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【 CASE REPORT 】

A Novel *SPTA1* Mutation in a Patient with Hereditary Spherocytosis without a Family History and Coexisting Gilbert's Syndrome

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Abstract:

Most patients with hereditary spherocytosis (HS) have a family history of disease, while those without such a history are difficult to diagnose. We herein report a case of HS with no family history harboring a novel heterozygous mutation of *SPTA1*, c.2161G>A (p.E721K), and a homozygous polymorphism of *UGT1A 1*6*. *In silico* analyses suggested that the mutation might contribute to the pathogenesis of HS. The coexistence of HS and Gilbert's syndrome increases the risk of gallstones. Therefore, splenectomy, alone or in combination with cholecystectomy, is recommended. The determination of genetic diathesis provides useful information for the management of hemolytic anemia.

Key words: hereditary spherocytosis, *SPTA1*, Gilbert's syndrome

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Introduction

Hereditary spherocytosis (HS) is the most common red blood cell (RBC) membrane disorder causing hereditary hemolytic anemia. Seventy-five percent of cases have a family history of HS (1), whereas cases without a family history are difficult to diagnose. Hereditary stomatocytosis (HSt) should be distinguished from HS because anemia and thrombosis in HSt patients are exacerbated by splenectomy (2). In contrast, splenectomy is a common treatment option for improving hemolysis in HS patients. Patients with HSt have a high mean corpuscular hemoglobin concentration, similar to that in patients with HS; therefore, it is difficult to distinguish HS and HSt using only biochemical tests.

Previous reports have shown that patients with HS have defects in the genes coding the structural organization of the RBC cytoskeleton, such as ankyrin (*ANK1*), band 3 (*SLC4A1*), protein 4.2 (*EPB42*), α-spectrin (*SPTA1*), and β-spectrin (*SPTB*) (1). The clinical utility of next-generation sequencing has been indicated, especially in neonates and atypical cases with undiagnosed hemolytic anemia (3).

We herein report an atypical case of HS without a family history harboring a novel mutation of *SPTA1* identified by target capture sequencing and *in silico* analyses. The patient also had *UGT1A1* polymorphism that resulted in Gilbert's syndrome and marked hyperbilirubinemia.

Case Report

A 15-year-old Japanese boy was admitted to our hospital because of jaundice for the past 3 days. On a physical examination, jaundice of the bulbar conjunctiva and whole skin was observed. Blood analyses showed a hemoglobin level of 9.3 g/dL, mean corpuscular hemoglobin concentration of 37.2 g/dL, and circulating reticulocyte count of 79‰. The serum levels of lactate dehydrogenase and indirect bilirubin were 283 IU/L (reference range, 124-224 IU/L) and 16.9 mg/dL (reference range, 0.1-0.8 mg/dL), respectively, and the concentration of urinary urobilinogen was elevated. Haptoglobin was undetectable, and direct and indirect Coombs' tests were negative. Computed tomography re-

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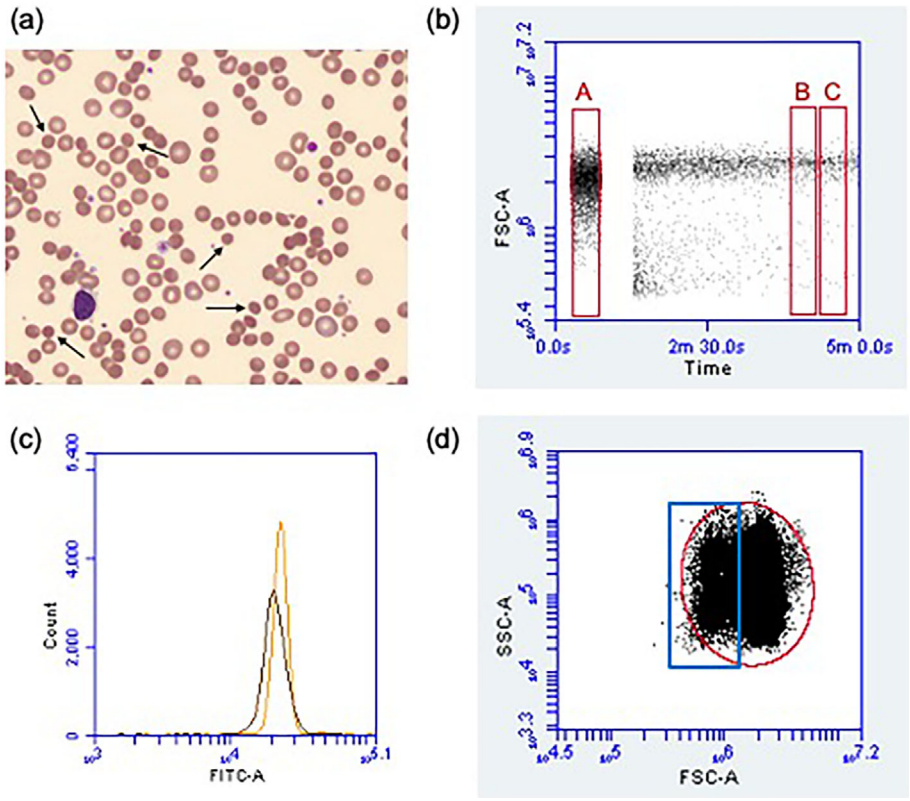

