Acute Kernicterus in a Neonate With O/B Blood Group Incompatibility and a Mutation in SLC4A1

We cared for a term female newborn, who at 108 hours of age, with a total serum bilirubin of 15.4 mg/dL, was discharged from the hospital on home phototherapy. At a return appointment 44 hours later, her total serum bilirubin was 41.7 mg/dL and signs of acute kernicterus were present. Maternal/fetal blood group O/B incompatibility was identified, with a negative direct antiglobulin test, which was positive on retesting. She had abundant spherocytes on blood smear, and these persisted at follow-up, but neither parent had spherocytes identified. A heterozygous SLC4A1 E508K mutation (gene encoding erythrocyte membrane protein band 3) was found, and in silico predicted to result in damaged erythrocyte cytoskeletal protein function. No mutations were identified in other red cell cytoskeleton genes (ANK1, SPTA1, SPTB, EPB41, EPB42) and the UGT1A1 promoter region was normal. Neurologic follow-up at 2 and 4 months showed developmental delays consistent with mild kernicterus.

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KEY WORDS
kernicterus, jaundice, neonate, hemolysis, ABO, hereditary spherocytosis

ABBREVIATIONS
DAT—direct antiglobulin test
HS—hereditary spherocytosis
TSB—total serum bilirubin

Dr Christensen is a physician involved in the care of the patient in the pediatric hematology clinic. He drafted the initial manuscript and organized the data. Dr Yaish, director of the pediatric hematology clinic, is the principal physician caring for the hematological problems of this patient. He contributed to the design of the report and to the writing of the manuscript. Drs Nussenzveig and Agarwal, research scientists at ARUP Laboratories, conceived of and established the flow-cytometric studies and the gene sequencing aspects that are central to this report. They wrote critical aspects of the manuscript. Dr Reading, a research scientist at ARUP Laboratories, supervised the promoter polymorphism studies, provided essential guidance in study design, and wrote critical aspects of the manuscript. Dr Eggert has been involved with this case since the first week. He devised and implemented the Intermountain Healthcare bilirubin screening program. He wrote aspects of the case report and critically reviewed the manuscript. Dr Prchal, a senior hematologist and scientist, has been involved as a consultant on the clinical case and supervises the ARUP scientists in aspects of hematology and genetics. He provided critical advice in case management, contributed to the design and writing of the report, and critically reviewed the manuscript. All authors approved the final manuscript as submitted.

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Several recent reports suggest that a high proportion of neonates who develop hazardous hyperbilirubinemia have no specific etiology discovered, and thus the jaundice is listed as “idiopathic.”1–3 We now report a neonate found to have a total serum bilirubin (TSB) of 41.7 mg/dL on day of life 6, accompanying signs of acute bilirubin encephalopathy, in which the family history and initial evaluation of the hyperbilirubinemia did not reveal the etiology. We hypothesized that hemolysis was an important contributor and used high-throughput gene sequencing to seek for mutations or polymorphisms that might have been relevant to hemolysis.

CASE

The patient was born by repeat cesarean delivery at 37 weeks 2 days of gestation to a 26-year-old Hispanic mother with blood group O (+). The parents were married and nonconsanguineous. The father was born in Pakistan and the mother in the United States of Puerto Rican ancestry. Neither parent nor siblings nor extended family members had a history of jaundice or anemia or gallstones. Birth weight was 3270 g (64th %), length was 52 cm (90th %), occipitofrontal circumference was 35.5 cm (89th %), and the Apgar scores were 8 and 9. She was sent to the well-infant nursery and fed Enfamil 20 kcal/oz formula.

The infant appeared normal, fed well, and when she was 51 hours old a serum bilirubin of 14.3 mg/dL was obtained as part of a universal TSB screening program. This result was identified as well above the 95th percentile hour-specific nomogram. The infant’s blood type was B (+) and the direct antiglobulin test (DAT; Coombs test) was negative. Phototherapy was begun by using a Giraffe Spot PT Lite (Ohmeda Medical, Laurel, MD) with a measured irradiance of 30 $\mu$W/cm$^2$/nm at 38 cm (15 inches) from the skin. TSB testing was repeated at 75 and 104 hours (Fig 1).

