Novel mutation in spta1 gene associated with severe hemolytic anemia

Abstract
Hereditary spherocytosis (HS), elliptocytosis (HE), and pyropoikilocytosis (HPP) are caused by mutations in the genes which encode erythrocyte cytoskeletal proteins. We report a patient with severe hemolytic anemia with a complex set of mutations, including a novel mutation predicted to cause abnormal splicing of SPTA1 gene, highlighting the utility of molecular diagnostics in patients with no identifiable family history of erythrocyte cytoskeletal disorders.

Keywords: spectrin, anemia, spherocytosis, elliptocytosis, hereditary

Introduction
Hereditary spherocytosis (HS), hereditary elliptocytosis (HE), and hereditary pyropoikilocytosis (HPP) are caused by mutations in five genes ANK1, SLC4A1, SPTA1, SPTB and EPB4 which encode for the erythrocyte cytoskeletal proteins, ankyrin, band 3, α-spectrin, β-spectrin and protein 4.2, respectively. These mutations and the resultant defective proteins lead to loss of red cell membrane surface area and reduced red cell deformity. Approximately 25% of patients will not have a clear family history. These autosomal recessive or de novo cases of severe hemolytic anemia pose a diagnostic challenge. We report a pediatric case of hemolytic anemia without a family history, where molecular diagnostics provided a definitive diagnosis of a red cell membrane disorder. The results of molecular diagnostics demonstrated a complex set of mutations, including a novel mutation in the SPTA1 gene, which assisted in counseling his parents that childhood immunizations and splenectomy would be the appropriate treatment.

Case presentation
The patient, of Caucasian ethnicity and lacking regular pediatric visits, presented at 3.5years of age with microcytic anemia, indirect hyperbilirubinemia, reticulocytosis, and splenomegaly (Table 1). The history was significant for hyperbilirubinemia in the newborn period. Workup included iron studies, liver function tests, chemistry panel, Coombs test, and flow cytometry for paroxysmal nocturnal hemoglobinuria, all of which were normal. The peripheral blood smear showed marked poikilocytosis of erythrocytes (Figure 1A–1C). Hemoglobin electrophoresis demonstrated 90.4% hemoglobin A (reference range 95.0 – 98.0%), 2.7% hemoglobin A2 (reference range 2.0 – 3.3), 6.9% hemoglobin F (reference range 0.0–2.0). No unstable variant was detected. Alpha-globin gene analysis was negative for deletions within the gene cluster. Osmotic fragility (OF) testing, prior to any packed red blood cell (PRBC) transfusions, was abnormal with increased red blood cell lysis suggesting the presence of spheroocytes. However, the eosin-5-maleimide (EMA) binding test showed a normal staining pattern. Red blood cell enzyme levels, evaluated prior to any PRBC transfusions, of glucose-6-phosphate dehydrogenase, pyruvate kinase, glucose phosphate isomerase, hexokinase, glutathione, adenine deaminase, adenylyl kinase, phosphofructokinase, phosphoglycerate kinase, and triosephosphate isomerase were normal or elevated. Parents, who are non-consanguineous and asymptomatic, declined to have blood work of their own drawn. A younger female sibling had normal complete blood count, reticulocyte count, and bilirubin level. Parents declined all childhood immunizations for their children. The patient’s hemoglobin intermittently decreased, coinciding with febrile episodes and temporary increase lysis. The parents consented to PRBC transfusions during these episodes (Figure 2A & Figure 2B). Over the course of 5years, the patient’s spleen continued to enlarge. Prior to packed red cell transfusions, serum ferritin levels were slightly increased, maximum 299mcg/L (reference 7–142mcg/L). MRI imaging to monitor for potential iron overload demonstrated a slight increase of average liver iron content over time, but not beyond the threshold that required iron chelation. Surveillance echocardiograms, which were initially normal, demonstrated dilated cardiomyopathy at 9years of age. Family permitted molecular diagnostic testing and chronic transfusions after cardiomyopathy developed.

