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Journal of Pediatrics and Neonatal Care

Case Report

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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

Volume 8 Issue 5 - 2018

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Received: January 29, 2018 | **Published:** February 29, 2018

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.[1] These mutations and the resultant defective proteins lead to loss of red cell membrane surface area and reduced red cell deformity.[2] Approximately 25% of patients will not have a clear family history.[3] 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 the 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 spherocytes. 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, adenosine deaminase, adenylate 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.

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).

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

	At diagnosis (3.5 years old)	2 years postsplenectomy	Reference Range
Hemoglobin	6.9	14.5	11.5 – 14.5g/dL
MCV	74.8	83.2	80.0 – 94.0fl
MCHC	34.6	36.2	32.0 – 37.0g/dL
Platelet	221	546	140 – 400x10^3/μL
Reticulocyte	395	No Data	40 – 100x10^3/μL
Total bilirubin	2.7	0.5	0.1 – 1.0mg/dL
Indirect bilirubin	2.5	No Data	0.1 – 0.6mg/dL

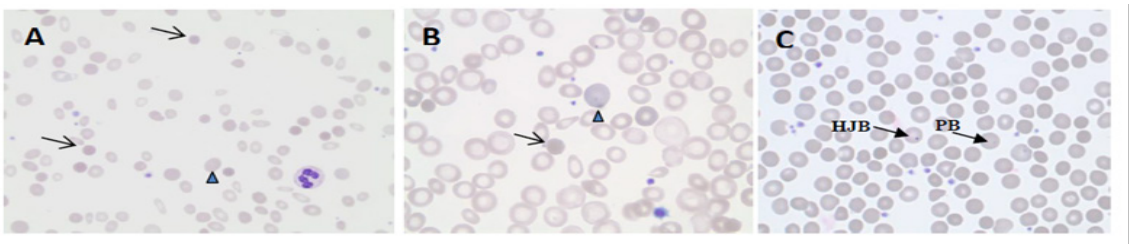

Figure 1A–1C Peripheral blood smears pre-splenectomy (A, 100x) and after red cell transfusion (B, 400x) show moderate to marked anisopoikilocytosis of erythrocytes with spherocytes (arrows) and reticulocytes (triangle). Post-splenectomy peripheral blood smear (C, 600x) show mild anisocytosis and polychromasia,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