She continued to feed well and was discharged from the hospital at 108 hours with a phototherapy blanket (Wallaby Phototherapy System; Fiberoptic Medical Products, Inc, Allentown, PA). The family was instructed in the use of the equipment and a return appointment was made for TSB testing within <48 hours after discharge. The parents were instructed to stop using the phototherapy blanket the morning of the recheck. They followed this schedule and returned to the pediatrician’s office 44 hours after discharge. The repeat TSB was 41.7 mg/dL and she was admitted to the regional children’s hospital for exchange transfusion.

On admission, she was severely jaundiced but appeared well hydrated and weighed 3100 g (95% of birth weight). She initially seemed alert and was feeding well, but under intensive phototherapy (5 Giraffe spot lights and 1 Wallaby bili blanket) while preparing for the exchange transfusion, she became hypertonic with opisthotonic posturing and a possible generalized seizure.

Before the exchange transfusion, her hematocrit was 25.1% and the mean cell volume was 94.1 fl. The mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin could not be reported because of markedly icteric plasma. The platelet count (250 000/$\mu$L) and leukocyte differential cell count were within the reference ranges for age but nucleated red blood cells were present (0.4/100 white blood cells). Her reticulocyte count was 2.4% and her blood film had a large proportion of spherocytes. Repeat DAT testing drawn before the double-volume exchange transfusion was positive and her urine contained free hemoglobin.

Brain MRI testing with and without spectroscopy were performed before discharge from the hospital on day of discharge.
life 10 and were interpreted as normal. At 2 months, she was evaluated in the pediatric neurology clinic where possible hypertonicity and poor nipple feeding were noted. Her mother reported that she did not focus on faces, raising a concern for a neurologic deficit. At that time, the DAT was repeated again and was negative.

At 3 months, she was evaluated in the pediatric hematology clinic. Her hematoctrit was 30.4%, mean cell volume 86.6 fl, mean corpuscular hemoglobin concentration 34.5 g/dL, reticulocytes 3.7%, and the blood contained ~10% spherocytes but no other abnormalities. Her parents were hematologically normal, with no spherocytes seen on blood smear. Erythrocyte osmotic fragility testing of the infant was normal, and UGT1A1 promoter genotyping was normal, with homozygosity for TA6.[6]

A flow cytometry–based test using eosin-5-maleimide dye showed decreased staining, typical of hereditary spherocytosis (HS). The dye binds stoichiometrically to erythrocyte membrane protein band 3. A decrease in mean fluorescence intensity of eosin-5-maleimide–tagged erythrocytes is associated with band 3 deficiency and red cell cytoskeleton disorders.[5,6]

Genetic analysis of erythrocyte membrane proteins was performed by using HaloPlex (Agilent Technologies, Santa Clara, CA) for targeted gene capture and sequencing on a HiSeq 2000 system (Illumina, San Diego, CA). Briefly, DNA was fragmented by using restriction enzymes and then denatured. A probe library was hybridized to the targeted fragments (Table 1). The probe also contains a method-specific sequencing motif incorporated during circularization and a barcode sequence. Probes are biotinylated and the targeted fragments retrieved with magnetic streptavidin beads for sequencing. Sequence analysis revealed a heterozygous GAG>AAG mutation, resulting in the amino acid substitution E508K in exon 13 of the SLC4A1 (band 3) gene. The E508K substitution is localized to the transmembrane 4 spanning domain of band 3 protein. This is predicted in silico to be a functionally significant mutation affecting band 3 protein. No mutations were identified in other erythrocyte cytoskeletal genes associated with HS,7 namely, ANK1 (ankyrin-1), SPTA1 (spectrin, α, erythrocyte 1), SPTB (spectrin, β, erythrocyte), EPB41 (band 3), or EPB42 (band 4.1), or in selected erythrocyte enzyme genes, mutations of which can cause hereditary hemolytic anemia (Table 1).

