Case Report

Transfusion support during childbirth for a woman with anti-U and the *RHD*weak D type 4.0* allele

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D– red blood cells (RBCs), always in short supply, and Rh immune globulin (RhIG) are not needed for patient care if D+ RBCs can safely be transfused. According to a recent work group recommendation, patients with the *RHD*weak D type 4.0* allele can be considered D+. We report an African American woman who presented for delivery at the end of the third trimester, at which time anti-U and a serologic weak D phenotype were recognized, requiring U–, D– RBC units. We obtained 3 U– RBC units, including 1 D– unit. Later, the *RHD*weak D type 4.0* allele was determined by *RHD* genotyping, only 6 days before delivery. The patient had an uneventful vaginal delivery of a D+ baby. No transfusion was needed for mother or baby. In this case, a pregnant woman with the *RHD*weak D type 4.0* allele can safely be managed as D+, relaxing the unnecessary D– restriction for the limited U– RBC supply. The procured U–, D– RBC unit was frozen with 14 days of shelf-life remaining. To conserve D– RBC units, not limited to U–, for patients with a definite need, we recommend molecular analysis of a serologic weak D phenotype before a transfusion becomes imminent. The best time to resolve a serologic weak D phenotype with *RHD* genotyping is early in a pregnancy. ***Immunohematology* 2021;37:1–4.**

Key Words: *RHD*weak D type 4.0* allele, RhIG, pregnancy

Hemolytic disease of the fetus and newborn is reliably prevented by proper management, based on antenatal D typing and screening for red blood cell (RBC) antibodies. Many hospital laboratories do not determine the *RHD* genotype of pregnant women with a serologic weak D phenotype, because these women are often managed as D–.[1] However, Rh immune globulin (RhIG) and provision of D– RBC units are unnecessary if D+ RBCs can safely be transfused. An Interorganizational Work Group on *RHD* Genotyping recommended in 2015[2] to phase-in *RHD* genotyping for patients with a serologic weak D phenotype. The same authors[3] specified their recommendation in 2020 to phase-out the reporting of a "serologic weak D phenotype" and resolve all weak D types with *RHD* genotyping.

We report a pregnant woman with anti-U and a serologic weak D phenotype. The clinical workup in this case illustrated the importance of molecular analysis of serologic weak D phenotypes early in the pregnancy to preserve rare D– RBC units and to eliminate the unnecessary administration of RhIG.

Case Report and Results

A 23-year-old African American woman (gravida 2, para 1) presented for childbirth. The woman had no history of blood transfusion. Testing of her blood sample showed her RBCs to be group B with a serologic weak D phenotype; anti-U was identified by the antibody screening process (Table 1). Without any molecular information for her *RHD* genotype, the woman was initially considered to be managed as D–. We decided to obtain 3 U– RBC units; however, only 1 was D–.

In the week before delivery, nucleotide sequencing of the *RHD* gene was performed on the mother and, later, on the neonate.[4] Based on the three amino acid substitutions (Table 2), we concluded that the mother carried the *RHD*weak D type 4.0* allele (Table 2) and was hemizygous for the *RHD* gene. The neonate was a compound heterozygote for the *RHD* gene with a *RHD*weak D type 4.0* allele from the mother *in trans* to a normal *RHD* allele from the father. A total of 12 and 13 nucleotide changes were confirmed in the mother and neonate, respectively (Table 2).

Zygosity testing for the *RHD* gene was done by a quantitative fluorescence–polymerase chain reaction (QF-PCR) assay.[5] The mother was hemizygous (one copy) and the neonate was homozygous (two copies) for the *RHD* gene. The QF-PCR is the preferred method for *RHD* zygosity testing in individuals of African descent, although it is known to have limitations in white individuals where a restriction fragment–length polymorphism (RFLP) assay may be the more reliable method.[6–8]

We still applied an RFLP assay that is designed to detect the standard downstream *Rhesus box,* indicative of the presence of an *RHD* gene (i.e., lack of the *RHD* deletion).[7] However, the mother who carried an *RHD* gene tested negative

Table 1. Clinical laboratory results for mother and neonate

Test	Results (normal range)
Maternal	
Transfusion medicine[†]	
ABO group	B
RhD phenotype	Serologic weak D phenotype: 2+ reaction strength[‡]
RhCE phenotype	C–E–c+e+
Antibody screen	Anti-U
DAT	Negative
Red cell genotyping[†]	
RHD allele	*RHD*weak D type 4.0*
RHD zygosity	Hemizygous
Hematology[†]	
Hemoglobin, g/dL	
Antepartum	9.8 (10.0–15.0)
Postpartum	8.9 (10.0–15.0)
Neonatal	
Transfusion medicine[§]	
ABO group	B
RhD phenotype	D+
RhCE phenotype	C–E–c+e+
DAT	Negative
Red cell genotyping[§]	
RHD allele	*RHD*weak D type 4.0* and normal *RHD*
RHD zygosity	Compound heterozygous
Hematology	
Hemoglobin, g/dL	17.6 (14.0–24.0)
Unconjugated bilirubin, mg/dL	
At birth	4.9 (<6)
4 hours after birth	5.5 (<6)
Reticulocyte count, %	4.46 (3.0–7.0)

[†]At the end of the third trimester.

[‡]Tested by the conventional tube method at immediate spin (Anti-D Blend, oligoclonal antibody mixture with clone numbers BS232, BS221, and H41 11B7; Bio-Rad, Hercules, CA).

[§]At birth.

