

# Blood Group Variants in an Egyptian Individual: A Case Study

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**ABBREVIATIONS:** ADT - antibody detect test, CBC - complete blood cell, EDTA - ethylenediaminetetraacetic acid, IRB - institutional review board, MCV - mean corpuscular volume, MLS - medical laboratory science, NGS - next-generation sequencing, PCR - polymerase chain reaction, RBC - red blood cell, SCD - sickle cell disease, SS - Sanger sequencing.

**INDEX TERMS:** immunohematology, blood group antigens, molecular genotyping, partial antigen expression.

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## INTRODUCTION

An ABO discrepancy was detected in the teaching laboratory of a medical laboratory sciences (MLS) program when a student performed a type and antibody detect test (ADT) on their own blood specimen as part of an educational exercise. Following the initial results, an institutional review board (IRB) application was submitted and approved to further investigate the discrepancy (IRB-AY23-24-299). Subsequent serologic and molecular analyses revealed the presence of additional rare blood group alleles. The participant was found to have the rare A<sub>2</sub>B blood type with anti-A1 antibodies, a partial c antigen, and the GATA-box silencing mutation, contributing to the limited literature on blood group diversity in the student's Egyptian ancestry.

Managing blood transfusion in individuals with rare blood types presents unique challenges. Individuals exhibiting certain ABO discrepancies can complicate compatibility testing and increase the risk of hemolytic transfusion reactions unless they are accurately detected and the underlying cause identified.<sup>1</sup> Additionally, inheritance of genetic variants, including partial c antigen expression, can lead to the formation of alloantibodies that complicate

the acquisition of compatible blood. This case study highlights the critical role of combining serologic and molecular testing in identifying blood group genetic variants, underscores the value of hands-on learning in MLS programs, and demonstrates how educational laboratory activities can lead to clinically significant discoveries.

## A<sub>2</sub>B With Anti-A1 Antibodies

Blood group discrepancies due to ABO subtypes can complicate pretransfusion testing.<sup>1-5</sup> These discrepancies may lead to delays in care because of the need for additional investigation and an increased risk of adverse transfusion reactions.<sup>6</sup> For instance, individuals with the A<sub>2</sub>B subtype may produce anti-A1 antibodies, which can contribute to these challenges.<sup>7</sup> The AB blood type in the Egyptian population has been reported to occur in 9.74% of individuals.<sup>8</sup> It is estimated that 22% to 35% of A<sub>2</sub>B individuals produce anti-A1 antibodies.<sup>9</sup> These antibodies typically develop in the absence of pregnancy or transfusion and are usually clinically benign, as they primarily react at room temperature or below.<sup>10</sup> In addition to analyzing the subject's ABO alleles, molecular testing was performed to investigate other blood group alleles, revealing the presence of a partial c antigen within the Rh blood group system.

## Partial Rh Antigens

The clinical significance of the partial c antigen includes the increased risk of alloantibody formation against missing c epitopes, the challenge of finding compatible blood because of the prevalence of the c antigen within the general donor population, and the potential for individuals with partial c antigens to produce antibodies that mimic anti-Rh17, further complicating transfusion management.

The inheritance of partial Rh antigens is more common in individuals of African descent, with partial D, C, and e antigens being the most prevalent.<sup>11</sup> To minimize the risk of alloimmunization, it is recommended to perform comprehensive Rh genotyping and transfuse phenotypically matched blood.<sup>12</sup> However, individuals with partial Rh antigens may still develop antibodies against the antigenic epitopes they do not possess, even when receiving antigen-matched transfusions.<sup>12,13</sup>

The partial C antigen can be found in approximately 33% of individuals with sickle cell disease (SCD) and in approximately 21% of individuals with thalassemia.<sup>13</sup> The partial D antigen is less common, with approximately

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17% of individuals with SCD expressing partial D variants compared with 8.4% of transfusion-dependent populations.<sup>13</sup> While individuals expressing partial D can be alloimmunized to form antibodies toward the epitopes they lack, the availability of Rh-negative blood facilitates compatible transfusions and precludes the necessity to identify the type of anti-D formed by these individuals.<sup>14</sup>

