

A Distinct Approach for Molecular ABO Typing: A Case of Serology-Genotype Discrepancy Within a Family

Moleküler ABO Tiplemesi İçin Farklı Bir Yaklaşım: Bir Ailede Seroloji-Genotip Uyumsuzluğu Olgusu

İD Zehra Narlı Özdemir¹, İD Fatma Liv¹, İD Özgür Şenol², İD Emre Can Gül¹, İD Aliye Arslan Gül¹, İD Ecegül Bengi¹

¹University of Health Sciences Türkiye, İzmir City Hospital, Transfusion Center, İzmir, Türkiye

²Dia Pro Medical Products R&D Center, Gebze, Kocaeli, Türkiye

To the Editor,

Accurate ABO blood group determination remains essential for safe transfusion practice. While serological methods constitute the standard approach in routine immunohematology, molecular techniques provide critical support when serological typing is inconclusive or discordant. They are particularly valuable in cases of ABO variants, the presence of autoantibodies, recent transfusions with mismatched blood groups, weakened or lost antigen expression in hematological malignancies, and the identification of novel alleles.

Here, we report the findings of a 7-year-old patient typed as the A3 phenotype and RhD-positive. The parents, both physicians, raised concerns because a child with an A subgroup appears biologically incompatible with two parents who are O RhD-positive under Mendelian inheritance rules. This clinical discrepancy prompted a comprehensive serological and molecular evaluation of all three family members.

Serological testing was performed using automated gel centrifugation (Across RBC, Dia Pro, Gebze, Türkiye), supplemented by anti-A1 and anti-H lectin testing. As shown

in Figure 1, the results revealed a discrepancy characteristic of weak A subgroups. According to the specific criteria outlined in the National ABO Variant Reference Table of the National Blood and Blood Components Preparation, Use, and Quality Assurance Guidelines [1], the A3 phenotype is explicitly defined by a characteristically weak or mixed-field reaction with anti-A, a completely negative reaction with anti-A1 lectin, and the possible presence of anti-A1 antibodies in the reverse grouping. Consistent with these national reference criteria, our patient demonstrated strong positive reaction with anti-H lectin, a negative reaction with anti-A1 lectin, and a negative reverse grouping for anti-A1 antibodies, thereby definitively confirming the diagnosis of the A3 phenotype.

To investigate further, molecular ABO genotyping was performed using end-point fluorescent polymerase chain reaction (RBC-FluoGeneNX assays, inno-train Diagnostik GmbH, Kronberg, Germany). The results, reported according to the International Society of Blood Transfusion ABO allele classification, supported the serological findings by demonstrating that all three individuals possessed A alleles at the genetic level (child: *ABO**A1.01/O.02; mother: *ABO**A1.01/O.04.02; father: *ABO**A1.01/O.02) (Table 1).

Table 1. Molecular ABO genotyping results and associated SNPs for the family.

Individual	Predicted phenotype	Genotype (ISBT)	Identified SNPs/mutations
Child	A3 phenotype (mixed-field)	<i>ABO</i> *A1.01/O.02	A1.01: 261G, 467C, 802G, 803G, 1061C O.02: 261delG, 467C, 802G, 803G
Mother	O phenotype (silenced A)	<i>ABO</i> *A1.01/O.04.02	A1.01: 261G, 467C, 802G, 803G, 1061C O.04.02: 261delG, 467T, 802G, 803G
Father	O phenotype (silenced A)	<i>ABO</i> *A1.01/O.02	A1.01: 261G, 467C, 802G, 803G, 1061C O.02: 261delG, 467C, 802G, 803G

Note on SNP alignment: All identified SNPs and allelic variants, including the maternal *ABO**O.04.02 allele characterized by the c.261delG frameshift mutation, were cross-referenced and found to be in strict alignment with the reference sequences documented in the dbRBC database of the National Center for Biotechnology Information (Bethesda, MD, USA) and the ABO blood group allele nomenclature tables of the International Society of Blood Transfusion (Amsterdam, the Netherlands).

SNP: Single-nucleotide polymorphism; ISBT: International Society of Blood Transfusion.

As detailed in Table 1, molecular analysis identified specific single-nucleotide polymorphism signatures, including the maternal *ABO**O.04.02 allele (c.261delG frameshift mutation) and the *ABO**A1.01 allele (confirmed by 261G, 802G, 803G, and 1061C). Although these A alleles were genetically present in all three individuals, antigen expression remained weak in the child and was entirely undetectable serologically in both parents (Figure 1). A pedigree chart (Figure 2) illustrates the inheritance pattern of these alleles.

The serological silence of the consensus *ABO**A1.01 allele in both parents represents an extraordinary phenotypic-genotypic discrepancy, as this allele typically drives robust A-transferase activity and strong A1 antigen expression. A major technical limitation of our study is that adsorption-elution testing was not performed for the parents' samples. Routine molecular typing alone is insufficient to definitively differentiate between complete genetic silencing and an ultra-weak, sub-threshold antigen density that eludes routine automated gel centrifugation.