Figure 1. **May-Giemsa staining of a blood smear showed red blood cells (RBCs) of different sizes, including microspherocytes (arrows, ×400) (a). A flow cytometric osmotic fragility test showed an extremely low number of remaining RBCs. Whole blood was added to hypotonic saline and incubated. Each gate represents the remaining RBCs at each time point within 5 minutes. The percentage of residual red cells was calculated based on the following formula: [(cell number of Gate B+C)/2]/cell number of Gate A ×0.6 (b). In the eosin-5´-maleimide binding ability test, fluorescent signals were decreased among all RBCs. The x-axis shows the signal strength of eosin-5´-maleimide-labeled RBCs. The histogram of the patient's sample is shown in black, and that of the normal control is shown in yellow (c). Small RBCs were gated by their different sizes (d), and their fluorescent signals were further diminished.**

vealed no splenomegaly or gallstones. A peripheral blood smear showed RBCs of various sizes, including microspherocytes (Fig. 1a). An osmotic fragility test using saline solution demonstrated no erythrocyte vulnerability to osmotic changes in fresh and stored blood at 37°C for 24 h. The patient was suspected of having HS because of the RBC morphology, although he did not have a family history of the disease. After the hemoglobin level reached a minimum of 6.5 g/dL, his anemia improved gradually with fluid therapy and folic acid supplementation.

To clarify the pathophysiology, we performed an RBC enzyme assay, flow cytometric osmotic fragility test, and eosin-5´-maleimide binding ability test. The activities of glucose phosphate isomerase, pyruvate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic acid dehydratase, and pyrimidine-5´-nucleotidase as well as the concentration of reduced glutathione were within normal ranges. The flow cytometric osmotic fragility test showed an extremely low number of remaining RBCs. The calculated percentage of residual red cells was also extremely low at 11.0% (reference range, 46.0-68.8%), which indicated decreasing osmolality resistance of RBCs (Fig. 1b). In the eosin-5´-maleimide binding ability test, fluorescent signals were decreased to 21.5 mg/dL RBC (reference range, 21.8-27.0 mg/dL RBC) among all RBCs (Fig. 1c). Upon gating smaller RBCs (Fig. 1d), their signals were even further diminished to almost 18.0 mg/dL RBC. Based on these findings, we proposed a likely diagnosis of HS.

Although the anemia had recovered and the hemoglobin level had remained in the range of 10.0-14.0 g/dL since the first hemolytic crisis, the patient experienced a second and third hemolytic crisis at 7 and 14 months after the first episode, respectively. We considered splenectomy to mitigate hemolysis; however, the patient had not been diagnosed with HS, and splenectomy was contraindicated for HSt. After obtaining his informed consent, we performed genetic analyses by target capture sequencing of 68 genes related to hereditary hemolytic anemia and hyperbilirubinemia (Table). Targeted gene capture and library construction for next-generation sequencing were performed using the HaploPlex HS custom panel (Agilent Technologies, Santa Clara, USA). The library was sequenced using 2×300 paired-end sequenc-