Partial c antigen inheritance is much less common than partial C, D, and e antigen inheritance, and procuring c-negative blood for transfusion can be more challenging, as the c antigen is expressed by approximately 80% of Caucasian blood donors and 98% of donors of African descent.<sup>14</sup> Furthermore, individuals with homozygous or hemizygous inheritance of partial c and partial e antigens can produce alloantibodies directed against the conventional RhCE protein.<sup>14</sup> This antibody mimics anti-Rh17 (Hr<sub>0</sub>), which makes transfusion very difficult because of the rarity of compatible blood lacking both c and e antigens.<sup>14</sup> However, on further investigation, these antibodies that appear to be anti-Rh17 can be shown to have a precise specificity, including CEST (RH57), CELO (RH58), or CEAG (RH59).<sup>14</sup>

Rh antigen frequencies in the Egyptian population have been reported as follows: D (85.6%), C (70.4%), E (41.7%), c (91.2%), and e (100%), with R1R1 (DCe/DCe) being the most prevalent Rh phenotype.<sup>15</sup> The frequencies of Rh blood group alleles encoding partial antigen expression within the Egyptian population are currently unknown. While the Rh blood group system plays a significant role in transfusion medicine, other blood group systems, such as Duffy, also hold important clinical relevance, particularly in relation to resistance to infection with certain malarial species, its physiologic function, alloantibody formation toward Duffy antigens, and insights into population genetics.

### The Gata Box Silencing Mutation

The Duffy transmembrane glycoprotein serves as a receptor for *Plasmodium vivax* and *Plasmodium knowlesi*; consequently, erythrocytes lacking expression of Duffy antigens are resistant to infection with these malarial species.<sup>16,17</sup> Additionally, the Duffy glycoprotein functions as a chemokine receptor and is thought to scavenge and remove harmful levels of proinflammatory chemokines from circulating blood.<sup>16,17</sup> Because of this function, it has been renamed the Duffy antigen receptor for chemokines (DARC). These chemokine antigen receptors are expressed in various tissues, including endothelial cells lining postcapillary venules and cerebellar Purkinje cells.<sup>17</sup> It is postulated that the DARC may also function in homeostatic processes in certain regions of the brain.<sup>17</sup>

The *FY\*02N.01* allele, a GATA box silencing mutation of the *FYB* gene, inhibits promoter activity in erythroid cells, preventing the expression of the Fy<sup>b</sup> antigen on red blood cells (RBCs) while permitting expression of Fy<sup>b</sup> on nonerythroid cells.<sup>16,17</sup> The Fy(a-b-) phenotype most

commonly found in individuals of West African descent is commonly associated with homozygosity of the GATA box promoter region mutation upstream of the *FY* allele, which disrupts the binding site for the GATA-1 transcription factor.<sup>16,17</sup> Consequently, individuals with this allele rarely produce anti-Fy<sup>b</sup> antibodies after exposure to Fy<sup>b</sup>-positive blood transfusions because the Fy<sup>b</sup> antigen, although absent on their erythroid cells, is present on other tissues.<sup>17</sup> Therefore, it is recommended that molecular analysis of the Duffy system genes should be performed in addition to serologic RBC typing to determine whether the Fy(a-b-) phenotype is due to the GATA box mutation prior to transfusing Fy<sup>b</sup> positive blood.<sup>17</sup> Although the Fy(a-b-) phenotype can be found in nearly 100% of individuals of West African ancestry, its prevalence in the Egyptian population is unknown.

### MATERIALS AND METHODS

After collecting the subject's blood in a tube containing ethylenediaminetetraacetic acid (EDTA), a 2% to 5% RBC suspension was made using physiologic saline and was washed once prior to testing to achieve optimal testing results. ABORh forward and reverse typing, *Dolichos biflorus* lectin testing, and the ADT were initially performed manually using tube technique with low ionic strength solution as the enhancement media as part of a routine educational activity.

