An Identification of B(A)02 subgroup blood

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Abstract: Objective To explore the necessity and feasibility of RBC blood typing by molecular technique for unidentified blood group. Methods The blood group was identified by serological test and molecular detection. Results One case of blood donor showed weakly positive reaction to anti -A, strongly positive reaction to anti-B and anti -H, weakly positive reaction to A 1 RBCs but no reaction to either B or O blood type. The sample was subjected to the detection of ABO genes and the results showed 9 mutants in Exons 6 and 7. The type B(A)02 was identified when comp a red with A101 allele. Conclusion Genetic detection is helpful for finding B(A) subgroup blood that cannot be identified by serological test.

Keywords: ABO subtypes, B (A) blood group, Alleles, Blood group genotyping

1. Introduction

Blood type identification is an essential part of clinical blood transfusion. All patients undergo blood type testing upon hospital admission. For critically ill patients in emergency rescue who need blood transfusion, the misidentification of ABO blood types and serious hemolytic transfusion reactions caused by blood type or subtype incompatibility have a high mortality rate, which has attracted the attention of clinical doctors and blood transfusion workers [1-5]. In routine work, the author found one case of a blood donor who had donated twice with inconsistent serological ABO forward and reverse typing. Molecular biology experiments were used for genetic typing and DNA sequencing verification, and the report is as follows.

2. Materials and methods

2.1. Sample source: blood donor

Male, 22 years old, born in June 1993, of Han ethnicity, with no history of blood transfusion, and meets the physical examination standards for blood donors. Due to limited conditions, no family investigation was conducted.

2.2. Main instruments and reagents

Main reagents include: Anti-A, Anti-B monoclonal serum (Changchun Bode Company, batch number 20140818); Anti-H monoclonal serum (Jiangyin Libo Medical Biotechnology Company, batch number 20140524); Microcolumn gel blood typing cards and cross-matching cards (Diana Company, batch number 15021.01); Coombs' reagent (Zhongshan Sheng Ke Reagent Co., Ltd., batch number 20150525); ABO reverse typing red blood cell reagents from our center

Instruments include: Fully automatic blood grouping and cross-matching system (GRIFOLS, made in Spain); Blood type-specific centrifuge (Taiwan Beso); PCR amplification instrument (BIO-RAD T100 Thermal Cycler); Benchtop high-speed centrifuge (Eppendorf Company); Gel imaging system (Tocan Ling Cheng Fully Automatic Gel Imaging System); Electrophoresis apparatus (BIO-RAD); Electrophoresis tank (BIO-RAD)

2.3. Blood group serological testing

Blood group identification was performed using the tube method, direct and indirect anti-human globulin tests, and antibody screening. Standard serological methods for blood grouping were followed [6].

https://doi.org/10.62852/csa/2025/111

2.4. Genomic DNA extraction

The TIA Namp Blood DNA Kit (Beijing Tian gen Biochemical Co., Ltd.) was used to extract genomic DNA from blood, strictly following the manufacturer's instructions.

2.5. Analysis of the sequences of exons 6 and 7 of the ABO gene

The primer sequences are as follows:6F: 5'-CGGGATCC-AGGGTGGCACCC-TGCC-3', 6R: 5'-CGGAATTCACTCGCCAC-TGCCTGGGTCTC-3';7F:5'-CGGGATCCCC-GTCCGCCTGCCTTGCAG-3', 7R: 5'-GGGCC-TAGGCTTCAGTTACTC-3'.Using the extracted genomic DNA as a template, the PCR amplification reaction has a total volume of 25μl, with the following components: LA Taq polymerase from Takara Bio (Dalian) (10mmol/L) 0.2μl,10× buffer 2.5μl,10mmol/L dNTP 2μl,Forward and reverse primers (10μmol/L) each 1μl,DNA template 1μl,H_2O 17.3μl.The amplification conditions are: 95℃ for 5 minutes, followed by 95℃ denaturation for 30 seconds; 62℃ annealing for 30 seconds, 72℃ extension for 45 seconds, for 35 cycles; and a final extension at 72℃ for 5 minutes. The PCR products were sent to Shanghai Sheng gong Biological Engineering Co., Ltd. for sequencing. The sequencing primers are the same as the primers used for amplifying exons 6 and 7 of the ABO gene, with both forward and reverse sequencing.

3. Results

3.1. Serological blood grouping results

The reaction pattern of this sample: Red blood cells showed weak agglutination (2+) with monoclonal anti-A serum, strong agglutination (4+) with monoclonal anti-B serum, no agglutination with anti-A1 serum, and strong agglutination (4+) with anti-H serum; the serum showed weak agglutination (2+) with Alc, and no agglutination with B c, O c, or autologous cells (Table 1).

