Identification of a novel ABO*O.01.01 allele with c.801G>T mutation in a Chinese A2 subtype individual

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Dear Editor,

ABO variant is the major factor for leading to discrepancy between forward and reverse typing and the difficulty in interpreting the results of serological method for ABO phenotyping[1]. Most ABO subtypes are caused by single nucleotide substitutions on exons 6 and 7, which encode for the catalytic domain of ABO glycosyltransferase[2]. ABO subgroups can be caused by different changes to the ABO gene, for example, single-nucleotide polymorphisms, deletions, or insertions, which are defined by weakly expressed A or B antigen, respectively, and result in typing anomalies[3]. The A2 is a very rare phenotype in the ABO blood group system in the Oriental population, which is weaker than the typical A antigen[4]. The presence of unusual O alleles is frequently associated with discrepancies in ABO blood group serologic typing results[5]. This study found a novel mutation on the ABO*O.01.01 allele with c.801G>T using molecular analysis, which was identified in a Chinese individual with an A2 phenotype.

The peripheral blood sample from a 62-year-old female patient diagnosed with multiple traumas was tested after obtaining informed consent. The ABO phenotypes were determined using monoclonal anti-A, anti-B, anti-AB, anti-Al, and anti-H by conventional standard gel matrix methods for forward typing. Reverse typing was detected by commercial A1, B, and O cells using standard gel matrix methods according to the manufacturer's instruction.

Genomic DNAs were extracted from peripheral blood white blood cells using a commercially available DNA isolation kit (Tianjin Super Development Co., Ltd, Tianjin, China). According to the ABO gene provided by GenBank (NCBI, Bethesda, MD, USA), we amplified and bidirectionally sequenced the whole coding region of the ABO gene as follows. The whole coding region (Exons 1-7) of the ABO gene was amplified and designed with sequence information (NG_006669.1). The sequencing primers were consistent with the amplification primers. Sanger sequencing was used the target fragment was amplified and sequenced directly. The amplification procedure was 96°C/2 min, 1 cycle; 96°C/20s, 68°C/60s, 5 cycles; 96°C/20s, 64°C/50s, 72°C/90s, 10 cycles; 96°C/20s, 61°C/50s, 72°C/90s, 25 cycles; 72°C/5 min, 1 cycle. Further, the haplotype sequencing was performed and sequencing data were analyzed with Chromespro (Technelysium Pty Ltd, South Brisbane, Australia) and DNAMAN software (ver. 8.0) (Lynnon Biosoft, San Ramon, CA, USA) and then manually compared with .ab1 reference and ABO gene sequence for reference (NG_006669.1) and Names for ABO (ISBT 001) blood group alleles v1.1 171023 acquired from NCBI dbRBC database and International Society of Blood Transfusion (ISBT)'s website.

