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Clinical and genetic diagnosis of two Turkish patients with hereditary spherocytosis

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ABSTRACT

Hereditary spherocytosis is a congenital disorder caused by defects in the erythrocyte membrane. It is characterized by hemolytic anemia, jaundice, splenomegaly, and cholelithiasis. The clinical presentation is variable. Especially in the neonatal period and cases without a family history, it isn't easy to diagnose with classical approaches. Here, we describe the genetic findings of a 1.5-month-old and a 2-month-old girl diagnosed with hereditary spherocytosis in Turkish families. Both cases presented with severe anemia and jaundice. Spherocytes were frequently seen in peripheral blood smears. Targeted next-generation sequencing (NGS) revealed that the 1.5-month-old girl was heterozygous for a novel frameshift mutation c.1617del (p.Leu540CysfsTer31) in exon 15 of the ANK1 gene, while the 2-month-old girl was heterozygous for a mutation c.1912C>T (p.Arg638Ter) in exon 13 of the SPTB gene, which leads to abnormal protein truncation. Parents did not carry these mutations. To our knowledge, the ANK1 mutation identified in a 7-month-old girl has not been reported previously. NGS may be helpful in diagnosing hereditary spherocytosis, especially in atypical cases.

Keywords: Hereditary spherocytosis, hemolytic anemia, neonatal jaundice, gene mutation

Hereditary spherocytosis (HS) is the most common inherited hemolytic anemia caused by gene mutations encoding erythrocyte cell membrane and skeletal proteins [1]. The prevalence of HS, which occurs in all racial groups and is especially common in individuals of Northern European origin, is reported as one case in 2000-3000 individuals [1, 2]. The prevalence in Turkey is unknown [3]. The clinical manifestations of HS are highly variable, ranging from almost asymptomatic disease to life-threatening anemia, severe splenomegaly, and/or severe bilirubinemia, even within the same family [2, 4, 5]. HS is most commonly associated with dominant inheritance (75%). The remaining cases represent autosomal recessive (OR) inheritance or de novo mutations in some sporadic cases [4, 5, 6]. HS is caused by defects in erythrocyte membrane proteins, including ankyrin, band 3, alpha-spectrin, beta-spectrin, and protein 4.2, encoded by the *ANK1, SLC4A1, SPTA1, SPTB,* and *EBP42* genes, respectively [7, 8]. *ANK1* and *SPTB* mutations constitute the most common causes of typical autosomal dominant HS, while autosomal recessive inheritance frequently involves *SPTA1* and *EBP42* gene variants [8, 9]. De novo *ANK1* and *SPTB* variants are relatively rare in HS [7, 9]. In this report, we have identified, through next-generation sequencing (NGS), a novel *ANK1* frameshift mutation causing HS in the first case and an *SPTB* mutation leading to abnormal truncation of the protein previously described in the literature in the second case.

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CASE PRESENTATION

Case 1

A 1.5-month-old baby girl with no other known disease was admitted with the complaint of decreased sucking and jaundice. The baby was born as a monozygotic twin at 34 weeks of gestation, weighing 2030 grams, and received phototherapy treatment after birth and erythrocyte suspension twice during her 3-week follow-up in the neonatal intensive care unit. There was no consanguinity between the parents, and there was no other blood disease in their family. Physical examination revealed pallor of the skin and icterus in the sclera. Laboratory investigations revealed moderate anemia, reticulocytosis, and indirect hyperbilirubinemia on a complete blood count. A peripheral smear was evaluated, and there was no significant increase in spherocytes. The direct Coombs test was negative. Hemoglobin electrophoresis revealed no abnormal hemoglobin variant. At 10 days of age, the os-

Table 1. Laboratory findings in Turkish cases with hereditary spherocytosis

Characteristics	Case 1	Case 2
Hemoglobin (g/dL) (reference range: 9.2-11.4)	5.9	7
MCV (fL) (reference range: 83.4-96.4)	82.7	85.8
MCH (pg) (reference range: 28-32.5)	27.6	29.5
MCHC (g/dL) (reference range: 32.5-34.9)	33.3	34.4
RBC ($\times 10^6$/ µL) (reference range: 2.9-3.8)	2.14	2.54
Reticulocytes (%) (reference range: 0.5-2)	12.76	4.28
Absolute reticulocyte count ($\times 10^9$/L), (reference range: 50-100)	273	109
Direct Coombs	Negative	Negative
Total bilirubin (mg/dL) (reference range: 0.3-1.2)	12.6	2.47
Direct bilirubin (mg/dL) (reference range: 0-0.2)	0.98	0.51
Serum iron (µg/dL) (reference range: 27-159)	114	71
Ferritin (µg/L) (reference range: 10-200)	179	197
Lactate dehydrogenase (U/L) (reference range: 0-247)	287	241
Glucose 6 phosphate dehydrogenase (U/g Hb) (reference range: 7-21)	13.6	10.3
Pyruvate kinase (U/g Hb) (reference range: 111-406)	267.4	389.9

MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration MCV=mean corpuscular volume, RBC=red blood cell.

