrocyte surface antigens, the phenotypes of ABO blood group system can be divided into four types: A, B, O and AB. Due to the possible mutation of each antigen coding gene, in practice, some subtypes and variants also come about. CisAB is an ABO subtype that exists at very low frequency in the world population. Its characteristic is that the coding product of the same allele has the activity of both A and B glycosyltransferase[1], which makes blood typing difficult. The serological and genetic analyses of a case of CisAB blood group

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in Ganzhou City Hospital was reported as follows.

MATERIALS AND METHODS

Patient sample

A 36-year-old male patient with cholelithiasis was admitted for surgery at the Hepatobiliary Department of Ganzhou City Hospital. The patient's EDTA anticoagulation blood sample was used for blood typing and blood preparation for selective surgery. The study was approved by the Ethics Committee of Ganzhou City Hospital.

Routine serological blood typing

The main reagents included anti-A, anti-B monoclonal antibodies (Millipore, USA); A and B reverse blood typing red cells (Jiangsu LIBO Medicine Biotechnology Co., Ltd. China); anti-A_1, Anti-AB, anti-H antibodies (Sanquin, Netherlands); anti-D monoclonal antibodies (Immucor, USA); ABO, RhD microcolumn gel blood group identification card, ABO microcolumn gel reverse type card (Jiangsu LIBO Medicine Biotechnology Co., Ltd. China). The serological operation was carried out according to National Procedures for Clinical Transfusion Testing.

ABO genotyping and sequencing

ABO genotyping and sequencing were carried out according to the manufacturer's instructions. ABO genotyping kit (Fluorescent real-time PCR method, Batch No. 20190426, Jiangsu ZOJIWAT Biomedical Co., Ltd. China) was used for ABO genotyping and sequencing. A nucleic acid extraction kit was used for nucleic acid extraction (Batch No. C011806R0, Dobe Qi Bio Technology (Xiamen) Co., Ltd. China).

PCR amplification

PCR amplification was performed at 95 °C for 20 s, 68 °C for 1min, 96 °C for 20 s, 66 °C for 50 s, 72 °C for 45 s, 96 °C for 20 s, 63 °C for 50 s and 72 °C for 45 s. The reaction was terminated at 72 °C for 2 min. The results were confirmed by the range of positive and negative Tm values.

Sequence analysis

Sequence analysis and sequencing of the coding gene were carried out in strict accordance with the instructions of human red blood cell ABO blood group exon 6 and exon 7 gene sequencing kit. The sequence primers were I6F (GTTCCCGCAGGTCC-AATGT) and I6R (GCTGCATGAATGACCTTTCC) for exon 6 sequencing, and E7F (TCTGCTGCTCTRA-GCCTTCC) and E7R (CTGCTAAAACCAAGGGCG) for exon 7 sequencing.

RESULTS

Serological blood typing results

The sample's forward serological result was AB_W, RhD positive, while the reverse serological result was AB by microcolumn gel method and saline method. The anti-B antibody in the sample's serum could be detected by the saline method under different temperature conditions, however the positive reaction was very weak, indicating that there was abnormal expression of B antigen in the red blood cells. The pattern of serological reaction was similar to that of A_2B_3 serotype (***Table 1***).

Table 1 **Serological test results of *CisAB01/O01***

		Forward typing								Reverse typing			
		Anti-A	Anti-B	Anti-D	Ctl	Anti-AB	Anti-A_1	Anti-H	Anti-H (OC control)	Ac	Bc	Oc	Self control
Microcolumn gel method		4+	1+	4+	0	/	/	/	/	0	0	/	0
Saline method	RT	4+	1+	4+	0	4+	0	4+	4+	0	1+	0	0
	4 ℃	4+	1+	4+	0	4+	0	4+	4+	0	1+	0	0
	37 ℃	4+	1+	4+	0	4+	0	4+	4+	0	1+	0	0

RT = Room temperature.

Genotyping results

The genotyping results showed that the sample was *A*/*O01* (***Fig. 1***).

Sequencing results

The sequencing results showed the deletion of coding gene at position 261 caused frameshift mutation. The two point mutations, C>T heterozygous mutation at 467 and G>T heterozygous mutation at 803 were also detected. The mutation caused a glycosyl-transferase peptide chain change from proline to leucine (P156L) at position 156, and glutamic to alanine (G268A) at position 268, which was a typical *CisAB01*. The other allele was *O01*, and the sample's genotype was *CisAB01*/*O01* (***Fig. 2***).