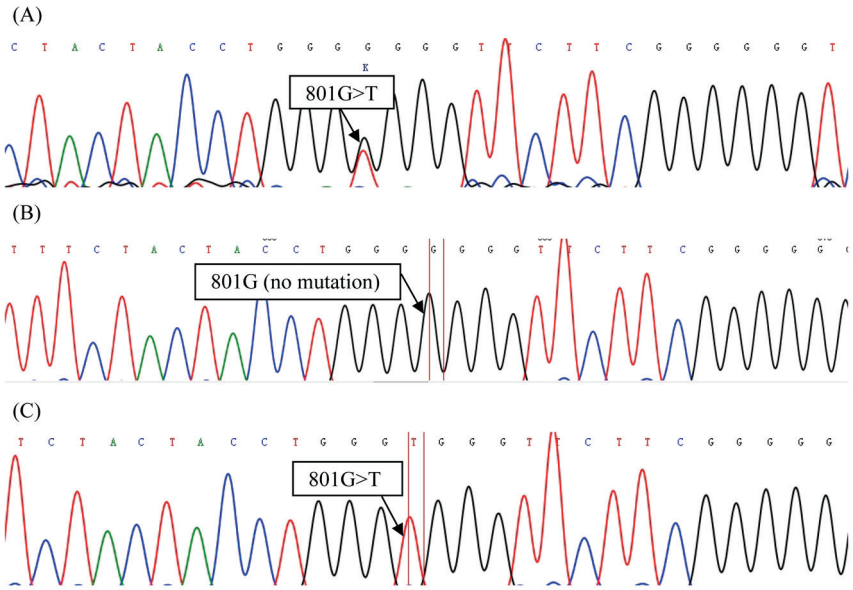

Figure 1 - Partial nucleotide sequence of Exon 7 region in ABO gene by direct sequencing and haplotype sequencing
(**A**) The c.801G>T heterozygous site was found by direct genomic sequencing. (**B**) The c.801G>T mutation was not found in ABO*A2.05 by haplotype sequencing. (**C**) The c.801G>T was determined in ABO*O.01.01 using haplotype sequencing. Each position labeled with arrow mark indicates each specific variant.

The results of serological testing showed a discrepancy between RBCs grouping and serum grouping of the patient. The RBCs of the case showed 3+ strength agglutination with anti-A, anti-AB and anti-H, not reactive with anti-B and anti-A1. The serum of the patient exhibited no agglutination with Oc, moderate (2+) agglutination with A1c and Bc. Therefore, the individual could be assigned as A2 phenotype based on its serologic characteristics.

The direct sequencing results demonstrated no mutations were detected in exons 1 to 5 in the A2 subtype patient. The sequencing analysis showed that the case was heterozygous for the ABO*A2.05 and ABO*O.01.01 alleles which did not completely match any known ABO allele combination, containing an additional nucleotide replacement novel mutation c.801G>T in Exon 7 of the ABO gene. Further, DNA haplotype sequencing of ABO gene revealed that the variation was located in the ABO*O.01.01 allele, which formed a novel O allele. In other words, the sequence of the novel allele had one nucleotide alteration (G>T) at position 801 on the background of the ABO*O.01.01 allele (**Figure 1**). The nucleotide sequence of the variant O allele has been submitted to GenBank nucleotide sequence database and the Accession Number (PQ206445) was assigned.

In conclusion, a novel O allele was identified in a Chinese patient with an A2 phenotype. This novel allele has the c.801G>T variant on the ABO*O.01.01 allele background by ABO blood group molecular typing, which introduced a p.Gly267Gly synonymous variant. Our next step is to conduct further investigation into the patient's family to determine if this mutation has genetic properties.

The Authors declare no conflicts of interest.

REFERENCES

1. Zhang J, Ying Y, Zhang B, Hong X, Zhu F. Identification of a novel A allele with a c.102C > A variation on the ABO*A1.02 allele. Transfusion 2023; 63: E15-E16. doi: 10.1111/trf.17257.
2. Deng D, Deng G, He Y, Yu Y. Identification of a novel A allele with c.478C>T missense mutation on the ABO*A1.02 background. Transfusion 2022; 62: E47-E48. doi: 10.1111/trf.17041.
3. Zhu Y, Liu L, Feng Z, Dong Y. A novel O allele in the ABO blood group system, a nine-base-pair deletion in an ABO*O.01.01 allele. Transfusion 2021; 61: E3-E4. doi: 10.1111/trf.16108.
4. Chen DP, Tseng CP, Wang WT, Sun CF. Identification of a novel A2 allele derived from the A transferase gene through a nucleotide substitution G539C. Vox Sang 2005; 88: 196-199. doi: 10.1111/j.1423-0410.2005.00608.x.
5. Zhang X, Zhou Z, Huang X, Li L, Li X, Li J. A c.1046_1048delAGG deletion causes a novel O allele on an ABO*O.01.04 allele in a Chinese Han blood donor. Transfusion 2023; 63: E34-E35. doi: 10.1111/trf.17362.