MEDICAL SCIENCE MONITOR

MEDICAL BIOCHEMISTRY

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21: 2781-2785
DOI: 10.12659/MSM.894021

Received: 2015.03.04
Accepted: 2015.04.25
Published: 2015.09.18

# Genetic Sequencing Analysis of A307 Subgroup of ABO Blood Group

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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**Source of support:** Departmental sources

**Background:** The aim of this study was to investigate the serology and gene sequence characteristics of the A307 subgroup of the ABO blood group.

**Material/Methods:** Monoclonal anti-A and anti-B antibodies were used to detect the ABO antigens of a proband whose positive blood type was not consistent with the negative blood type of the ABO blood group. Standard A-, B-, and O-negative typing cells were used to test for ABO antibodies in the serum. Additionally, polymerase chain reaction with sequence-specific primer (PCR-SSP) was used to confirm the genotype, and subsequently, exons 6 and 7 of the ABO gene were detected by gene sequencing. Samples from the wife and daughters of the proband were also used for serological and genetic testing.

**Results:** Red blood cells of the proband showed weak agglutination reaction with anti-A antibody, while anti-B antibody was detected in the serum. Moreover, PCR-SSP detected A307 and O02 alleles, while gene sequencing revealed mutation of c.745C>T in exon 7, which produced a polypeptide chain p.R249W. The A307 gene of the proband was not inherited by his daughters.

**Conclusions:** A mutation (c.745 C>T) in exon 7 of the ABO blood group gene resulted in low activity of α-1,3-*N*-acetyl-galactosaminyl transferase, producing A3 phenotype.

**MeSH Keywords:** ABO Blood-Group System • Receptor, Adenosine A3 • Sequence Analysis, Protein

**Full-text PDF:** http://www.medscimonit.com/abstract/index/idArt/894021







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## Background

The A allele encodes glycosyltransferase that binds to α-N-acetylgalactosamine at the d-galactose end of the H antigen, thus producing the A antigen. The glycosyltransferase gene determining the ABO blood type is located on chromosome 9, position 9q34.1-q34.2, consisting of 7 exons and 6 introns [1]. Exons 6 and 7 encode the catalytic domain of ABO glycosyltransferase. Gene encoding domains of A101, B101, and O01 are highly conserved, with up to 99% homology [2,3], and the gene was the common ABO allele in human. Differences in only a few bases leads to different antigen specificity of the ABO blood group system, wherein A differs from B blood type by virtue of 6 single-nucleotide transitions (467C>T, 526C>G, 657C>T, 703G>A, 796C>A, and 803G>C) in exon 7. In addition, arginine, glycine, leucine, and glycine in the A allele are determined by 526C, 703G, 796C, and 803G in exon 7 and important factors contributing to α-1,3-*N*-acetyl-galactosaminyl transferase to produce the A antigen [4]. Furthermore, 4 bases also form the basis of the A antigen activity. Usually, glycosyltransferase activity is affected by a change in the glycosyltransferase gene, which results in a weak A antigen. With the development of genetic techniques, molecular basis of subgroups has gained popularity among researchers [5–8]. For example, isoforms such as A2, A3, Am, Ax, Ael, Aint, B3, Bm, Bx, CisAB, and B(A) exhibited the positive and negative stereotyping inconsistency serologically, which often presented difficulties in blood typing and cross-matching of clinical transfusion, and even caused the hemolytic transfusion reactions. In this study, we investigated the serology and gene sequencing characteristics of the ABO blood grouping system and found a new ABO blood type subgroup, A307, using polymerase chain reaction with sequence-specific primer (PCR-SSP) and gene sequencing methods.

## Material and Methods

### Subject

The proband, male, aged 57 years old, was admitted to our hospital for treatment of a tibial plateau fracture. He had no history of blood transfusion, hepatitis, renal disease, or drug allergy. His red blood cell count was $4.23\times10^{12}$/L, hemoglobin level was 120.0 g/L, hematocrit was 0.38, and platelet count was $149\times10^{5}$/L. ABO typing unconformity was identified by right and inverse blood grouping methods; therefore, further study was performed. Blood specimens from the wife and daughters of the proband were collected and analyzed. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Wenzhou Medical University. Written informed consent was obtained from all participants.

Right and inverse ABO blood grouping was performed using the tube method. Monoclonal anti-A and anti-B antibody reagents were purchased from Changchun Institute of Biological products Co., Ltd. and Shanghai Hemo-Pharmaceutical & Biological Co., Ltd. Anti-A1 and anti-H reagents were purchased from Shanghai Hemo-Pharmaceutical & Biological Co., Ltd. Negative cells of ABO blood types were obtained from our lab, which contained mixed cells from more than 3 individuals with the same ABO blood type. The DNA extraction kit was obtained from Shanghai Generay Bio-tech Co., Ltd, and A-subgroup genotyping kit was purchased from Tianjin Super Biotechnology Developing Co., Ltd.

