Molecular characterization and multidisciplinary management of Gerbich hemolytic disease of the newborn

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Abstract
Gerbich (Ge) antigens are high frequency red cell antigens expressed on glycophorin C (GYPC) and glycophorin D. Hemolytic disease of the fetus and newborn (HDFN) due to Gerbich antibody is rare and presents a clinical challenge, as Gerbich negative blood is scarce. We report a case of HDFN due to maternal Ge3 negative phenotype and anti-Ge3 alloimmunization, successfully managed by transfusion of maternal blood. Molecular testing revealed that the mother has homozygous deletion of exon 3 of GYPC, the father is homozygous wildtype for GYPC, and the infant is obligate heterozygote expressing Ge3.

KEYWORDS
anti-Ge3, Ge, Gerbich, GYPC, HDFN, HDN, hemolytic disease of the fetus and newborn, maternal alloimmunization, maternal blood transfusion

1 | INTRODUCTION

Maternal alloantibodies can cause severe hemolytic disease of the fetus and newborn (HDFN), Rh disease being the most familiar.¹ Rare causes of HDFN include antibodies against Gerbich (Ge) antigens, present in 99.9% of the population. Gerbich antigens are due to glycosylation sites on red blood cell (RBC) membrane proteins glycophorin C (GYPC) and glycophorin D, both encoded by GYPC.² Three Ge phenotypes have been identified serologically: Gerbich, Ge:-2,-3,4, Leach, Ge:-2,-3,-4, and Yus Ge:-2,3,4, reflecting different combinations of Gerbich antigens.³ Ge negative blood is nearly impossible to procure.

We describe a case of HDFN due to maternal Ge:-2,-3,4 phenotype and anti-Ge3 alloimmunization that was molecularly investigated and successfully managed by transfusion of maternal blood.

Abbreviations: DOL, day of life; Ge, Gerbich; GYPC, glycophorin C; HDFN, hemolytic disease of the fetus and newborn; Hgb, hemoglobin; retic, reticulocyte count

2 | CASE

A 34-year-old G3P1021 woman of Paraguay origin, with prior terminated pregnancies, presented at 30 weeks gestation with anti-Ge3. She was D- and Ge:-2,-3 by antigen typing. Since she was negative for anti-D, RhoGAM was administered at week 33. Maternal blood collection was scheduled near her due date for possible neonatal transfusion, but was precluded by spontaneous delivery at 37 weeks, at which time maternal anti-Ge3 titer was 256. Cord blood was direct Coombs positive for IgG; the eluate confirmed anti-D and anti-Ge3. Birth hemoglobin (Hgb) was 12.6 g/dl, reticulocyte count (retic) was 8.6%, and bilirubin 5.9 mg/dl. The infant was discharged on day of life (DOL) 4 with Hgb 10.6 g/dl.

On DOL 7, the infant was referred by a pediatric nurse practitioner to pediatric hematology for lethargy and poor feeding, with Hgb 7.4 g/dl, retic 2.6%, and bilirubin 6.6 mg/dl. Blood smear showed polychromasia and giant platelets. The infant needed transfusion, but
RESULTS AND DISCUSSION

Ge3- blood was unavailable despite searching rare donor registries. Fortunately, the mother was blood group B, Rh+, suitable for donating blood to her B, Rh+ infant. Maternal blood collection was expedited, requiring obstetrics authorization due to the mother’s Hgb of 10.9 g/dl postpartum, below the donor criterion of Hgb 12.5 g/dl. An aliquot of maternal cells was washed twice with saline and subjected to centrifugation at room temperature to remove maternal antibodies, then transfused to the infant (15 ml/kg) within 24 hr of his presentation, at which time his Hgb had dropped further to 6.1 g/dl. Post-transfusion, his Hgb was again 7.1 g/dl. Another transfusion was needed. By this time, the rest of unwashed, unmanipulated maternal blood had hemolyzed. A second maternal unit was collected, although mother’s Hgb was 9.9 g/dl. The infant received his third transfusion of 23 ml/kg of washed maternal blood (Table 1).

On DOL 45, the infant had Hgb 9.2 g/dl, bilirubin 0.7 mg/dl, retic 1.7%, and anti-Ge3 titer in his serum was 2. No further transfusion was necessary. At 9 months follow-up, the infant was thriving with Hgb 11.7, and maternal anti-Ge3 titer was 64 while anti-D was undetectable.