Comparison of sample sequencing results and reference sequences

The sample's exon 6 and exon 7 sequencing results

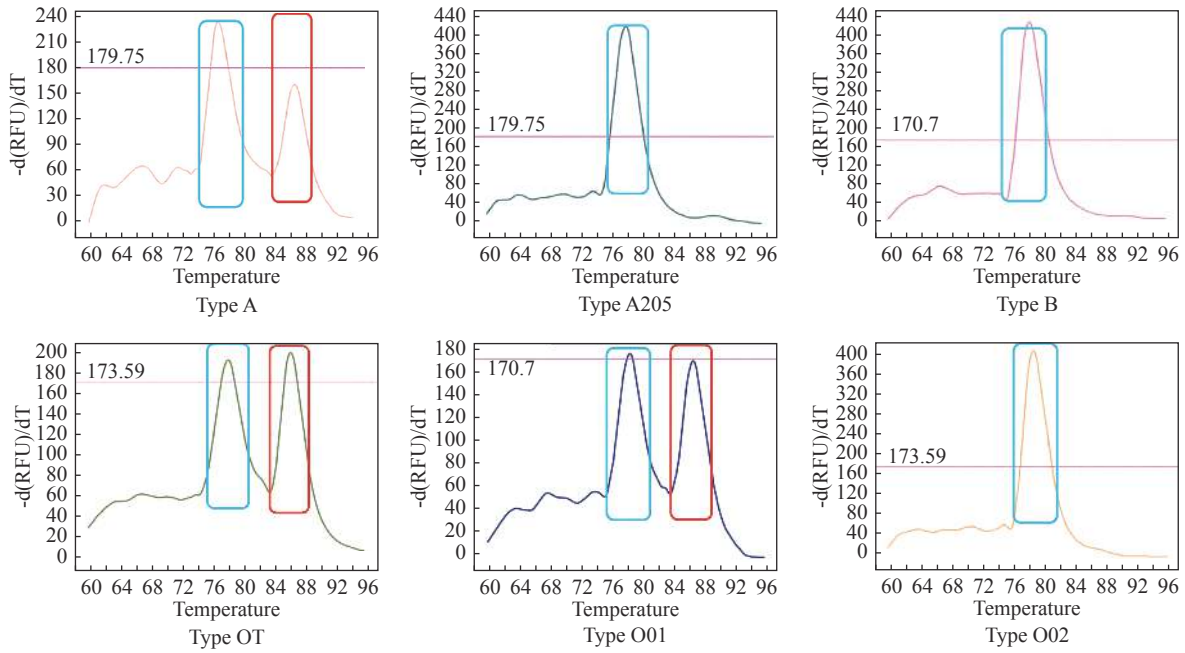

Fig. 1 **Genotyping results.** The blue box represents the internal control fluorescence peak, while the red box shows the fluorescence peak of the tested sample.

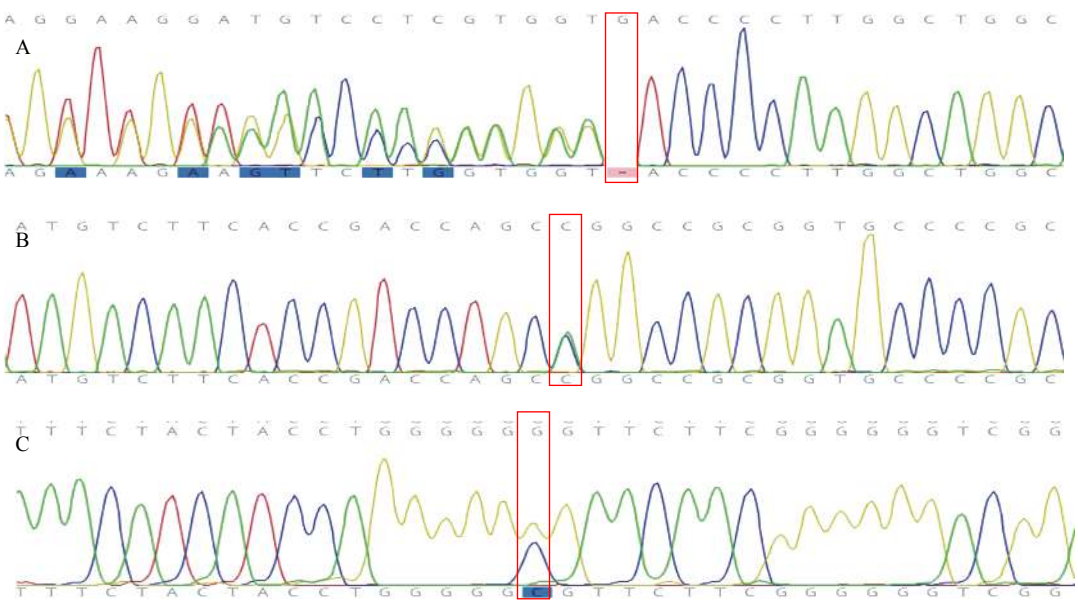

Fig. 2 **Sample sequencing results.** A: Deletion of a 261 lead to frame shift mutation. B: 467 C>T heterozygous mutation. C: 803 G>T heterozygous mutation.

were compared with reference sequences. The results showed that the sample had a typical *CisAB01*/*O01* mutation sequence (***Table 2***).