### PCR-SSP assay

The PCR-SSP A subtype blood genotyping kit (Tianjin Super Biotechnology Developing Co., Ltd.) was used with human growth hormone gene as the internal reference control. The kit had a total of 46 wells, with the primers coated in the well, and the volume of amplification reaction system in each well was 25 μl, which contained 2 μl 10×PCR buffer (Shanghai Generay biological Engineering Co., Ltd.), 80–100 ng sample DNA, and 0.8U LA TaqDNA polymerase (Dalian Takara company). The final concentrations of dNTP and MgCl2 were 0.2 mmol/L and 2.5mmol/L, respectively (Shanghai Generay biological Engineering Co., Ltd.). The PCR amplification instrument was a US ABI's 9700. The PCR conditions included initial denaturation at 96°C for 5 min, 30 cycles of denaturation at 96°C for 40 s, annealing at 62°C for 50 s, and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. The PCR products were cooled at 10°C and separated by 2.5% agarose gel electrophoresis for 15 to 20 min. Electrophoresis was continued until the control band separated completely from the positive band, and results were observed under an ultra-violet imaging system. The results were compared with the A subtype blood genotyping kit to confirm the genotype.

### Sequencing analysis of exons 6 and 7 of ABO gene

In brief, the procedure was the following. 1) PCR amplification: PCR primers were designed using the Oligo6 software according to sequence information from NCBI GenBank. The whole length of the amplified product was about 2749 bp, including exon 6, intron 6, and exon 7. The E67 forward primer used was 5'-ctcaaggggctgttctgaag-3' and the reverse primer used was 5'-gcgattgcgtgtctgtgtat-3'. The total volume of the amplification reaction was 50 μl, and the PCR conditions used were as described above. 2) Purification of the PCR products: 1 U alkaline phosphatase (Promega, USA) and 10 U excision enzyme (TaKaRa Company, Dalian) was added into the PCR tubes to remove the extra PCR primers and dNTPs. Next, the thermal cycle program of the PCR auto-amplification analyzer (ABI9700, USA) was performed as follows: 37°C for 30 min





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**Table 1.** Serological result of ABO blood type of proband.

| Temperature | Right blood typing | | | | Negative blood typing | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Anti-A | Anti-$A_1$ | Anti-B | Anti-H | $A_1$ cell | $A_2$ cell | B cell | O cell | Own cell |
| 4°C | mf | 0 | 0 | 4+ | 0 | 0 | 4+ | 0 | 0 |
| RT | mf | 0 | 0 | 4+ | 0 | 0 | 4+ | 0 | 0 |
| 37°C | mf | 0 | 0 | 4+ | 0 | 0 | 4+ | 0 | 0 |

Agglutinated grades were gradually increased from (+) to (4+); mf presented agglutination in mixed field.

and then 80°C for 15 min, after which it was cooled to 4°C. 3) Sequencing: PCR products were sequenced using the BigDye sequencing kit (ABI Company, USA) as per the manufacturer's instructions. The A101 allele sequence (AF134412, Genbank) was used as the template to confirm the genotype.

## Results

### Serological results of the ABO blood typing of the proband

Red blood cells of the proband showed weak A antigen and agglutinated anti-H but not anti-A1, and even presented mixed agglutination reaction during microscopic observations (Table 1). Therefore, we considered it as the A3 subgroup of ABO blood type, but this required further analysis at the genetic level.

### Gene typing results of A subgroup

The results of gel electrophoresis showed that the 46$^{th}$ negative control well exhibited the positive band of internal control. The 15$^{th}$,31$^{st}$,42$^{nd}$ and 45$^{th}$ well exhibited the positive results, compared with the A subtype genotyping assay kit. The above genes were A307, Ax07, A/O and O02 gene. The 31$^{st}$ well would often have a false-positive result; therefore, the Ax07 allele was not considered temporarily. The genotyping results revealed that the proband might have A307 and O02 alleles (Figure 1), which still needed to be confirmed by the gene sequencing results.

### Sequence analysis of ABO gene

Direct sequences of exon 6 and 7 showed 261delG, 297A>G, 646T>A, 681G>A, 771C>T, 829G>A, 745C>T, and 467C>T, where exon 7 had mutation at 745 C>T to cause a substitution of arginine > tryptophan at position 249 (Figure 2). This point mutation was in accordance with A307 characteristics [9] and the other allele of the ABO blood type of the proband was O*02* (Figure 3); therefore, we confirmed that the genotype of the proband was A307/O*02*. This figure shows sequencing of exon 7 of ABO genes; the arrow indicates the point mutation (c.745C>T).