3 | RESULTS AND DISCUSSION

To determine the genetic alteration responsible for this HDFN case, PCR reactions diagnostic of specific GYPc deletions were performed (Figure 1). GYPc has four exons, with exons 2 and 3 being homologous. Deletions of one or more exons, with flanking introns, give rise to the various Ge negative phenotypes (Figure 1B). ‘Diagnostic’ PCRs designed to amplify bands when wildtype DNA is present but no band when DNA is deleted, showed a homozygous deletion in maternal DNA spanning intron 2, exon 3, and intron 3 (Figure 1A, top panel, lanes 4–6), confirmed by the presence of the expected deletional PCR product for the Gerbich02 deletional allele (Figure 1A, top panel, lane 14).

For infant DNA, all wildtype sequences were detected (Figure 1A, lower panel, lanes 1–8), but the PCR for the Gerbich02 deletional allele was also positive (Figure 1A, bottom panel, lane 14), consistent with the infant having both wildtype and Gerbich02 deletional alleles. The father’s DNA was positive for all PCRs for wildtype DNA and negative for all deletional PCRs (data not shown). Thus, the mother was homozygous for GYPc exon 3 deletion (ISBT: Ge*01.-03/Ge*01.-03), the father was homozygous wild type (Ge*01/Ge*01), and the infant was heterozygous for exon 3 deletion (Ge*01/Ge*01.-03) (Figure 1B).

Gerbich alleles encode high frequency antigens; consequently, Ge3 associated HDFN is rare, making diagnosis and management complex. In this case, the mother was sensitized to Ge but not to D, due to RhoGAM in previous pregnancies. Both anti-D and anti-Ge3 were eluted from the infant’s RBCs. Anti-D disappeared soon postpartum, while anti-Ge3 persisted, consistent with anti-D being passive and maternal anti-Ge3 being causative of the infant’s severe hemolysis.

The infant was the first liveborn for the mother, but his HDFN was severe, due to maternal Ge alloimmunization from previous pregnancies. The birth Hgb (12.6 g/dl) and retic (8.6%) indicated mild intrauterine hemolysis while erythropoiesis was suppressed, consistent with GYPc antibodies causing erythroid progenitor apoptosis. Thrombocytopenia can occur and Gerbich antigens are found on neutrophils. In this infant, the lowest platelet count was 74,000 and absolute neutrophil count stayed above 1000.

Maternal blood was the only source of Ge3- blood for this infant. The mother was adopted, precluding testing relatives for antigen negative blood. Expediting maternal blood collection resulted in a good outcome. Had the mother and infant been ABO incompatible, transfusion of mismatched maternal blood to the infant should still have worked in theory, since neonates do not have anti-A or anti-B; however, literature search did not find reports of such practice. Maternal transfusion for Gerbich HDFN has been reported; in that case, both mother and infant were blood group O.