Table. **List of Genes for Target Gene Capture Sequencing.**

1	*ADA*	18	*EPB42*	35	*SEC23B*	52	*CFB*
2	*ALDOA*	19	*SLC4A1*	36	*KIF23*	53	*THBD*
3	*AK1*	20	*SPTA1*	37	*KLF1*	54	*CFHR2*
4	*ENO1*	21	*SPTB*	38	*ATP11C*	55	*CFHR4*
5	*G6PD*	22	*ANK1*	39	*COL4A1*	56	*CFHR5*
6	*GPI*	23	*GYPC*	40	*GATA1*	57	*DGKE*
7	*GPX1*	24	*EPB41*	41	*PIGA*	58	*PLG*
8	*GSR*	25	*STOM*	42	*UGT1A1*	59	*ULK1*
9	*GSS*	26	*RHAG*	43	*ATP7A*	60	*BECN1*
10	*GCLS*	27	*SLC2A1*	44	*ATP7B*	61	*PIK3C3*
11	*HK1*	28	*PIEZO1*	45	*ADAMTS13*	62	*AMBRA1*
12	*NT5C3A*	29	*KCNN4*	46	*C3*	63	*PRKAA1*
13	*PFKM*	30	*ABCG5*	47	*CD46*	64	*ATG9A*
14	*PGD*	31	*ABCB6*	48	*CFH*	65	*TBC1D5*
15	*PGK1*	32	*ABCG8*	49	*CFI*	66	*BNIP3L*
16	*PKLR*	33	*CDAN1*	50	*CFHR1*	67	*BNIP3*
17	*TPI1*	34	*C15orf41*	51	*CFHR3*	68	*FUNDC1*

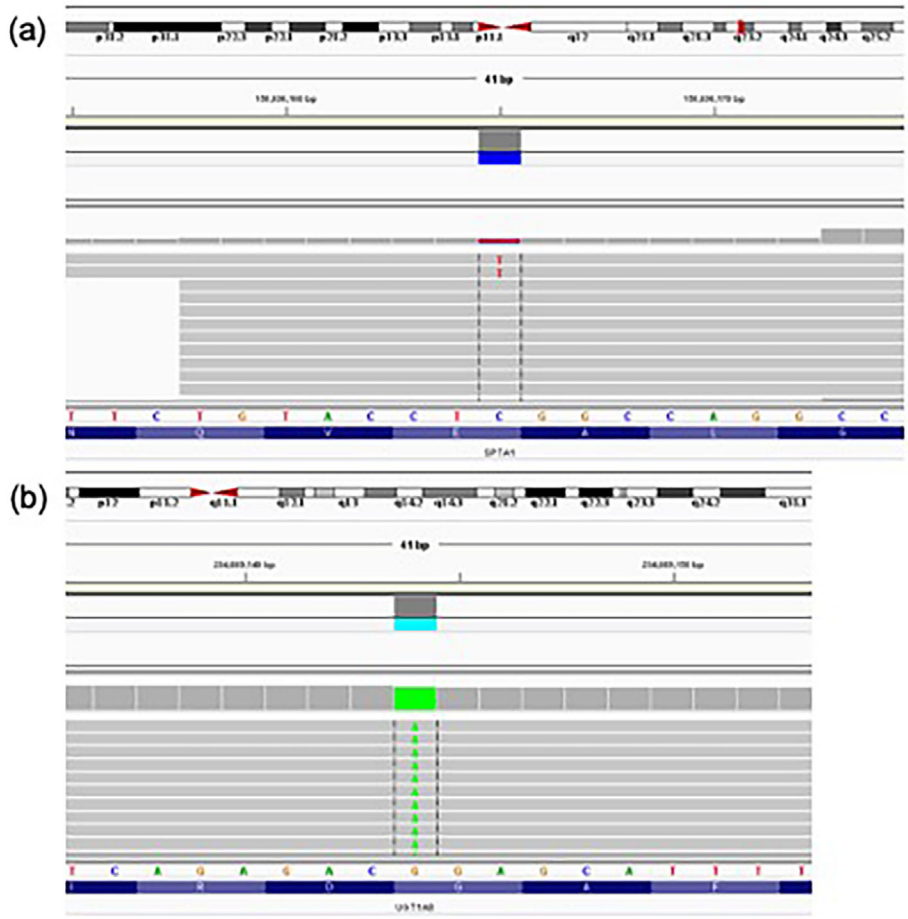

Figure 2. **A heterozygous mutation of c.2161G>A (p.E721K) in *SPTA1* was identified (a). A homozygous polymorphism of c.211G>A (p.G71R) in *UGT1A1* (*UGT1A1*6*) was also identified (b).**

ing on the Illumina MiSeq (San Diego, USA). To quantify and prioritize the list of variants, the annotating software programs SureCall (Agilent Technologies) and wANNOVAR were used. Missense mutations were evaluated by the Conservation Score from Genomic Evolutionary Rate Profiling at http://mendel.stanford.edu/SidowLab/downloads/gerp/. The relevant data from this report are hosted at the Human Genome Variation Database at http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html.