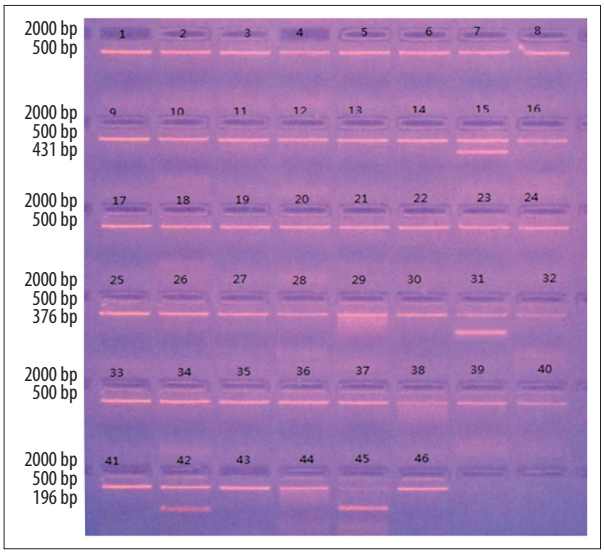


**Figure 1.** Gene typing result of A subgroup.

### Blood type of family members

Genealogical analysis indicated that the blood phenotypes of the proband's wife and 2 daughters were A, O, and A blood type, while the genotypes were A101/O*02*, A101/O*02*, and O*02*/O*02*, respectively. The A307 gene of proband was not inherited by his daughters.

## Discussion

Since the discovery of blood typing in 1990, at least 29 blood group systems have been tested, employing more than 240 blood group antigens. Besides such normal ABO phenotypes as A, B, AB and O, there still exist some rare subtypes. The A3 subgroup is a rare subtype, and presents a mixed field in microscopic observations, implying that there are several groups of agglutinated red blood cells surrounded by free non-agglutinated red blood cells after the reaction between red blood cells and anti-A reagent. However, this weak agglutination is usually ignored for subjective and objective reasons. A few studies have suggested that a low level of A antigen combined with an absence of extended, branched glycolipids may be responsible for the mixed-field





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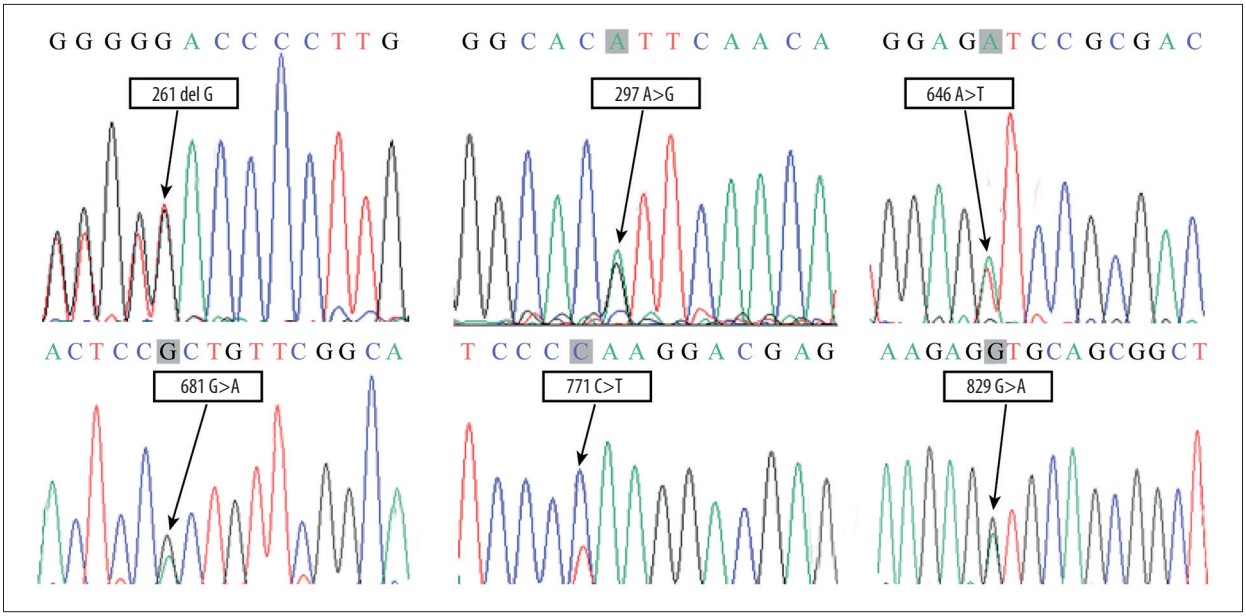