DAT = direct antiglobulin test.

(seemingly no copies) in this RFLP assay, and the neonate who carried two copies of the *RHD* gene tested hemizygous for the *RHD* gene (seemingly only one copy). These discrepancies are explained by variations in the downstream *Rhesus box* of individuals of African ancestry and are a known limitation of the RFLP assay in these individuals.[6,8,9]

The mother, with an unexplained hemoglobin (Hb) concentration of 9.8 g/dL prepartum, had an uneventful vaginal delivery. Her Hb dropped by 0.9 g/dL, and she did not require transfusion (Table 1). The neonate's blood sample typed as group B, D+ with normal clinical laboratory results (Table 1), and no treatment was required. The 2 U–, D+ RBC units were returned and used in the care of another pregnant woman with anti-U. The unnecessarily procured U–, D– RBC unit had to be frozen, however, with only 14 days of shelf-life remaining.

Discussion

The present clinical report exemplifies the advantage of *RHD* genotyping in expectant mothers to identify *RHD* alleles that allow the mothers to be safely treated as D+.[3] The molecular analysis should be performed early in a pregnancy. This approach, which was missed at the first-time maternity visit in our patient, would have allowed for an efficient organization of RBC genotyping with or without antibody identification. Most hospitals would typically send samples to an immunohematology reference laboratory. In our case, while birth was imminent, the shipping and testing was accomplished within 5 days, including a weekend. The extra cost inflicted by this time constraint could surely have been avoided with better planning of the required tests during the pregnancy. Complex serologic and molecular testing in immunohematology are more prone to errors when performed under extreme time constraints and thus should be avoided.

The blood supply in transfusion service is often limited, especially for patients with the D– phenotype, and more so if antibodies to high-prevalence RBC antigens are also present.[10] For the expectant mother in our study, a compatible donor with a U–, D– phenotype is extremely rare, representing <0.1 percent of the African American population.[11] U–, D+ RBC units are also very rare, but there are five to ten times more donors if the D– restriction can be removed.

Supporting every patient who is D+ due to *RHD*weak D type 4.0* with D– RBC units to prevent anti-D would be a burden and is discouraged.[3,12] D– RBC transfusion and RhIG administration may be considered during pregnancy in an abundance of caution, although several health care systems are considering moving to an exclusively D+ transfusion management policy.[3,13,14] Pregnant women with the *RHD*weak D type 4.0* allele, who were never shown to produce an alloanti-D with adverse clinical outcome, could falsely be diagnosed of carrying an alloanti-D that is actually

Table 2. Single nucleotide variants detected in the *RHD* gene

				***RHD* genotype**	
Location	**Nucleotide change***	**dbSNP reference number**	**Protein residue change†**	**Mother‡**	**Neonate**
Promoter	−368a>g	rs28710826	NA	g/g	a/g
Intron 2	336−76_−75−>insTGAA	rs112473736	NA	insTGAA/insTGAA	insTGAA/−
Intron 3	487−414a>g	rs28586271	NA	g/g	a/g
	487−316t>g	rs28572396	NA	g/g	t/g
Exon 4	602C>G	rs1053355	Thr201Arg	G/G	C/G
Exon 5	667T>G	rs1053356	Phe223Val	G/G	T/G
Intron 5	801+219t>g	rs28510210	NA	g/g	g/g
	801+395g>a	rs145236797	NA	a/a	g/a
	802−16c>t	rs201120463	NA	t/t	c/t
Exon 6	819G>A	rs150606530	Ala273Ala	A/A	G/A
Intron 6	939+295c>a	rs112222730	NA	c/c	c/a
Intron 7	1073+94g>a	rs533903485	NA	a/a	g/a
	1073+311g>c	rs3118453	NA	c/c	c/c

*Nucleotide substitutions are shown relative to the reference sequence (NG_007494.1). Nucleotide positions are defined using the first nucleotide of the coding sequence of NM_016124.4 isoform as nucleotide position 1. The uppercase nucleotides are located in the coding sequence, and the lowercase nucleotides are located in the non-coding sequence of the *RHD* gene.

†Relative to the National Center for Biotechnology Information (NCBI) Reference Sequence NP_057208.2.

‡The nucleotide sequence of the *RHD*weak D type 4.0* allele comprising 8572 base pairs, detected in the mother hemizygous for one *RHD* gene, has been deposited in GenBank as accession number MT900842.

dbSNP = Single Nucleotide Polymorphism Database; NA = not applicable.

due to RhIG administration.[13,14] This passively acquired anti-D can mislead the results of compatibility testing, when RBC units are crossmatched in preparation for delivery. Pitfalls of mistaking passive anti-D for active immunization can be avoided by obtaining a history and performing an anti-D titer.[15] In summary, we decided to treat the current patient as D+ for transfusion purposes and recommended against RhIG administration during pregnancy and after the birth of the D+ baby boy.

Studies on cost and financial implications explored the economic aspect of *RHD* genotyping for pregnant women with a weak D phenotype.[16,17] If the personal health information is properly maintained and shared, particularly in highly developed countries like the United States, *RHD* genotyping would add only a one-time testing cost for each pregnant woman with a weak D phenotype, while providing a rationale for the transfusion strategy during the rest of a woman's life.[18] This strategy could prevent unnecessary costs and risks associated with RhIG administration and follow-up scheduling during the current and every subsequent pregnancy.[3] The best time to resolve a serologic weak D phenotype with *RHD* genotyping is early in the first pregnancy.[2]

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