Case Report

Two cases of the variant *RHD*DAU5* allele associated with maternal alloanti-D

J.A. Duncan, S. Nahirniak, R. Onell, and G. Clarke

Rh is a complex blood group system with diverse genotypes that may encode weak and partial D variants. Standard serologic analysis may identify clinically significant D variants as D+; nevertheless, individuals with these D variants should be managed as D– patients to prevent antibody formation to absent D epitopes. Variant identification is necessary during pregnancy to allow for timely and appropriate Rh immune globulin (RhIG) prophylaxis for hemolytic disease of the fetus and newborn (HDFN) as D alloimmunization can occur with some D variants. Here, we describe two cases of the *RHD*DAU5* allele associated with maternal alloanti-D in patients of African ancestry. Two obstetric patients were initially serologically classified as D+ with negative antibody detection tests on routine prenatal testing. Repeat testing at delivery identified anti-D in both patients with no history of RhIG administration or transfusion. DNA sequencing revealed that both patients possessed the *RHD*DAU5* allele. Cord blood testing on both infants revealed positive direct antiglobulin test (DAT) results with anti-D eluted from the red blood cells (RBCs) of one of the infants. Despite the positive DAT, neither infant experienced anemia or hyperbilirubinemia. We document two cases of pregnant women whose RBCs expressed a partial D variant and were classified as D+ on the basis of standard serologic testing, resulting in subsequent failure to provide RhIG prophylaxis. Both cases were associated with alloanti-D formation but without significant HDFN. To our knowledge, these are the first reported cases of maternal alloanti-D associated with the *RHD*DAU5* partial D variant. ***Immunohematology* 2017;33:60–63.**

Key Words: Rh blood group system, partial D, *DAU5,* anti-D, alloimmunization

The Rh blood group system is the most complex and polymorphic blood group system; after ABO, it is the most clinically significant system in transfusion medicine. This complexity is primarily due to the Rh system being encoded by two homologous genes, *RHD* and *RHCE,* whose close proximity allows for conversion to occur between the two genes and the subsequent creation of polymorphic proteins that are responsible for the plethora of variants observed in the Rh system.[1]

The Rh system contains several highly immunogenic antigens. The D antigen in particular provokes antibody production in up to 80 percent of D– volunteers transfused with D+ blood.[2] However, more recent retrospective analyses of D– patients who received D+ red blood cells (RBCs) have shown lower rates of antibody formation.[3,4] D is also a significant cause of hemolytic disease of the fetus and newborn (HDFN), in which D– women with a D+ fetus may be alloimmunized during pregnancy or at delivery. Maternal alloimmunization and subsequent HDFN can be prevented in the vast majority of women with timely Rh immune globulin (RhIG) prophylaxis during pregnancy and after delivery. For this reason, the appropriate assignment of D antigen status is required for proper selection of blood products and perinatal management.

Although the majority of individuals can be correctly classified as D+ or D– using routine serologic methods, there are D variants that differ from wild-type D that create a gray area between these two categories. More than 460 *RHD* alleles have been identified with mutations that result in qualitative or quantitative changes in D expression.[5] These variations in D are broadly, but somewhat artificially, categorized on the basis of serologic studies as weak D and partial D phenotypes.

Weak D variants involve changes in the D protein where at least one amino acid substitution occurs in the transmembrane or intracellular portions of the protein.[6] By serologic definition, a weak D will give no or weak (≤2+) reactivity with immediate spin testing and will show stronger agglutination using an indirect antiglobulin test for detection. Prevalence of weak D varies by race and ethnicity, and current data suggest that individuals with the most common weak D types (types 1, 2, and 3), which make up the majority of weak D individuals found in Europeans,[7] are not at risk of alloimmunization by D.[8] These patients could safely receive D+ blood components and would not require RhIG prophylaxis. Unfortunately, the identification of patients with exceptional weak D types who are susceptible to D alloimmunization is not through serologic testing, but only through molecular analysis of the *RHD* gene.

The term "partial D" has been used to describe qualitative variants where amino acid substitutions in the extracellular portion of the D protein, or hybrid alleles, result in altered or missing epitope expression.[6] This group can be very difficult to characterize serologically because of extensive variability in testing with different anti-D reagents. Consequently, RBCs with partial D variants may react strongly with initial

testing and be identified as D+, resulting in the individual not receiving RhIG prophylaxis during pregnancy or receiving D+ RBC transfusions. The frequency of anti-D formation occurring in partial D variants under these circumstances is not known. There are multiple case reports of obstetric patients with a partial D genotype who have formed anti-D as a complication of their pregnancy and have had infants demonstrating HDFN.[9,10] Despite the potential for severe HDFN in these infants, the clinical manifestations have been mild in the majority of cases.[11]

