Spherocytosis in Newborn Secondary to Novel Heterozygous Mutation in SPTB Gene

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Abstract

This case report describes a novel mutation of the *SPTB* gene as a potential pathogenic cause of spherocytosis. A three-week-old male presented with clinical and laboratory signs consistent with hemolytic spherocytosis (HS), including jaundice, hyperbilirubinemia, anemia, reticulocytosis, negative Coombs test, no ABO or Rh incompatibility, and a peripheral blood smear notable for numerous spherocytes. His lab work demonstrated persistent anemia despite daily folate prompting next-generation sequencing (NGS) which revealed a novel mutation in the *SPTB* gene resulting in a non-functioning protein product. Correlation of the genetic finding with clinical presentation may help guide management for this and future patients.

Introduction

Hereditary Spherocytosis (HS) is a common type of congenital hemolytic anemia, seen in 1 in every 2 000 patients. It is caused by genetic variations in erythrocyte membrane and cytoskeleton proteins such as spectrin, ankyrin, band 3, and band 4.2, which then cause the red blood cell (RBC) to have an abnormal shape. This can present in patients as pallor and jaundice. Patients may have anemia, abnormal peripheral blood smears, reticulocytosis and hyperbilirubinemia. HS is important to identify to ensure that the patient receives timely treatment to prevent serious outcomes of this disease, such as kernicterus in neonates, cholelithiasis and hemolytic crises.[1]

Results

A male neonate born at 40w4d via spontaneous vaginal delivery presented to the neonatal intensive care unit (NICU) at 38-hours-old with hyperbilirubinemia on phototherapy. His vital signs showed a blood pressure was 54/31 mmHg, pulse of 140 beats/minute, body temperature of 37.2°C (99°F), respiratory rate of 70 breaths/minute, and oxygen saturation of 99% on room air. The patient's head circumference, length, and weight were all within one standard deviation of average for his age range and sex. Physical examination revealed diffuse jaundice and scleral icterus. Both of his parents identified as African American, and his family history was negative for hemoglobinopathies and hemolytic anemia.

His lab work was notable for a total bilirubin of 20.2 mg/dL (normal range, 0-8.2), direct bilirubin of 0.4mg/dL (0.0-0.6), hemoglobin of 17.2 g/dL (14.7-18.6), mean corpuscular hemoglobin concentration (MCHC) of 26.0 g/dL (31-34.2), mean corpuscular volume of 98.6fL (97.3-109.8), red cell distribution width of 21.9% (12.0-15.2), and a reticulocyte count of 9.2% (2.5-6.5). These findings in combination with the patient's jaundice on exam and down trending hemoglobin to 12.9 g/dL suggested a hemolytic process. The differential diagnosis focused on both intrinsic and extrinsic causes of hemolytic anemia, including membrane defects, enzyme deficiencies such as G6PD and pyruvate kinase deficiencies, hemoglobinopathies, as well as autoimmune diseases.[2]

On consultation with the pediatric hematology-oncology team, additional labs were suggested, revealing a negative Coombs test and no ABO or Rh incompatibility. He was found to have a G6PD Quantity of 22.1 U/g[Hb] (7.0-20.5) and a Pyruvate Kinase Enzyme Activity of 11.9 U/g[Hb] (5.5-12.4). Additionally, a hemolytic anemia screening panel was ordered on day of life (DOL) 6. This panel revealed increased osmotic fragility, decreased Eosin 5-Maleimide binding, and decreased Protein band 3, suggesting a membrane-related disease, such as HS or hereditary elliptocytosis (HE).

Peripheral blood smears were also used to investigate the cause of the patient's symptoms. A smear from DOL 2 showed polychromasia with normocytes, occasional spherocytes, and rare schistocytes in the absence of bite or helmet cells. White blood cells (WBCs) and platelets showed normal morphology. At this point, the differential included HS or HE, as the smear was significant for spherocytes on blood smear, and the patient had reticulocytosis in the absence of an immune cause. HS and HE can present similarly on peripheral smears early in life although they have different disease courses to prepare for.[3]

The patient was discharged in stable condition from the NICU after 8 days with daily outpatient folic acid and vitamin D3. He continued to have significant reticulocytosis and indirect hyperbilirubinemia. Because of the persistently abnormal lab values, the negative family history, and with a goal of determining the underlying cause and inheritance risk in her siblings and in his own offspring, a NGS panel was ordered.

The patient was evaluated using a Hereditary Hemolytic Anemia Panel, which revealed a heterozygous out-of-frame deletion in Exon 2-3 of the*SPTB* gene, resulting in an abnormal and non-functioning spectrin protein product. Loss-of-function mutations of the *SPTB* gene are known to cause abnormally shaped erythrocytes and are implicated in both HS and HE.[4] However, the specific deletion present in this patient has not been reported in literature and is thus potentially pathogenic.