Table 1 Laboratory values at diagnosis and 2years post-splenectomy At diagnosis 2years post- splenectomy reference range

<table>
<thead>
<tr>
<th></th>
<th>(3.5 years old)</th>
<th>2 years postsplenectomy</th>
<th>Reference Range</th>
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</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>6.9</td>
<td>14.5</td>
<td>11.5 – 14.5gm/dL</td>
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<tr>
<td>MCV</td>
<td>74.8</td>
<td>83.2</td>
<td>80.0 – 94.0fl</td>
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Table Continued...

<table>
<thead>
<tr>
<th>Test</th>
<th>(3.5 years old)</th>
<th>2 years postsplenectomy</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCHC</td>
<td>34.6</td>
<td>36.2</td>
<td>32.0 – 37.0g/dL</td>
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<tr>
<td>Platelet</td>
<td>221</td>
<td>546</td>
<td>140 – 400x103/μL</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>395</td>
<td>ND</td>
<td>40 – 100x103/μL</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>2.7</td>
<td>0.5</td>
<td>0.1 – 1.0mg/dL</td>
</tr>
<tr>
<td>Indirect bilirubin</td>
<td>2.5</td>
<td>ND</td>
<td>0.1 – 0.6mg/dL</td>
</tr>
</tbody>
</table>

Figure 1A–1C Peripheral blood smears pre-splenectomy (A, 100x) and after red cell transfusion (B, 400x) show moderate to marked anisopoikilocytosis of erythrocytes with spheroocytes (arrows) and reticulocytes (triangle). Post-splenectomy peripheral blood smear (C, 600x) show mild anisocytosis and polychromasia, with post-splenectomy expected findings of rare Howell Jolly bodies (HJB) and moderate Pappenheimer bodies (PB). Wright Giemsa stain.

Figure 2A & 2B Time from diagnosis vs hemoglobin levels. Boxed inset in A is shown in greater detail in B. PRBC: packed red blood cell.

Methods
Massively parallel sequencing was performed using a diagnostic panel of 28 genes encoding erythrocyte cytoskeletal proteins, enzymes, and UGT1A1 polymorphisms. Targeted gene capture and library construction was performed using Sure Select kit (Agilent Technologies, Santa Clara, USA).

Results
Three variants in SPTA1 gene were identified by massively

parallel sequencing. A novel pathogenic mutation (c.7134+2T>G, p.?) was identified in addition to heterozygous low expression variants in the αLEPRA and αLELY alleles. A fourth heterozygous variant of unknown significance was found in the SPTB gene (c.4564-4G>A). Given the genetic testing results, the parents consented to immunizations, splenectomy and cholecystectomy. Four months after splenectomy, the patient’s hemoglobin improved to 15.2g/dL and echocardiogram changes were resolving.

**Discussion**

HS and HE are the most common red cell membrane disorders, often associated with an autosomal dominant inheritance.1 In patients with clinical features of hemolytic anemia and a family history of HS, no additional diagnostic test is required.2 Severe forms of HE, known as HPP, and autosomal recessive HS are often due to autosomal recessive inheritance or de novo mutations, and these patients may lack a clear family history of hemolytic anemia.3,2 Autosomal dominant HS is often associated with ankyrin 3, band 3, or β spectrin gene mutations, whereas de novo mutations leading to recessive disease are associated with mutations in ankyrin and β spectrin genes.2 Alpha spectrin mutations are rare in HS. Splenectomy ameliorates the clinical symptoms associated with hemolysis in HS, HE, and HPP, but may be more beneficial in spectrin-deficient and ankyrin-deficient cases compared to band 3-deficient cases.3 In these non-dominantly inherited patients, additional laboratory testing may be necessary. Tests available to aid in the diagnosis of a patient with suspected defects in erythrocyte cytoskeletal proteins includes OF testing, glyceral lysis test, cryohemolysis test, osmotic gradient ektacytometry, EMA binding test, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Table 2). Each test has their limitations, and a combination of tests may be necessary.4 According to the guidelines published by the British Committee for Standards in Haematology, the EMA binding test or cryohemolysis test are the recommended screening methods for the diagnosis of hereditary spherocytosis in cases that are not clear.2 SDS-PAGE can be used to detect the defective protein if screening tests are non-diagnostic.2 Confirmatory testing using molecular diagnostic technique may be warranted when tests are non-diagnostic or the role of splenectomy may be questioned.1 Spectrin deficiency is the most frequent membrane defect in HS patients diagnosed during childhood.5 Spectrin molecules, composed of α- and β-spectrin heterodimers, are critical in maintaining the erythrocyte membrane, shape, and function.6,8 In normal erythroid cells, α-spectrin chains are produced in three to four-fold greater number than β-spectrin chains.9,30 Thus, a single mutation in β-spectrin gene is sufficient to cause a disease phenotype, whereas α - spectrin defects are clinically relevant only if they are inherited with a pathogenic allele either as homozygous or compound heterozygous.3,32 Alpha-spectrin mutations occur in 5% of HS patients.2 A α-spectrin expression reduction to less than 25% of the normal yield is necessary to cause the symptoms of spherocytosis.32