Partial D variants vary with race and ethnicity. Current serologic D typing strategies in North America and the UK are based on detecting partial D phenotypes more commonly found in white populations.[8] For example, licensed anti-D reagents are required to react as D– in the setting of a partial DVI variant, which is the most common clinically significant partial D in this group. In black populations, the occurrence of partial D variants is more frequent, however, as is the frequency of anti-D in pregnancies with D+ mothers.[12]

The *DAU* allele cluster has been described in individuals with African ethnicity,[13] and several *DAU* variants have been shown to have variable reactivity when tested with common commercially available anti-D reagents.[14] *DAU* is a phylogenetically related cluster of alleles with *DAU0* postulated as the primordial allele. The *DAU5* allele is defined by a mutation (F223V, E233Q, T379M) in *RHD* in exons 5 and 8, resulting in recombination of *DAU0* and DVI. The *DAU5* partial D allele has not been associated with anti-D resulting in HDFN through October 2016.[15]

In this case report, we describe two obstetric patients whose RBCs with a partial D variant were classified as D+ during standard prenatal serologic testing resulting in subsequent failure to provide RhIG prophylaxis and subsequent development of alloanti-D at time of delivery but without HDFN.

Case Report

Patient A, a 34-year-old woman (gravis 6, para 2), who had emigrated from the Congo, had routine prenatal serologic testing at 13 weeks' gestation completed at a perinatal testing laboratory. Testing for D included monoclonal blend Series 4 and Series 5 anti-D reagents (ImmucorGamma, Norcross, GA) on the Galileo Neo automated solid-phase testing platform (Immucor). Antibody screening was also performed on the Galileo Neo platform using Immucor 2 Cell Capture-R Ready Screen. All testing was completed in accordance with the manufacturer's instructions. Patient A's blood typed as group A, D+ (4+ reactivity with both Series 4 and Series 5 anti-D), and the antibody detection test was negative. No prior testing results were on record for this patient. The pregnancy was uneventful, with normal ultrasounds reported at 14, 21, and 32 weeks' gestation.

The patient was admitted to the hospital at 40 weeks' gestation in labor, and repeat serologic testing was performed at that time. In-hospital testing was performed on the Galileo Echo platform (Immucor) with Series 4 and 5 anti-D reagents. Her blood type was confirmed as group A, D+, with 4+ reactivity with both Series 4 and Series 5 anti-D reagents and 3+ reactivity with manual tube D testing with Series 5 anti-D. Her antibody detection test, also performed on the Galileo Echo using 2 Cell Capture-R Ready Screen (ImmucorGamma), was positive (3+ reactivity in both cells) with subsequent identification of the antibody as anti-D. The direct antiglobulin test (DAT) on the patient's RBCs was negative. There was no record of RhIG administration or transfusion for the patient.

This specimen was tested at a reference laboratory with single nucleotide polymorphism (SNP) array analysis and subsequent DNA sequencing. Molecular analysis included genotyping/SNP analysis performed on the ID Core XT (Progenika-Grifols, Derio, Spain). Sanger sequencing was performed by use of the following: Progenika-Grifols (Medford, MA) using genomic DNA extracted from EDTA-whole blood, specific primers to PCR-amplify the 10 *RHD* exons and flanking introns, a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) to resolve the extension products by capillary electrophoresis, and SeqScape software (Applied Biosystems) to analyze data by comparison with the National Center for Biotechnology Information (NCBI) reference sequence. Genomic DNA sequencing detected the presence of the silenced *RHD*Pseudogene* and a partial D variant *RHD*DAU5*. The patient's genotyping results on the ID CoreXT for the other Rh antigens predicted the RBCs to be C–, c+, E–, and e+, and a marker for r'^{S} was not detected.

This patient delivered a healthy girl with APGAR scores of 7 and 9 at 1 and 5 minutes, respectively. In-hospital testing showed the infant's cord blood type was group A, D+ with DAT reactivity of 1+. An eluate of the cord RBCs contained anti-D. The newborn's plasma bilirubin levels over the next 2 days ranged from 102 to 151 μmol/L (normal: <200 μmol/L), thus prophylactic phototherapy was not required.

Patient B, a 31-year-old woman (gravis 3, para 1) originally from West Africa, had routine prenatal testing at 12 weeks' gestation also at the same perinatal testing laboratory. Her blood type was group O, D+ (4+ reactivity with Series 4 and Series 5 anti-D), and her antibody detection test was negative,

consistent with historical records from previous testing in 2010. Her ultrasounds at 12 and 20 weeks' gestation were normal, and her pregnancy progressed without complication. Repeat serologic testing was performed in-hospital predelivery at 38 weeks' gestation when the patient had a caesarian section for partial placental abruption. Her blood type was confirmed as group O, D+ (4+ reactivity with Series 4, 3+ with Series 5 anti-D). The patient's antibody detection test was positive (3+ reactivity in two screening cells) with antibody identification confirming anti-D. DAT was negative. There was no record of RhIG administration or blood transfusion for the patient. This maternal specimen was also forwarded for molecular analysis. Genomic DNA sequencing detected the presence of a hybrid *RHD*DIIIa-CE(4-7)-D* and a partial *RHD*DAU5* allele. The patient's genotyping results on the ID CoreXT for the other Rh antigens predicted her RBCs to be c+, E–, e+, with the hybrid *RHD*DIIIa-CE(4-7)-D* encoding variant (partial) C antigen expression. The patient's phenotype was confirmed as C+ serologically.