**Figure 3.** Sequencing diagram of the other allele of the ABO blood type of the proband (*O02* allele).

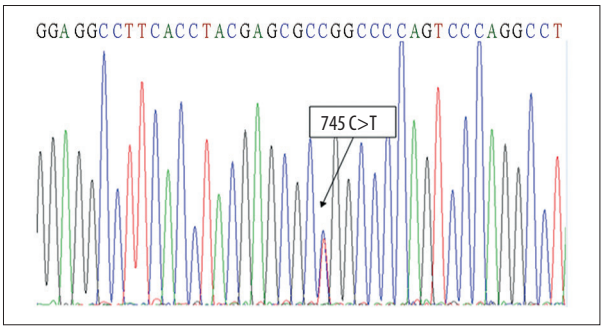


**Figure 2.** Sequencing diagram of proband.

agglutination phenomenon [10]. Svensson et al. [10] reported that the A3 detection rate was less than 1:1000. Furthermore, Yamamoto et al. reported that 2 white individuals with A3B type of 4 A3 subgroups were found to have a mutation at 871A>G, which caused Asp substituted by Asn at position 291 in the glycosyltransferase polypeptide chain [11]. Li et al. [9] found 3 single-nucleotide transitions (745C>T, 820G>A, and 860C>T) in 9 A3/A3B cases in Taiwan. Takahashi et al. found that point mutations of enhancer sequences at +5893 and +5909 affected cellular transcription, leading to weak expression of antigens to form A3, but there are few reports of A3 found in China [12–14]. In this study, we analyzed samples from an individual with the A3 subgroup. First, we used an anti-serum reagent to confirm the blood type of the proband and the results showed that agglutinating red blood cells were not seen under the microscope. Positive ABO blood typing showed O blood type, while negative blood typing showed A blood type. Next, we used another anti-serum reagent that resulted in weak agglutination, shown by the mixed-field result; therefore, we suspected that this was subgroup A3. All anti-serum reagents used in this study were monoclonal reagents and different manufacturers used a different antigen site. Therefore, we further verified our results by using anti-serum reagents from different manufacturers. Currently, serology is the mainstay during clinical transfusion. On one hand, serological testing is easily affected by subjective factors, but on the other hand, serological testing only detects surface antigens of the red blood cell and it is difficult to conclude when the surface antigen is weak. Within the serological identification, the false or missed identification might result in the inconsistency of classic serology and molecular biological classification, and the development of molecular biological techniques opened a new detection method for identifying the ABO subtypes. For example, the absorption-irradiation test could be done towards the suspected isoforms, which could combine with the serological and molecular biological technique to identify the right blood type, thus avoiding the false or missed identification of subtypes, and ensuring the safety of clinical transfusions [15]. In this study, the A gene subtype test revealed the presence of 2 A307/O02 alleles in the proband, while direct sequencing analysis of exons 6 and 7 of the ABO gene showed exon 7 mutation (c.745C>T), which resulted in 249 arginine > tryptophan (p.R249W). Moreover, 745C>T reduced the catalysis of α-1, 3-*N*-acetyl-galactosaminyl transferase and produced weak A antigen. Activities of serum corresponding to A transferase still needed further study. Furthermore, Genealogical analysis indicated that the proband did not pass on the A307 allele to his daughters.

At present, more than 200 ABO allele genes have been found, formed by mutations such as base insertion, omission, point mutation, gene recombination, and exchange [6,15–21]. Furthermore, the database for blood group antigen gene





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mutations has recorded 118 subgroup alleles of A phenotype, which include 11 A3 phenotypes. Although the frequency of ABO subtypes in the population was not high, because of the large amount of clinical testing, it might have ABO subtype-caused difficulties of typing or blood matching in clinical practice. This study confirmed that the proband had A307 subgroup after unconformity of ABO typing was identified by right and inverse blood grouping methods. Therefore, we should focus on this phenomenon and combine molecular biology with serological methods to confirm the correct blood type [22]. Presently, the domestic blood transfusion principles towards ABO isoforms still lack clear description, but basically follow the principle of same type or compatible infusion. As for the A3-subtype individual in this study, judging from the existing serological properties and molecular mechanisms, the best source was the same A3 subtype blood, but the source was very difficult, and almost impossible. Therefore, the clinic would use O type rinsed red blood cells for the transfusion, or mobilize the family members for mutual transfusion. Additionally, from the domestic established blood banks, the research data entered could be used to look for the mutual donor with the same type. For the patients that met the criteria of autologous transfusion, long-term refrigeration of autologous blood and red blood cells might also be considered to ensure blood transfusion safety.

## Conclusions

Results of the present study show that blood serological identification and family genetic molecular mechanism of ABO subtype was important for the study of rare subtypes and determining the correct clinical transfusion strategy.

### Conflicts of interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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