After IRB approval was obtained, additional EDTA specimens were collected and labeled with fictitious data to protect the anonymity of the subject. All serologic testing was repeated for confirmation using well-controlled antisera in a local hospital's immunohematology laboratory, and all results were concordant with previous testing. Separate refrigerated whole-blood specimens collected in EDTA were sent to the New York Blood Center and LifeShare Blood Services for ABO and additional blood group genetic testing, respectively.

Genetic testing was performed using Sanger sequencing (SS) to determine the subject's *ABO* alleles on a refrigerated specimen of whole blood collected in EDTA. After extracting the DNA from the whole-blood specimen, the DNA was amplified using polymerase chain reaction (PCR) to target the *ABO* gene. The final genotype was determined by examining the *ABO* promoter, enhancer, exons 1 to 7, and flanking intron regions.

Additional blood-group genotyping using targeted next-generation sequencing (NGS) was performed to detect the single-nucleotide polymorphisms. The DNA was extracted from a refrigerated whole-blood specimen and then amplified using targeted multiplex PCR. The individual reactions were combined to create an NGS library. After quantification, the NGS library was loaded on a sequencer for data acquisition, and the data were analyzed using specialized software designed to assign genotypes and generate the predicted phenotypes.

Capture solid phase technology (Immucor/Werfen) was used to determine whether the anti-A1 antibody isotype was completely IgG or if it contained an IgG component. The procedure was performed according to the manufacturer's instructions using a semi-automated Capture workstation (Immucor/ Werfen). Reagent A<sub>1</sub> cells were immobilized to the bottom of the microstrip wells, and a drop of the manufacturer's positive and negative control serum were added to their respective cells. The subject's plasma was collected from an EDTA specimen and was added to 3 microstrip wells to perform the isotype identification in triplicate to ensure reproducibility. After performing the subsequent steps according to the manufacturer's instructions, an illuminated light box was used to read the reactions. The presence of a smooth monolayer of cells indicated a strongly positive reaction, and tight cell buttons demonstrated a negative reaction.

## RESULTS

The subject's ABORh-forward type demonstrated an AB-positive blood type, while the reverse type displayed an unexpected 2+ positive hemagglutination reaction with the A<sub>1</sub> cells using the manual tube technique. The RBCs were then tested with 2 different lots of *D. biflorus* lectin, and the cells were nonreactive, indicating the absence of A<sub>1</sub> antigens.

Additionally, the subject's plasma consistently demonstrated 2+ positive reactions when tested with 2 additional lots of A<sub>1</sub> cells and negative reactions with 2 different lots of A<sub>2</sub> cells using the manual tube technique. Subsequently, the ADT demonstrated all negative reactions at room temperature, ruling out cold reactive unexpected antibodies.

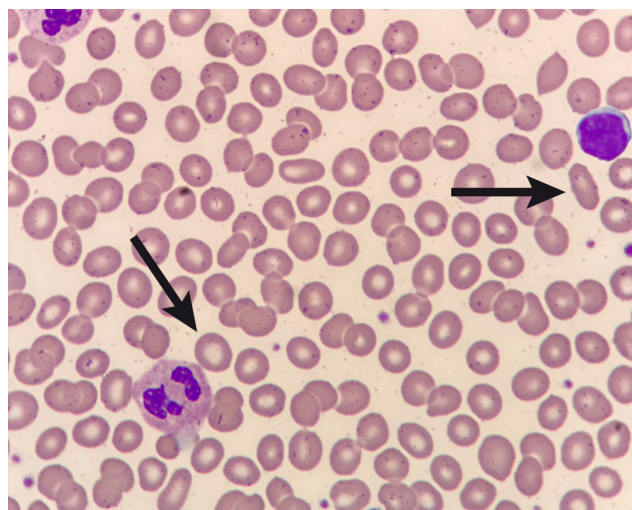
The subject's ABO genotype, *ABO\*A2.01/B0.1*, was determined with SS. In addition, the extended blood group genotype was found to be *RHCE\*01.20.01/RHCE\*02*. C+, E-, e+, V+, VS+ and partial c+, and the *FY\*02N.01* GATA box mutation was detected.