The patient delivered a healthy boy with APGAR scores of 9, 10, and 10 at 1, 5, and 10 minutes, respectively. Cord specimen testing showed blood group O, D+. DAT was positive with weak reactivity, and a subsequent eluate performed on cord RBCs was negative. The infant had a transcutaneous bilirubin of 147 μmol/L after 24 hours and was discharged home without prophylactic phototherapy.

Discussion

Here, we have described two cases of the partial D variant *DAU5* associated with maternal anti-D in unrelated African patients. One mother's *RHD* genotype was *RHD*Pseudogene/RHD*DAU5* and the *RHD* genotype of the other mother was *RHD*DAU5/RHD*DIIIa-CE(4-7)-D*. In both cases, the patients were classified as D+ on the basis of concordant strong reactivity with two different anti-D reagents. As a result, neither mother received RhIG, and both were subsequently alloimmunized for D during their pregnancies.

These cases emphasize the importance of being able to recognize and identify weak and partial D phenotypes that place women of child-bearing potential at risk of forming alloanti-D so that subsequent RhIG prophylaxis can be provided as well as avoiding transfusion of D+ RBCs.

To avoid this complication, it has been recommended to test samples from obstetric patients and potential transfusion recipients with two specifically selected monoclonal anti-D reagents, that have dissimilar specificities, in an attempt to increase the likelihood of identifying variant D expression and to potentially identify those in which genotyping may be useful.[16] The cases we describe did not exhibit the serologic discrepancies in D typing that would suggest the presence of a D variant, a phenomenon that has been reported previously.[17] The epitopes altered by the mutation remained fully serologically reactive with standard commercial reagents used on both automated and manual testing platforms. These cases may represent variations in the particular source and specificity of antisera, resulting in failure to detect this variant antigen.

Fortunately, neither infant in the reported cases experienced significant hyperbilirubinemia. The infants did not develop anemia nor require phototherapy, and both were discharged from the hospital without an extended stay.

Obstetric patients with a partial D phenotype are known to have the potential to form anti-D during pregnancy, and their infants are at risk for HDFN. Despite the potential for severe HDFN due to anti-D, the clinical manifestations in patients with partial D have been mild in the majority of cases except for those in mothers with DVI variants.[11]

The reason for partial D variants to be associated with a lesser degree of morbidity when compared with HDFN in D– individuals with alloanti-D is unknown. One hypothesis proposes that because D variants do express numerous epitopes of the D antigen, the associated anti-D may not have a broad specificity and hence has diminished hemolytic potential compared with anti-D in a D– individual.[18]

These two cases highlight the limitations of routine serologic testing to detect some partial D variants. A commentary by Sandler et al.[19] of the AABB–College of American Pathologists (CAP) Working Group proposed selective integration of *RHD* genotyping in routine obstetric and pre-transfusion testing to improve the accuracy of D typing results, to reduce unnecessary RhIG administration in women with serologic weak D phenotypes, and to overall decrease transfusion of scarce D– RBCs to patients with weak D phenotypes. The AABB-CAP Working Group did not address management of partial D phenotypes, except in the management of discordant D typing results, in which case they recommend *RHD* genotyping.[19] The incorporation of genotyping into prenatal testing may offer a solution, although the significant cost of this methodology limits use in routine testing. The implementation of genotyping of D+ women of childbearing age who are of African descent may offer a more feasible solution to this issue.

To our knowledge, these are the first reported cases of maternal alloanti-D associated with the *RHD*DAU5* partial D variant. Neither infant experienced clinically significant

anemia nor hyperbilirubinemia although the RBCs of both infants reacted in the DAT.

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Jennifer A. Duncan, MD, Hematopathology Resident; Susan Nahirniak, MD, Hematopathologist; Rodrigo Onell, MD, Hematopathologist, Alberta Health Services, Edmonton, Alberta, and Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton Alberta, Canada; and Gwen Clarke, MD (corresponding author), Hematopathologist, Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada, and Canadian Blood Services, Canadian Blood Services, Medical Office, 8249-114th Street, Edmonton, AB T6G 2R8, Canada, gwen.clarke@blood.ca.