Table 1: Serological ABO Forward and Reverse Typing Reaction Patterns of the Sample

Reagent	ABO Forward Typing				ABO Reverse Typing			
	Anti-A	Anti-B	Anti-A1	Anti-H	A1c	B c	O c	Autocontrol
Result	2+	4+	θ	4+	2+	θ	θ	θ

3.2. Molecular biology identification results

Sequencing of the donor's ABO gene exons 6 and 7 was performed using A101 as the standard for sequence comparison. Base point mutations were found, and the genotype was ultimately determined to be B(A)02. The polymorphic sites identified were c.297A>G in exon 6 and c.526C>G, c.657C>T, c.700C>G, c.703G>A, c.796C>A, c.803G>C, c.930G>A, and c.1096G>A in exon 7 (Figure 1). There are six reported B(A) subtypes, among which the B(A)02 allele is characterized by a single missense mutation at nt700, and the other four sites exhibit characteristics of the B allele. Therefore, the proband's genotype is determined as B(A)02 blood type.

4. Discussion

The human ABO gene is located at 9q34.1-9q34.2 and consists of 7 exons, spanning a genomic DNA length of 18kb. The size of the exons ranges from 28 to 691bp, with the majority of the coding sequences located in exons 6 and 7. The A and B genes encode α (1,3) N-acetylgalactosaminyltransferase and D-galactosyltransferase, respectively, both composed of 353 amino acids, with a molecular weight of approximately 46Kd. The single and multiple nucleotide sequences of each ABO subtype are based on the alleles, with one or more nucleotides mutated, leading to changes in the activity of the glycosyltransferase. This results in alterations in the quality or quantity of A or B antigens expressed on the surface of red blood cells, ultimately causing discrepancies in serological forward and reverse blood typing [7,8]. The B(A) blood type is a rare ABO subtype, with an occurrence frequency of only 0.005% in the population, first

https://doi.org/10.62852/csa/2025/111

discovered in 1985, and first reported in China in 2004 with genetic blood type testing [9]. From a serological perspective, B(A) red blood cells contain a small amount of A antigen activity and nearly normal B antigen specificity, with H antigen content close to that of O-type cells, significantly higher than that of normal B-type cells; whereas B(A) serum contains anti-A, which can agglutinate A1 cells and some A2 cells. It is inferred that the mechanism of B(A) formation may be a single base mutation in the B allele on the basis of the normal B gene sequence. The mutated B gene may have the ability to encode a bifunctional active enzyme, hence, in addition to exhibiting B antigen specificity, it also shows a small amount of A antigen specificity [10,11].

5. Discussion

There are currently six reported subtypes of the B(A) blood group, namely B(A)01, B(A)02, B(A)03, B(A)04, B(A)05, and B(A)06. In China, four B(A) alleles have been reported: B(A)02, B(A)04, B(A)05, and B(A)06, with B(A)02 and B(A)04 being the predominant alleles [12-14]. Compared to the B101 allele sequence, the B(A)02 allele has a mutation at position 700C>G in the exon 7 region, which causes the proline at position 234 to mutate to alanine, thereby affecting the spatial conformation of the important residue for blood type identification, such as histidine at position 233, methionine at position 266, and alanine at position 268. This mutation leads to changes in the amino acid structure, thus altering the specificity of ABO-related enzymes [12-14]. The rare and difficult blood types detected through routine serological tests and the related antibodies encountered in blood cross-matching can be identified at the molecular level by designing primer sequences for different identification purposes. This not only saves costs and improves accuracy but also makes the primer sequences easy to prepare and store. It solves the problems of limited sources, high prices, low titers, and short shelf lives of monoclonal/polyclonal sera used for detecting high-frequency antigens and antibodies. Moreover, molecular biology methods are not affected by autoantibodies, irregular antibodies, and the diseases the tested individuals may have [15].

In this study, a blood donor who donated twice was found to have inconsistent results between the ABO forward and reverse typing upon reconfirmation compared to the initial B type identification. The reason was that the monoclonal anti-A serum used in the first test did not cover certain sites recognized by high-titer monoclonal anti-A (clone MHO4), hence no agglutination occurred, and it was identified as B type [13]. The second blood type identification revealed a discrepancy between forward and reverse typing (the monoclonal antibody serum used this time and the one used in the first identification were from the same company but different batches). Through molecular biology methods, the donor's ABO gene exons 6 and 7 were sequenced, and by comparing the sequence with A101 as the standard, base point mutations were found, ultimately determining the genotype as B(A)02.