### TABLE 1  Clinical parameters for infant with Ge3 HDFN

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Hgb g/dl</th>
<th>Retic %</th>
<th>Platelet x 10^5/μl</th>
<th>Bilirubin mg/dl</th>
<th>DAT</th>
<th>Ge3 titer maternal plasma</th>
<th>Ge3 titer infant’s plasma</th>
<th>Transfusion maternal blood</th>
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<tr>
<td>Birth</td>
<td>12.6</td>
<td>8.6</td>
<td></td>
<td>5.9</td>
<td>Positive</td>
<td>256</td>
<td>–</td>
<td>–</td>
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<tr>
<td>7</td>
<td>7.4</td>
<td>2.6</td>
<td>87</td>
<td>6.6</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
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<tr>
<td>8</td>
<td>6.1</td>
<td>–</td>
<td>99</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>15 ml/kg</td>
</tr>
<tr>
<td>9</td>
<td>10.8</td>
<td>–</td>
<td>115</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>15</td>
<td>7.1</td>
<td>–</td>
<td>192</td>
<td>2.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20 ml/kg</td>
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<tr>
<td>16</td>
<td>11.7</td>
<td>1.8</td>
<td>153</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>28</td>
<td>7.8</td>
<td>0.7</td>
<td>261</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8 23 ml/kg</td>
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<td>0.7</td>
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<td>56</td>
<td>9.2</td>
<td>3.3</td>
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<tr>
<td>287</td>
<td>11.7</td>
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<td>295</td>
<td>0.6</td>
<td>Negative</td>
<td>64</td>
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</table>
Figure 1  (A) Diagnostic PCRs for Gerbich alleles. Lanes 1–8 are reactions designed to detect the presence of wildtype sequences. Regions spanned by the PCR primers in lanes 1–8 are shown as shaded bars 1–8 in (B). Lanes 9–15 are reactions designed to detect deletions represented by dotted lines 9–15 in (B). Top panel: maternal DNA. Expected bands in lanes 4–6 are missing, showing the absence of wildtype sequences in intron 2, exon 3, and intron 3. Maternal DNA is positive for the exon 3 deletion represented by the 577 bp amplified fragment in lane 14. Her serum contains anti-Ge3 and her red cell phenotype is GE: -2, -3, 4; her genotype is homozygous (GE*01.-03/GE*01.-03). Bottom panel: infant DNA. The infant’s DNA shows the presence of all wildtype sequences tested in lanes 1–8, as well as a positive 577 bp band in the deletional PCR, in lane 14, for the exon 3 deletion represented by the dotted line 14 in (B). His genotype is heterozygous (GE*01/GE*01.-03); his red cells have the wildtype GE:2,3,4 phenotype. Arrow: 1000-bp GH1 control band. The concentration of control primers is 80 nM and the concentration of primers for specific diagnostic amplicons is 800 nM. Control primers will amplify a 1000-bp product of GH1 in all negative reactions to ensure that PCR worked, but amplification product may not be present in reactions in which specific diagnostic amplicons are generated, due to lower control primer concentration and competition between the two amplifications for enzyme and substrates. M: molecular length markers of 50, 100, 200, 400, 800, and 1500 bp. PCR primer sequences, concentrations and combinations, reaction conditions, and setup are described in detail in Ref. 3 (table SI, section D and E, of supplement 002, and materials and methods section). (B) GYP C gene structure, diagnostic PCR design, and deletional alleles.3 e1–e4: exon 1–exon 4. Ge4, Ge2, and (Ge2) Ge3 are designations for phenotypes correlated with exon 1, exon 2, and exon 3 deletions. Shaded bars 1–8 represent regions spanned by PCR reactions 1–8. Dotted lines 9–15 represent known deletions named Yus01, Yus02, Yus03, Yus04, Gerbich01, Gerbich02, and Gerbich03. If a deletion is present, PCR primers flanking the deletion would amplify short PCR products in reactions 9–15 in (A). Reprinted from Br. J. Haematol 2017 May;177(4):630–640 with permission

Molecular workup utilizing newly developed diagnostic PCRs revealed that the mother harbors a recently characterized homozygous GYP C exon 3 deletion.3 As the father is homozygous wildtype (GE*01/GE*01), all offspring are obligate heterozygotes expressing Ge3 antigen (GE*01/GE*01.-03) and susceptible to HDFN.

Severe hemolysis is expected in subsequent pregnancies, and intrauterine transfusion will likely be necessary. ABO mismatch is not a significant risk in intrauterine transfusions and repeated blood donation for antigen-negative blood from the pregnant mother has been reported.11

GYP C stabilizes the RBC membrane and Ge3- cells are known to be unstable ex vivo. While maternal Hgb prior to pregnancy was normal (12–13 g/dl), collected maternal blood did not survive storage beyond 2 weeks, necessitating repeat blood collection from the mother while she was still anemic, although both collections were uncomplicated. Alternative approaches include collecting less blood from the mother each time, returning unused blood to the mother, or performing exchange transfusion in the infant to provide more Ge3- blood while lowering alloantibody load to decrease the need for frequent transfusions.

This unusual case highlights: (1) the importance of early recognition of rare alloantibodies in pregnancy, and the need to have a system in place when antigen-matched blood is not readily available; (2) the value of molecular diagnosis in rare alloimmunizations; and (3) the collaboration of multiple disciplines for successful management of rare HDFN, including primary care pediatrics, pediatric hematology,
obstetrics, blood bank, blood donor center, and transfusion medicine reference laboratories, which were, in this case, BloodCenter of Wisconsin, American Red Cross, and Blood Transfusion Service Zurich, Swiss Red Cross.

ACKNOWLEDGMENT
Pediatric nurse practitioner Jan Cantrell is credited for recognizing severe anemia of HDFN in this infant and immediately referring the infant to pediatric hematology.

CONFLICT OF INTEREST
The authors have no conflict of interest.

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