If absolute genetic silencing occurred in the parents, several literature-supported mechanisms could explain this transcriptional or translational blockade:

Epigenetic hypermethylation: Epigenetic modifications, specifically hypermethylation of the *ABO* proximal promoter, are known to disrupt the binding of critical transcription factors such as Sp1, effectively silencing an otherwise functional *ABO**A1.01 allele [2,3].

Cis-enhancer variations: Mutations or deletions within the key +5.8-kb cis-enhancer region can drastically reduce or eliminate

ABO gene transcription, leading to a silent or weak phenotype despite an intact coding region [2].

Modifier genes and cis/trans interactions: Unidentified modifier genes or silent mutations in regulatory elements outside exons 6 and 7 may exert compressive cis or trans regulatory environments, suppressing A-transferase enzyme synthesis or functional stability [2,3].

Interestingly, the unexpected manifestation of an A3 phenotype in the offspring suggests a potential inheritance dynamic where these repressive epigenetic or regulatory environments were remodeled or relieved during germline transmission. This potential "epigenetic escape" or modification in the next generation likely elevated A-transferase expression just enough to cross the detection threshold, manifesting as a weak subgroup [2,3]. While the exact underlying mechanism remains unresolved in this case, further advanced studies utilizing whole-gene sequencing (including regulatory and promoter regions), promoter methylation analysis, or transcriptional assays are required to definitively elucidate the molecular basis of this phenotype.

This case highlights several important points. Molecular ABO typing offers higher accuracy, reproducibility, and broader detection capability compared to serology [4]. It effectively identifies allelic variants and silent alleles in cases where antigen expression is weak, mosaic, or masked [5,6]. Additionally, it provides reliable results in multi-transfused patients and in the presence of autoantibodies. DNA-based methods also benefit from improved sample stability and compatibility with

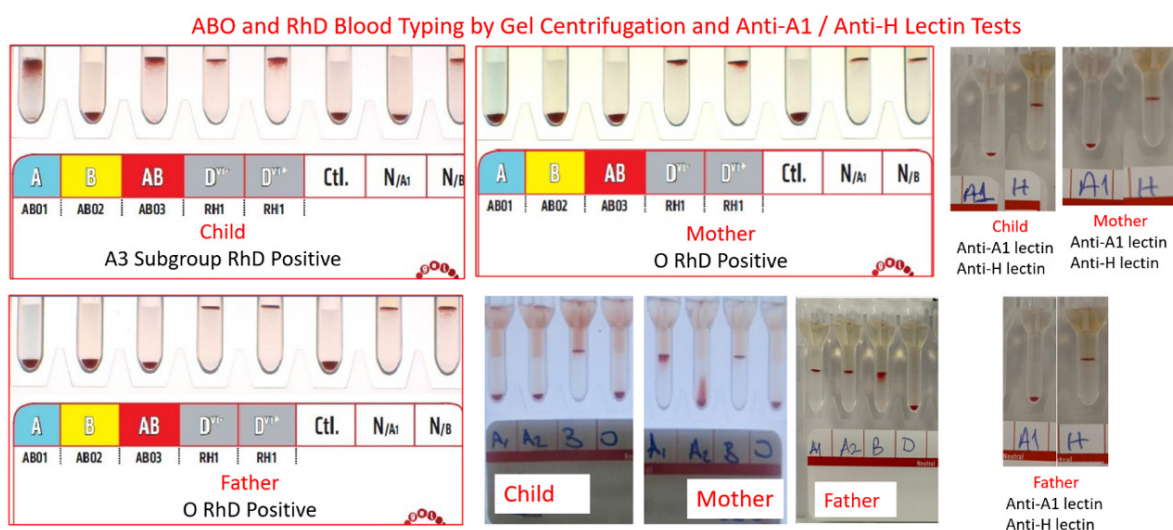


Figure 1. Forward-reverse ABO/RhD grouping and lectin test results obtained by gel centrifugation. The child demonstrated mixed-field agglutination in A and AB microtubes (forward grouping), while both parents showed negative reactions. Anti-H lectin showed strong agglutination (4+) in all three individuals, consistent with the expected high H-antigen density in weak A variants and O groups. Critical distinctions between A1-related results were observed as follows: Top right (antigen testing) - The negative reaction with anti-A1 lectin confirmed the absence of the A1 antigen on the child's red blood cells (forward grouping). Bottom right (antibody testing) - The test confirmed that no anti-A1 antibodies were present in the child's serum.