motic fragility test (OFT) result was negative. The glucose-6 phosphate dehydrogenase and pyruvate kinase enzyme levels were within normal limits (Table 1). Alpha and beta-globin genetic analyses excluded alpha and beta-thalassemia. Abdominal ultrasonography showed no splenomegaly. Genetic analysis was performed by NGS to determine the cause of unexplained hemolysis. Genetic analysis revealed that she was heterozygous for a c.1617del (p.Leu540CysfsTer31) frameshift mutation in exon 15 of the *ANK1* gene. It was determined that neither of her parents carried this mutation. Our patient received her first transfusion at 12 days of age and has required a total of 6 red blood cell transfusions to date. Our patient is now 1.5 years old, and the last red blood cell transfusion was given at 14 months of age. Initially, an average of one transfusion per month was performed, and the transfusion rates gradually decreased during follow-up. Her hemoglobin levels have ranged between 9 g/dL and 10 g/dL. She is receiving folate supplements and is being followed up intermittently in our clinic with supportive measures without a splenectomy.

Case 2

A term infant with a birth weight of 3000 grams was admitted on the 2nd postnatal day because of skin jaundice. There was no consanguinity between the parents, and there was no family history of hereditary hematologic disease. Investigations revealed anemia and reticulocytosis. The direct Coombs test was negative. Total bilirubin was 22.5 mg/dL and direct bilirubin was 0.7 mg/dL, and she was hospitalized in the neonatal intensive care unit because of indirect hyperbilirubinemia. Phototherapy treatment was started. In the follow-up, Hb decreased to 7 g/dl, and erythrocyte suspension was given. The glucose-6 phosphate dehydrogenase and pyruvate kinase levels were normal. No abnormal variant was found on hemoglobin electrophoresis. A peripheral smear showed few spherocytes, polychromasia, and rare nucleated red blood cells. At 7 days of age, the OFT test result was negative (Table 1). Abdominal ultrasonography showed no splenomegaly. NGS of genomic DNA obtained using periferic blood samples demonstrated heterozygosity for SPTBc.1912C>T (p.Arg638Ter). Her parents were screened for mutations, and none of them had mutations. The first blood transfusion was performed at 15 days of age in the neonatal period, and a total of 13 times red blood cells were administered. Our patient is now 3 years old, and the last red blood cell transfusion was given at 30 months of age. Initially, blood transfusion requirements were more frequent, but transfusions' frequency gradually decreased during follow-up, and Hb levels ranged between 9 g/dL and 10 g/dL. Folic acid supplementation is continued.

DISCUSSION

Hereditary spherocytosis is rarely encountered in the neonatal period, and the diagnosis might become difficult if there is no known family history [8, 10]. Here, we describe two cases with no family history, one confirmed by NGS in the *ANK1* gene and the other in the *SPTB* gene.

Hereditary spherocytosis is usually diagnosed in childhood. Anemia, jaundice, and splenomegaly are the main clinical features [5, 11]. The diagnosis is usually made on clinical suspicion, including family history and typical biological signs of HS (increased MCHC>36 g/dL, spherocytic cells in blood smears, non-immune hemolytic anemia, positive osmotic fragility test) [5, 12, 13]. Although increased spherocytes in the blood smear are useful in the diagnosis of HS, they can also be seen in immune hemolytic anemia, sepsis, ABO incompatibility, and glucose-6 phosphate dehydrogenase enzyme deficiency [5, 10, 14]. In addition, approximately 10% of patients with HS may not have spherocytes in the peripheral smear, and as a result, may be misdiagnosed [11]. Furthermore, although the OFT test is a confirmatory test for diagnosing HS, its sensitivity and specificity are low [14]. False-negative results may be observed in OFT tests in patients with iron deficiency and obstructive jaundice. The OFT test may also be false positive in hereditary elliptocytosis and autoimmune hemolytic anemia [10, 14, 15]. The OFT test also has some pitfalls when used in neonates. In neonates, HS erythrocytes are more sensitive to osmotic lysis than normal erythrocytes due to the reduced membrane surface area. Therefore, it is recommended to use neonatal osmotic fragility curves instead of adult curves. In addition, the OFT test can't distinguish spherocytes seen in HS from spherocytes resulting from other causes, such as ABO incompatibility [16, 17]. In cases with an atypical course and no family history of HS, genetic studies

identify the clinical presentation of hereditary blood diseases [6, 11, 13]. The two cases in our study have had unexplained hemolysis and indirect hyperbilirubinemia since the neonatal period. There was no family history. OFT tests were negative. The glucose-6 phosphate dehydrogenase and pyruvate kinase tests were negative. Therefore, we used the NGS panel to identify the responsible genes for hematologic disorders in these patients. In our first patient, we found a de novo *ANK1* c.1617del (p.Leu540CysfsTer31) frameshift mutation, which had not been previously described in the literature. In the other patient, we detected the *SPTB* c.1912C>T (p.Arg638Ter) mutation previously defined in the literature and reached the diagnosis this way [18, 19].

Molecular genetic testing can be used as an effective way to reach an accurate clinical diagnosis. For this purpose, NGS-based genetic tests have provided an alternative to conventional tests, especially in the diagnosis of genetic disorders showing phenotypic and genetic heterogeneity, and the use of the tests has begun to increase gradually [10, 20].