A repeat peripheral smear performed at 10 months of age showed RBC morphology as normocytic, normochromic with significant polychromasia. Numerous spherocytes with occasional acanthocytes and echinocytes were noted. WBCs and platelets had a normal appearance (Fig. 1). This confirmed a diagnosis of HS, specifically moderate-severe HS. The patient had hemoglobin between 6-8, as well as a reticulocyte count between 6-10%.[5] Additionally, the parents were offered a NGS test to discover if this is a de novo or an inherited mutation.

Discussion

This case expands the base of knowledge in the HS field by identifying and tracking the impact of a deleterious heterozygous *SPTB*loss-of-function mutation not previously reported in the literature. Mutations in beta-spectrin, a principal cytoskeletal protein of the intracellular side of the plasma membrane, often show an autosomal dominant pattern of inheritance; however, this patient developed HS in the absence of a positive family history.[6] Patients with *SPTB* mutations typically present with mild to moderate-severe phenotypes/symptoms, with the possibility of blood transfusions and splenectomy.[7] This novel mutation guides us in tracking the patient's clinical course over time. This also elucidates the class of HS and special considerations for future patient's evaluation.

One limitation of this study is that the parents have not yet completed genetic testing. It is therefore unknown if this mutation has an autosomal recessive pattern or if it is a de novo mutation. The parents have been counseled about the risk of HS in future offspring at this time.

Conflict of Interest statement

All authors, Daphna Varadi, Benjamin Caplan, Dr. Maria Scarano, and Dr. Rafat Ahmed, declare that they have no conflicts of interest.

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Table

TABLE 1 Graph demonstrating results timeline

Age	Event / Results (normal in parenthesis)
DOL[1] 1	*Physical Exam*: notable for jaundice, scleral icterus *Lab work*: Direct Antiglobulin Test, Anti-IgG Negative
DOL[1] 2	*Physical Exam*: notable for jaundice, scleral icterus *Lab work*: Total Bilirubin maximum- 20.2 mg/dL (0-8.2); Reticulocyte Count- 9.2% (2.5-6.5) *Blood Smear*: polychromasia with normocytes, occasional spherocytes, and rare schistocytes in the absence of bite or helmet cells *Consultants*: Pediatric Hematology-Oncology Team Consulted
DOL[1] 7	*Lab work*: Hemolytic Anemia Panel Elevated Osmotic Fragility Decreased Band 3 Decreased Eosin 5-Maleimide binding
2 Months	*Lab work*: G6PD Quantity- 22.1 U/g[Hb] (7.0-20.5) Pyruvate Kinase Enzyme Activity- 11.9 U/g[Hb] (5.5-12.4) *Consultants*: Seen in Pediatric Hematology Office and started on folic acid and vitamin D3
10 Months	*Blood Smear*: Numerous spherocytes, polychromasia
18 Months	*Lab work*: Hemoglobin- 10.6 g/dL (10.3-13.3) MCHC[2]- 34.0 g/dL (31.9- 35.2)

[1]DOL: Day of Life

[2]MCHC: Mean Corpuscular Hemoglobin Concentration

Figures

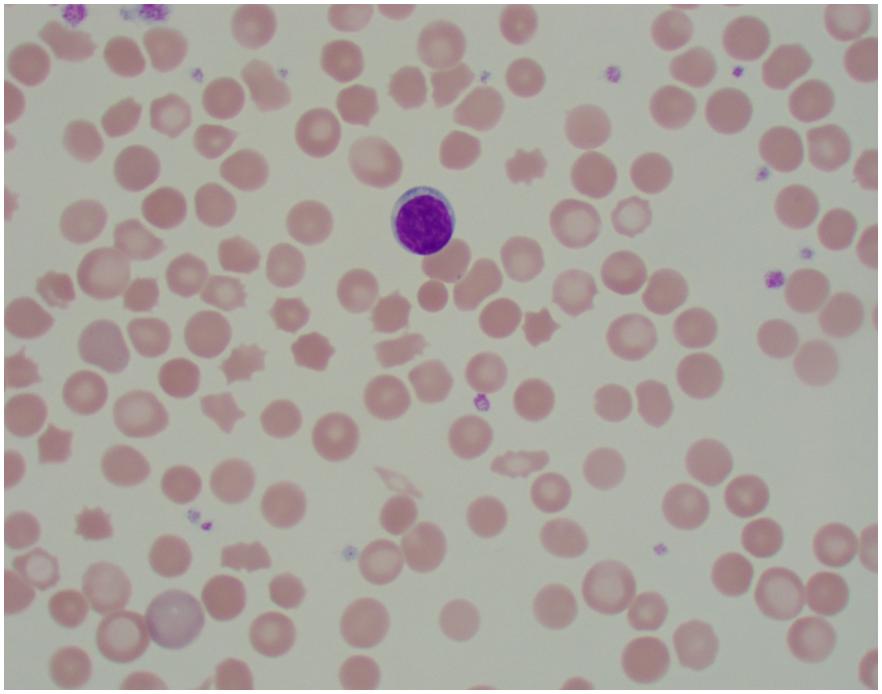

FIGURE 1 Peripheral smear at 10 months and 27 days of age.

polychromasia

spherocytes