On completion of the peripheral blood smear, hypochromia was noted, along with few ovalocytes and acanthocytes (Figure 1). Additionally, the automated complete blood cell (CBC) results generated from a Sysmex XS-1000i, indicating that the mean corpuscular volume (MCV) is slightly less than the lower limit of the reference interval, corresponding with the mild microcytosis observed (Table 1).

Because the capture solid phase method used in this study detects IgG only, the lack of reaction of the subject's anti-A1 antibodies indicates that the antibodies do not contain an IgG component and are, therefore, presumed to be predominantly of the IgM isotype.

## DISCUSSION

This case study, examining an ABO discrepancy discovered in an educational exercise, emphasizes the importance of



**Figure 1.** Peripheral blood smear. Left arrow indicates a hypochromic RBC, and the right arrow indicates an ovalocyte.

**Table 1.** Automated CBC results

CBC Results		
Category	Value	Female Reference Interval
WBC	$8.8 \times 10^3/\mu\text{L}$	$4.5\text{--}12.5 \times 10^3/\mu\text{L}$
RBC	$5.3 \times 10^6/\mu\text{L}$	$4.0\text{--}5.3 \times 10^6/\mu\text{L}$
HGB	13.6 g/dL	12.1–15.2 g/dL
HCT	42%	36%–47%
MCV	77 fL	81–97 fL
MCH	25.6 pg	26.7–33.4 pg
MCHC	33 g/dL	32–36 g/dL
PLT	$386 \times 10^3/\mu\text{L}$	$140\text{--}400 \times 10^3/\mu\text{L}$
RDW-SD	40.5 fL	38.4–49.5 fL
RDW-CV	14.9%	11.3%–15.6%
MPV	10.3 fL	6.6–12.0 fL
Abnormalities seen	1+ ovalocytosis, 1+ poikilocytosis, 1+ acanthocytosis, 1+ hypochromia	

Abbreviations: CBC, complete blood cell; CV, coefficient of variation; HCT, hematocrit; HGB, hemoglobin; MBV, mean platelet volume; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration; PLT, platelet count; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

implementing pretransfusion testing protocols in clinical curriculum. This analysis exemplifies a practical scenario that has the potential to be encountered in a routine laboratory setting and yields blood grouping results that are underexplored in the Egyptian population. Genotype testing revealed the subject to be A<sub>2</sub>B and Rh positive with the expression of a partial c antigen. The absence of prior pregnancies or transfusions, combined with the characterization of anti-A1 antibodies as wholly or partially IgM, suggests natural antibody stimulation and adds to the

limited published data regarding the tendency of A<sub>2</sub>B individuals forming non-RBC immune anti-A1 antibodies. Reported ABO blood group distributions across various North African countries are similar to those observed in Egypt. For example, the frequency of the AB-positive type is reported as 5.00% in Tunisia, 4.28% in Algeria, and 5.00% in Sudan.<sup>18-20</sup> Although expression of the partial c antigen is uncommon and poorly documented in the literature, it poses a potential risk for transfusion incompatibility because partial c antigen expression is associated with a higher risk of alloimmunization.<sup>13</sup> Furthermore, the frequency of the *FY\*02N.01* allele within the Egyptian population is also currently unknown.

## CONCLUSION

This case study highlights the importance of investigating blood group genetic variations across populations. The data collected provide preliminary insight into potential causes of discrepancies encountered in the immunohematology laboratory. In addition, this case highlights the utility of combining traditional serologic testing with molecular methods to achieve comprehensive blood typing. Currently, blood group allelic frequencies within the Egyptian population are not well documented. Further research is needed to underscore the significance of our findings. Expanding the sample size would enhance the reliability and applicability of our results, particularly in addressing ABO discrepancies and partial Rh antigen expression among different ethnic groups.

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