**DISCUSSION**

Among 126 neonates with kernicterus reported in the US Kernicterus Registry, 69 (55%) had no etiology identified.[1,2] Similarly, we reported that during a 10-year period at Intermountain Healthcare, 32 neonates had a TSB ≥30.0 mg/dL and 66% of these had no etiology identified; 112 had a TSB of 25.0 to 29.9 mg/dL and 86% of these had no etiology identified.[5] We speculate that many of the unfortunate neonates with hazardous hyperbilirubinemia had unrecognized hemolysis.[3,8] We speculate further that many neonates who develop kernicterus have more than 1 mechanistic explanation. As detailed by Watchko and colleagues,[9,10] Kaplan et al,[11] and Stevenson et al,[12] sometimes 2 or more factors, each with a minor effect on the serum bilirubin concentration, can interact to result in a high bilirubin level. For instance, an excessive load of bilirubin from hemolysis can be compounded by a reduced uptake and/or conjugation of bilirubin in hepatocytes. This type of pathologic synergy has been observed in neonates who develop kernicterus and had HS and coexpression of UGT1A1 promoter polymorphisms (Gilbert syndrome).[13] Also, Kaplan et al14,15 reported this pathologic synergy in neonates coexpressing glucose-6-phosphate dehydrogenase deficiency and Gilbert syndrome.

The early hyperbilirubinemia in our patient (14.3 mg/dL at 51 hours) was consistent with a hemolytic process. Thus, she had a risk factor for severe jaundice, but she lacked others. The family history and DAT were negative and she was formula feeding well. Once the high TSB was found on day of life 6, spherocytes were seen on the blood film and presumed to be from O/B alloimmune hemolysis. The repeat DAT was positive and anti-B antibodies were eluted from her erythrocytes. However, the extremely high TSB and large proportion of spherocytes that persisted on future blood film examination long after the DAT test normalized were not consistent with O/B alloimmunization. Later testing revealed a heterozygous mutation in the gene encoding erythrocyte membrane protein band 3. Mutations of this gene are a well-known cause of HS.[7,16,17] We speculate that the extremely high rate of rise in TSB between days 4 and 6 may have involved...

**TABLE 1** Genes Sequenced in This Patient in Search of Mutations Contributing to the Extreme Neonatal Jaundice That Occurred With No Family History of Anemia or Jaundice

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANK1</td>
<td>Ankyrin-1</td>
</tr>
<tr>
<td>EPB41</td>
<td>Erythrocyte membrane protein band 4.1</td>
</tr>
<tr>
<td>EPB42</td>
<td>Erythrocyte membrane protein band 4.2</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GPI</td>
<td>Glucose-6-phosphate isomerase</td>
</tr>
<tr>
<td>HIF1A</td>
<td>Hypoxia-inducible factor-1α</td>
</tr>
<tr>
<td>HK1</td>
<td>Hexokinase 1</td>
</tr>
<tr>
<td>NTSGB</td>
<td>5′-nucleotidase, cytosolic III</td>
</tr>
<tr>
<td>PIN1</td>
<td>Peptidylprolyl cis/trans isomerase, NIMA-interacting 1</td>
</tr>
<tr>
<td>PKLR</td>
<td>Pyruvate kinase, liver, and red blood cell</td>
</tr>
<tr>
<td>SLC4A1</td>
<td>Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3)</td>
</tr>
<tr>
<td>SPTA1</td>
<td>Spectrin, α, erythrocyte 1</td>
</tr>
<tr>
<td>SPTB</td>
<td>Spectrin, β, erythrocyte</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>UDP-glucuronosyltransferase promoter region</td>
</tr>
</tbody>
</table>
both O/B hemolytic disease and hemolysis from HS. Obviously, neither the DAT-positive situation nor the HS were known on day of life 4 when it was decided to discharge her on home phototherapy with a return TSB check <48 hours later.

Preventing kernicterus can be a goal of well-infant care.18,19 This is so even for mild cases of kernicterus, which can cause intellectual as well as physical impairment. To accomplish this, means are needed to identify those few neonates who have a high risk for kernicterus based on factors unknown or unknowable to the pediatrician at the time of hospital discharge. One potential example might be the use of rapid, noninvasive, end-tidal CO measurement to assess bilirubin production rate in selected neonates.20 For instance, this could be applied to neonates going home on phototherapy, or those who have ABO incompatibility, even if the DAT is negative, or to those with a family history of severe jaundice even if their TSB is not above a cutoff value. Whether end-tidal CO measurement of this neonate at the time of the initial hospital discharge would have been of value is unknown.

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