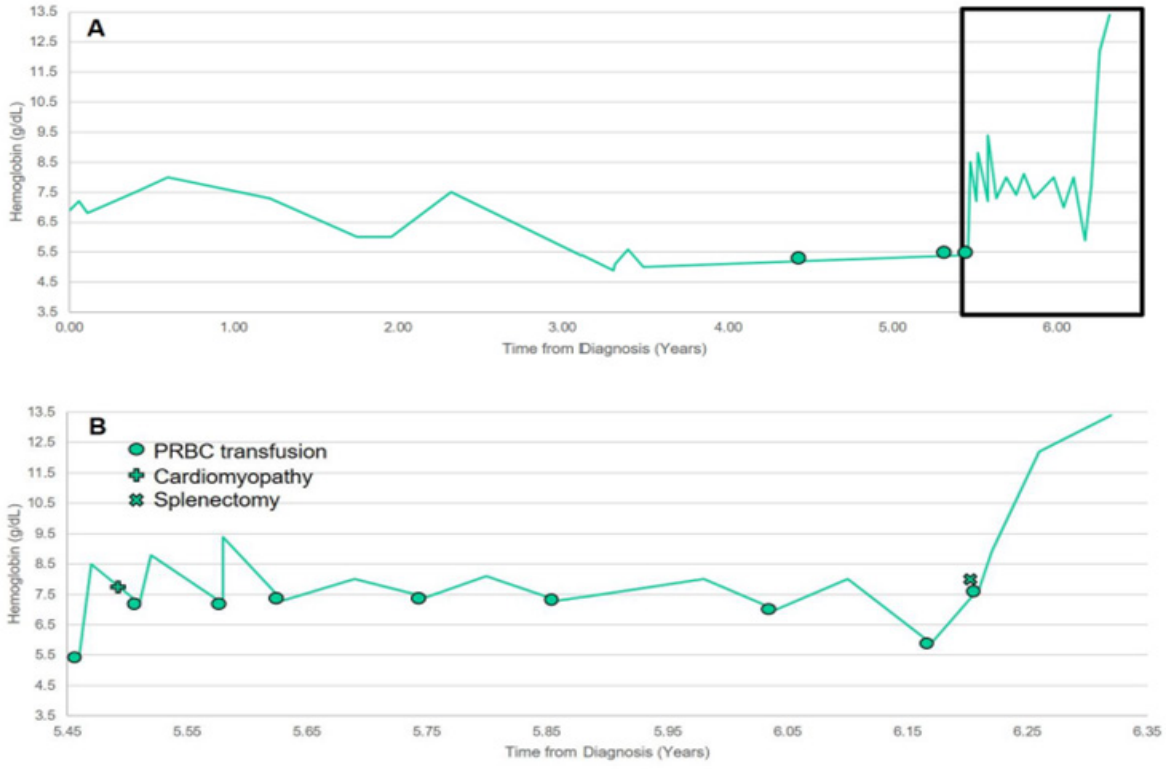

Figure 2A & 2B Time from diagnosis vs hemoglobin levels. Boxed inset in A is shown in greater detail in B. PRBC: packed red blood cell.

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 α^{LEPRA} and α^{LELY} 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.

Citation: Polega J, Stumph J, Agarwal A, et al. Novel mutation in *SPTA1* gene associated with severe hemolytic anemia. *J Pediatr Neonatal Care.* 2018;8(5):250–253. DOI: 10.15406/jpnc.2018.08.00351

Discussion

HS and HE are the most common red cell membrane disorders, often associated with an autosomal dominant inheritance.[4] In patients with clinical features of hemolytic anemia and a family history of HS, no additional diagnostic test is required.[3] 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.[3,4] 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.[3] 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.[2] In these non-dominantly inherited patients, additional laboratory testing may be necessary.

Tests available to aide in the diagnosis of a patient with suspected defects in erythrocyte cytoskeletal proteins includes OF testing, glycerol 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.[5] 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.[3] SDS-PAGE can be used to detect the defective protein if screening tests are non-diagnostic.[3] Confirmatory testing using molecular diagnostic technique may be warranted when tests are non-diagnostic or the role of splenectomy may be questioned.[4]

Spectrin deficiency is the most frequent membrane defect in HS patients diagnosed during childhood.[6] Spectrin molecules, composed of α- and β-spectrin heterodimers, are critical in maintaining the erythrocyte membrane, shape, and function.[7–9] In normal erythroid cells, α-spectrin chains are produced in three to four-fold greater number than β-spectrin chains.[10,11] 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.[2,12]

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.[1] 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 α^{LEPRA} (LEPRA: Low Expression PRAgue)[13,14] and α^{LELY} (LELY: Low Expression LYon).[15,16] α^{LEPRA} 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.[11,13] The α^{LELY} mutation, which causes partial skipping in exon 46 in 50% of the transcripts, results in chains unsuitable for dimerization with beta spectrin.[11,15] Patients are clinically asymptomatic if they are heterozygote for α^{LEPRA} or α^{LELY} as the chain produced by the normal allele will preferentially dimerize with the beta chain.[15–18] In contrast, patients have severe hemolytic anemia when α^{LEPRA} or α^{LELY} are paired in *trans* to a pathogenic *SPTA1* allele, as in our patient. This is particularly the case with α^{LEPRA} as it is weak enough to manifest hematological disease whereas α^{LELY} may generate a sufficient supply of α-spectrin.[11] Compound heterozygotes for α^{LEPRA} and a second defective α-spectrin allele are more severely affected than patients who are homozygous for α^{LEPRA} allele.[12,13,14]

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.[2] 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.[20] 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.[21] 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.

Table 2 Laboratory tests

Test	Comments
Osmotic Fragility	Low sensitivity and specificity [5, 25]
Glycerol Lysis Test	Limited availability in US; low sensitivity and specificity [5, 25]
Cryohemolysis	Limited availability in US; conflicting sensitivities and specificities [5, 6, 26, 27]
Osmotic Gradient Ektacytometry	Limited availability; analysis must be performed within 48 hours of obtaining the blood sample [4]
Eosin-5-Maleimide Binding	High sensitivity, specificity, positive and negative predictive values [3-5]
SDS – PAGE	Cut-off to define abnormal is debated [4]
	May not classify 10% of non-splenectomized patients, particularly spectrin and ankyrin deficient patients [6, 28]
	Limited availability [29]

US: United States, SDS – PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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.[22] Predictive models are available to determine the potential clinical significance of an individual genetic variant.[23,24] However, the complex interactions between multiple variants may not be fully elucidated using these predictive models. Continued molecular analyses and reporting on complex, atypical patients are necessary to determine how clinically relevant

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these variants are to counsel patients on treatment options, including splenectomy.[30] Further analyses, including spectrin content and molecular diagnostics on our patient and his family members will determine the effects of this suspected pathogenic novel *SPTA1* variant and how it interacts with α^{LEPRA}, α^{LELY}, and *SPTB* variant to cause severe hemolytic anemia.

Acknowledgements

None.

Conflict of interest

Authors declare that there is no conflict of interest.

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