As a result of these analyses, a heterozygous mutation of c.2161G>A (p.E721K) in the *SPTA1* gene was identified (Fig. 2a). We also identified homozygous polymorphism of

*UGT1A1*6* by sequencing (Fig. 2b). Resequencing of *SPTA1* confirmed the presence of c.2161G>A and identified a heterozygous mutation of c.5665-24T>C. To our knowledge, this single nucleotide *SPTA1* mutation has not been reported previously (3-8); therefore, we performed *in silico* analyses using sorting intolerant from tolerant (SIFT) and combined annotation dependent depletion (CADD) algorithms to determine the functional impact of the mutation. SIFT is an algorithm that predicts the potential impact of amino acid substitutions on protein function, and CADD is a tool for scoring the deleteriousness of single-nucleotide variants as well as insertion/deletions variants in the human genome. The calculated SIFT and CADD scores were low at <0.05 and high at 19.3, respectively, and were determined to be deleterious. These results suggested that the *SPTA1* mutation affected the function of α-spectrin protein and caused HS in this case.

Discussion

In this case, using target capture sequencing, we identified a novel single-nucleotide mutation in *SPTA1* coexisting with homozygous polymorphism of *UGT1A1*. An *in silico* analysis suggested that the *SPTA1* mutation was responsible for developing HS.

Generally, a heterozygous missense mutation in *SPTA1* is not sufficient to cause HS, and another factor may be needed. Alternative splicing resulting in a marked decrease in α-spectrin production is a common cause of severe HS, in combination with a pathogenic *SPTA1* mutation. In our case, resequencing of *SPTA1* revealed no well-known polymorphic alleles, such as α-LEPRA or Prague, which provoke alternative splicing (9). However, we detected a heterozygous mutation of c.5665-24T>C in intron 40. Although this mutation has not been reported previously, its pathogenesis may involve an abnormal splicing mechanism, such as a frameshift. Considering the laboratory findings, we assume that HS in this case was caused both by the c.2161G>A missense mutation, which was determined to be deleterious by computational analyses, and abnormal splicing. *SPTA1*-associated HS has autosomal dominant inheritance. Because of the low frequency of 0.003 of the *SPTA1* mutation in the Japanese population and high conservation score of 4.55, it is likely that the mutation is *de novo* in cases in which the patient's parents do not have the mutation.

*UGT1A1*6* reduces the activity of UGT1A1 protein and results in Gilbert's syndrome (10). This syndrome causes unconjugated hyperbilirubinemia, and its prevalence is 3-10% in the normal population (11). Because coexistence of HS and Gilbert's syndrome increases the risk of gallstones from 15% to 50% (12), a routine abdominal ultrasound scan is needed. In the present case, the high level of hyperbilirubinemia might have been caused by the coexistence of HS and Gilbert's syndrome. Splenectomy and/or cholecystectomy are recommended in cases with exacerbation of hemolysis or gallstones. According to published guidelines (1), this patient should undergo regular ultrasound examinations of the biliary system. If his hemolysis becomes exacerbated to severe HS, splenectomy should be performed. Concomitant cholecystectomy will be recommended if there are symptomatic gallstones, due to the reduction in the risk of gallstones after splenectomy.

In conclusion, we report *SPTA1*-associated HS without a family history coexisting with Gilbert's syndrome, diagnosed by multiple biochemical assays, target capture sequencing, and bioinformatics analyses. In atypical HS cases, physicians should exclude other etiologies of hemolysis in which splenectomy is contraindicated. Determination of genetic diathesis provides useful information for deciding treatment options, the prognosis, and the heredity of hemolytic anemia.

The authors state that they have no Conflict of Interest (COI).

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