DISCUSSION

Until present, 43 blood group systems and more than 370 blood antigens have been identified. Among these blood group systems, the ABO blood group system is the most important for clinical transfusion. Accurate ABO blood group typing is of primary importance during pre-transfusion testing[2]. The distribution of ABO blood types shows different distribution characteristics among different ethnicities. The ratio of the ABO subgroup is about 0.047% in Chinese[2]. The identification of ABO subgroups

Table 2 **Comparison of sample sequencing results and reference sequences**

Nucleotide position	Exon 6		Exon 7										
	261	297	467	526	646	657	681	703	771	796	803	829	930
A101(AJ920329.1)	G	A	C	C	T	C	G	G	C	C	G	G	G
A102(AF134413.1)	-	-	T	-	-	-	-	-	-	-	-	-	-
B101(AF134414.1)	-	G	-	G	-	T	-	A	-	A	C	-	A
O01(AF134415.1)	del	-	-	-	-	-	-	-	-	-	-	-	-
O02(AF134416.1)	del	G	-	-	A	-	A	-	T	-	-	A	-
CisAB01	-	-	T	-	-	-	-	-	-	-	C	-	-
S*	del/G	AA	CT	CC	TT	CC	GG	GG	CC	CC	CG	GG	GG
S**CisAB01*	-	-	T	-	-	-	-	-	-	-	C	-	-
S**O01*	del	-	-	-	-	-	-	-	-	-	-	-	-

"S*" stands for sample ABO gene; "S**CisAB01*" stands for one of the sample ABO genes (*CisAB01* haplotype); "S**O01*" stands for the other ABO gene of the sample (*O01* haplotype); "del" stands for missing, and "-" represents consistency with *A101*.

requires additional experiments to assist in the interpretation[3], as forward and reverse ABO typing are inconsistent among ABO subgroup samples[4]. Therefore, genetic analyses of ABO coding genes, as well as molecular typing are required when ABO subgroups are encountered, where serological typing could not determine the sample blood type.

CisAB was first reported in 1964. In the early 1980s, Japanese scholars proposed two possible CisAB mechanisms. One was the unequal exchange of chromosomes, while the other was a point mutation in the coding gene[5−6]. More than ten years later, it was confirmed that there were 467 C>T and 803 G>T mutations in the CisAB blood group coding gene[7]. In 2003, Xu *et al*. reported for the first time the molecular mechanism of CisAB in the Chinese population[8]. As *A101* and *B101* are highly homologous, there are only seven nucleotide differences in the coding region between *A101* and *B101*. Among them, 297A>G, 657C>T, and 930G>A are synonymous mutations, only 526C>G, 703G>A, 796C>A, and 803G>C cause the substitution of four amino acids (R176G, G235S, L266M, and G268A). Seto *et al*. labeled *A101* containing 526C, 703G, 796C, and 803G as AAAA, and *B101* containing 526G, 703A, 796A, and 803C as BBBB. Different clones of ABBB, AABB and AAAB were constructed, the glycosyl-transferases encoded by them were expressed and purified in *E. coli*, and their catalytic activity was determined *in vitro*. It was confirmed that these four coding genes play a pivotal role in the determination of glycosyltransferase activity[9−10]. Until now, there are 11 *CisAB* alleles named by ISBT. A large sample survey conducted in Gwangju revealed that the gene frequency of *CisAB* in the South Korea population was 0.0354%, while in Japanese it was 0.0012%[11]. A large sample survey of *CisAB* gene frequency in China has only been found in blood donors in Shanghai[12]. The results showed that the gene frequencies of *CisAB01*, *CisAB02* and *CisAB06* were 0.66×10^5, 0.12×10^5 and 0.06×10^5 respectively. The remaining reports have mostly focused on individual cases. In 2003, there was a family investigation of seven cases, which were serologically typed as CisAB. Unfortunately, no further genetic analysis was carried out, which affected the reliability of the results[13]. Another analysis involving 10 samples from one family showed that 4 of them were *CisAB* genotypes, which proved that *CisAB* gene has stable genetic characteristics[14]. In an investigation of 12 Chinese CisAB samples, the researchers found that *CisAB01* accounted for 66.7% of the total samples, and confirmed the existence of *CisAB05* which retained the 803G locus of *A101* in the background of *B101*[15]. The analysis of the *CisAB01* intron sequence showed that CisAB01 was not caused by simple point mutation on the genetic background of *A101*, and two homozygotes of *CisAB01* were found in 12 cases of CisAB variant. It is worth mentioning that there is heterogeneity of serological phenotypes in CisAB. The same genotype may produce different serological phenotypes. Similarly, the internal molecular mechanism of the same serological phenotype may be different. Therefore, it is particularly important to classify samples on serotype in combination with genotype.

Here, we reported a case of *CisAB01*/*O01* confirmed by sequence analysis. One of the proband's ABO blood group system genes was *CisAB01*, while the other was *O01*. The serological phenotype of CisAB is different between different combinations of genes. For example, *CisAB01*/*O01*(*O02*) often displays A_2B_3 or A_2B_w phenotype, and *CisAB01*/*A101*(*A102*) often shows A_1 phenotype. Here, the proband showed A_2B_3 phenotype with discrepancy between forward and reverse blood group determination.

Although the frequency of CisAB in the population is very low, once present, it has the potential to cause a good deal of difficulty for blood group identification and cross matching. Therefore, clinicians should be aware of its potential implications for clinical blood transfusion. For the samples with similar CisAB reaction pattern in serological test, the gene typing and sequencing analysis should be carried out as far as possible, so as to better ensure clinical blood transfusion safety.

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