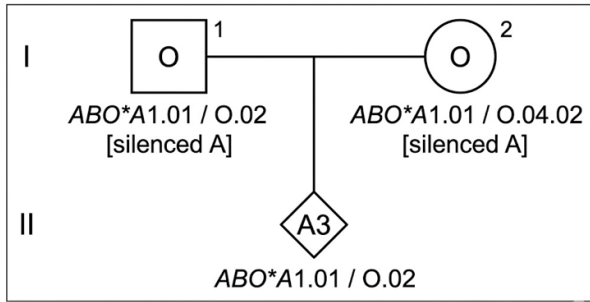


Figure 2. Pedigree chart illustrating the inheritance pattern of the ABO alleles. Squares represent male family members and circle represents female family member. The parents (I-1 and I-2), both demonstrating a serological O phenotype, are heterozygous carriers of the silenced ABO*A1.01 allele. The child (II-1) inherited the ABO*A1.01 allele from one parent and the ABO*O.02 allele from the other, resulting in a serologically detectable weak A3 phenotype. Genotypes identified by FluoGeneNX assay are indicated below each individual in strict compliance with International Society of Blood Transfusion nomenclature guidelines

automated workflows, reducing operator dependency and turnaround time [7].

In conclusion, this case underscores the value of molecular ABO genotyping as a complementary tool to serology, particularly in suspected ABO variants or serological discrepancies, ultimately enhancing transfusion safety. From a clinical management perspective, individuals presenting with an ultra-weak subgroup, such as the A3 phenotype observed in the child of this case, pose a challenge for routine blood banking. For future transfusion strategies, while this patient can molecularly synthesize A transferase, the lack of anti-A1 antibodies provides a wider margin of safety, though a cautious approach is still warranted. To maximize transfusion safety and prevent potential adverse reactions, it is clinically recommended that this individual receive Group O packed red blood cells [1,8]. By integrating molecular workflows into routine laboratory management, clinical centers can significantly reduce turnaround time and operator dependency, moving beyond the mere resolution of biological discrepancies toward proactive, personalized transfusion safety.

Keywords: ABO typing, Serology, Genotyping, ABO variant, A3 phenotype

Anahtar Sözcükler: ABO tiplendirme, Seroloji, Genotiplendirme, ABO varyant, A3 fenotip

Ethics

Informed Consent: Informed consent was obtained from each participant without any obligation to complete the study if they did not want to.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.Ş., E.C.G., A.A.G., E.B.; Concept: Z.N.Ö.; Design: Z.N.Ö., F.L.; Data Collection and Processing: F.L., E.B.; Analysis or Interpretation: Ö.Ş.; Literature Search: Z.N.Ö.; Writing: Z.N.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. General Directorate of Health Services, Department of Blood and Blood Products, 201. Ulusal Kan ve Kan Bileşenleri Hazırlama, Kullanım ve Kalite Güvencesi Rehberi 2016. Ankara. Available at <https://shgmkanhizmetleridb.saglik.gov.tr/TR-71523/ulusal-kan-ve-kan-bilesenleri-hazirlama-kullanim-ve-kalite-guvencesi-rehberi-2016>.
2. Ogasawara K, Sano R, Kominato Y. Review of ABO expression and variations based on transcriptional regulation of the ABO blood group gene. *Transfus Med Hemother*. 2024;51:210-224.
3. Kominato Y, Sano R, Takahashi Y, Hayakawa A, Ogasawara K. Human ABO gene transcriptional regulation. *Transfusion*. 2020;60:860-869.
4. Li H, Feng CC, Chen Q. [The advances and application of ABO blood group genotyping technology --review]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2022;30:622-626.
5. Usmani A, Morris GP, Murphey C. The increasing need for ABO blood group genotyping and quality assurance implications for laboratory implementation. *Hum Immunol*. 2024;85:110766.
6. Yamamoto F. A historical overview of advances in molecular genetic/genomic studies of the ABO blood group system. *Glycoconj J*. 2022;39:207-218.
7. Gorakshakar A, Gogri H, Ghosh K. Evolution of technology for molecular genotyping in blood group systems. *Indian J Med Res*. 2017;146:305-315.
8. Li G, Li F, Liu F. Serology and gene sequence analysis of rare subgroup A3 blood group. *Blood and Genomics* 2022;6:37-42.



Address for Correspondence/Yazışma Adresi: Zehra Narlı Özdemir, M.D., University of Health Sciences Türkiye, İzmir City Hospital, Transfusion Center, İzmir, Türkiye
E-mail: zehranarli@hotmail.com ORCID: orcid.org/0000-0003-3237-320X

Received/Geliş tarihi: April 3, 2026

Accepted/Kabul tarihi: July 1, 2026

Epub: July 2, 2026

DOI: 10.4274/tjh.galenos.2026.98522



©Copyright 2026 by Turkish Society of Hematology Turkish Journal of Hematology, Published by Galenos Publishing House.
Licensed under a Creative Commons Attribution-NonCommercial (CC BY-NC-ND) 4.0 International License.