Our patient was found to have three variants in SPTA1 gene identified by massively parallel sequencing. A novel mutation (c.7134+2T>G, p.?) was identified in addition to heterozygous low expression alleles αLEPRA (LEPRA: Low Expression Praague)13,14 and αLELY (LELY: Low Expression Lyon).15,16 αLEPRA mutation activates an alternative splice site in SPTA1, resulting in a shorter and less abundant protein product, approximately 1/5 of the full length of α-spectrin protein and 16% of the total product, compared to normal allele.10,13 The αLELY mutation, which causes partial skipping in exon 46 in 50% of the transcripts, results in chains unsuitable for dimerization with beta spectrin.10,15 Patients are clinically asymptomatic if they are heterozygote for αLEPRA or αLELY as the chain produced by the normal allele will preferentially dimerize with the beta chain.15,16 In contrast, patients have severe hemolytic anemia when αLEPRA or αLELY are paired in trans to a pathogenic SPTA1 allele, as in our patient. This is particularly the case with αLEPRA as it is weak enough to manifest hematological disease whereas αLELY may generate a sufficient supply of α-spectrin.19 Compound heterozygotes for αLEPRA and a second defective α-spectrin allele are more severely affected than patients who are homozygous for αLEPRA allele.13,15,14

**Table 2 Laboratory tests**

<table>
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<tr>
<th>Test</th>
<th>Comments</th>
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<tr>
<td>Osmotic Fragility</td>
<td>Low sensitivity and specificity 4, 22</td>
</tr>
<tr>
<td>Glycerol Lysis Test</td>
<td>Limited availability in US; low sensitivity and specificity 4, 22</td>
</tr>
<tr>
<td>Cryohemolysis</td>
<td>Limited availability; conflicting sensitivities and specificities 4, 5, 23, 24</td>
</tr>
<tr>
<td>Osmotic Gradient Ektacytometry</td>
<td>Limited availability; analysis must be performed within 48 hours of obtaining the blood sample</td>
</tr>
<tr>
<td>Eosin-S-Maleimide Binding</td>
<td>High sensitivity, specificity, positive and negative predictive values 1, 2, 4</td>
</tr>
<tr>
<td>SDS – PAGE</td>
<td>Cut-off to define abnormal is debated 1</td>
</tr>
<tr>
<td></td>
<td>May not classify 10% of non-splenectomized patients, particularly spectrin and ankyrin deficient patients 5, 25</td>
</tr>
</tbody>
</table>

Variants in SPTB are known to cause HS through mutations which introduce mRNA transcript instability or truncation of the produced beta-spectrin protein.19 HS caused by defects in SPTB is most often autosomal dominant in nature.3 Our patient demonstrated a SPTB variant (c.4564-4G>A), that has been previously reported in an individual with HS and reduced SPTB mRNA level.26 A previously reported computational study predicted this SPTB variant would result in abnormal splicing of SPTB gene, however, to date, there have been no experimental studies which have demonstrated the effect of this mutation.31 This variant may be inherited from either of his asymptomatic parent with resultant severe disease in our patient when present with the three SPTA1 variants. Alternatively, this may be a de novo mutation.22-26

**Conclusion**

This case report is significant for two reasons: first, a new SPTA1 variant, suspected to be pathogenic is found and second, this patient harbors a complex genotype suggesting that the interactions between the variants, including SPTA1 and SPTB gene mutations, may have resulted in the severe phenotype.27 Predictive models are available.
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References