https://doi.org/10.62852/csa/2025/111

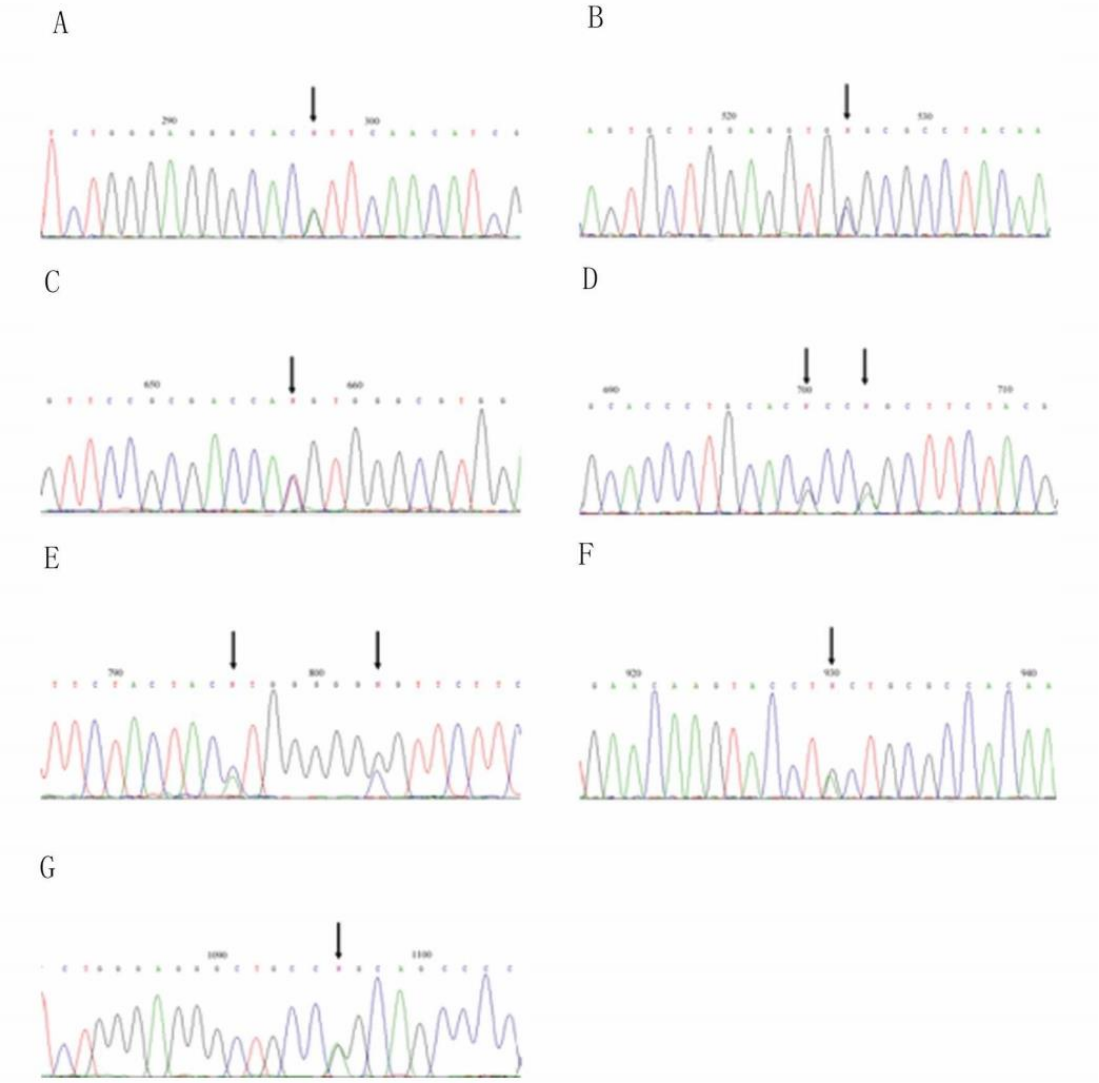

Figure 1: Sequencing of PCR Products for Exons 6 and 7 of the A B O Gene

6. Conclusion

In summary, in clinical identification, serological preliminary identification serves as the routine method for clinical blood transfusion and forms the foundation for molecular biology. Molecular biology provides the basis for serological identification; the two are not mutually exclusive but rather complementary and indispensable. This experiment first used serological methods for preliminary identification and combined it with molecular biology identification results to make the identification more accurate.

(Thanks to Professor Xiang Dong and Professor Jin Sha from the Blood Group Reference Laboratory of Shanghai Blood Center, and other teachers for their guidance and assistance.)

7. References

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https://doi.org/10.62852/csa/2025/111