Five genes have been identified as responsible for HS (*SPTA1, SPTB, ANK1, SLC4A1*, and *EPB42*) (4, 15). *ANK1* mutations are the most common among this group and account for approximately half of all HS. Park *et al*. [21] confirmed that heterozygous *ANK1* mutations account for 52% of all Korean patients. Nakanishi *et al*. [22] found that *ANK1* mutations are involved in approximately 31% of Japanese HS patients. The majority of reported mutations, including nonsense, end-joining, and frameshift mutations, were predicted to result in protein truncation [1, 10]. More than 60 mutations have been identified in *ANK1* [23]. However, this is the first case of HS caused by a frameshift mutation in exon 15 of the *ANK1* gene.

Another protein that plays an important role in erythrocyte membrane stability is beta-spectrin encoded by the *SPTB* gene [23]. The *SPTB* mutation is the second most common pathologic mutation in HS after the *ANK1* mutation, and this mutation is responsible for approximately 20% of HS cases [1, 12]. In a Korean study, 25 patients with HS were reported to carry mutations in *ANK1* (n=13) or *SPTB* (n=12). In another study conducted in China, 13 mutations in *ANK1* and 10 mutations in *SPTB* were observed in 23 patients. In a study conducted in Japan, *ANK1* variants were the most common and were observed in 46% (6/13) of patients, while *SPTB* variants were identified in 31% (4/13). To date, most gene defects in *SPTB* (splicing, nonsense variants, and frameshift variants) usually result in exon skipping, mRNA transcript instability, or truncated synthesis of beta-spectrin proteins [7, 99. In a recent study, 6 frameshift, 5 nonsense, and 1 insertion error mutations were reported in the SPTB gene [12]. In the second case, we described a mutation that abnormally shortens the protein in the 13th exon of the *SPTB* gene, which was previously reported in the literature.

The genotype-phenotype correlation of HS is currently unclear [10, 13]. The complexity of mutations and gene regulation may explain the heterogeneity of clinical manifestations [13]. Patients with *ANK1* gene mutations are more prone to anemia and have a higher reticulocyte count compared to those without this mutation. Clinical findings may also vary according to ANK1 mutation sites. It has been suggested that spectrin-binding or regulatory mutations in the *ANK1* gene may be associated with more severe anemia. However, co-inheritance of mutations in iron and bilirubin metabolism and erythrocyte defects may affect phenotypic variability in HS. This phenotypic variability may depend on age and race [24]. As a result, the severity of the disease may differ between individuals even if the site is the same [10, 13]. Therefore, we should combine clinical, erythrocyte morphology, biochemical, and genomic data in the diagnosis of HS [23]. In this article, we demonstrated two different mutations that may explain the clinical picture in two patients with no family history, who received the first transfusion in the neonatal period and continued to require transfusion in the follow-up. Although severe anemia was observed in the early period in both patients, the frequency of transfusion decreased with age.

Because of HS's phenotypic and genetic heterogeneity, it is difficult to diagnose, especially in atypical cases. Genetic diagnosis has gained more importance since traditional diagnostic tests miss the diagnosis of HS due to late results.

The NGS method is now becoming prominent in suspected erythrocyte membrane disorders, both in terms of reaching a diagnosis in a shorter time and in terms of efficiency, and the use of NGS is becoming increasingly widespread [11, 13, 14]. This genetic technology also provides information for potential ge-

netic counseling and future research [13, 14].

The first line of treatment is supportive care. Phototherapy is often given to newborns, and transfusions are given in severe cases. In the older age group, blood transfusions may be required during hemolytic crises. Splenectomy may be useful in cases of HS and prolongs the life span of erythrocytes [4, 13]. However, the risk of infection is a disadvantage of splenectomy in childhood. The diagnosis should be confirmed by a genetic diagnosis before splenectomy. In addition, folate supplementation is recommended for moderate-to-severe forms of HS [20]. Both of our patients received phototherapy treatment, and erythrocyte suspension support in the neonatal period and folate treatment was started in both cases.

CONCLUSION

In conclusion, mutations related to HS were identified, and the diagnosis was reached in two patients who had no family history and had required intermittent blood transfusions since the neonatal period. The lack of traditional diagnostic methods, especially in newborns with unclear clinical features and no family history, shows that the use of NGS is necessary for diagnosis. Molecular diagnosis and genetic counseling can predict the prognosis of young patients with HS.

Informed Consent

Parents were informed about the purpose of the case report, and informed consent was obtained from both families for this publication.

Authors' Contribution

Study Conception: ÇC; Study Design: ÇC; Supervision: ÇC; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ÇC; Statistical Analysis and/or Data Interpretation: ÇC; Literature Review: ÇC; Manuscript Preparation: ÇC and Critical Review: ÇC.

Conflict of interest

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REFERENCES

1. Da Costa L, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev. 2013;27(4):167-178. doi: 10.1016/j.blre.2013.04.003.
2. An X, Mohandas N. Disorders of red cell membrane. Br J Haematol. 2008;141(3):367-375. doi: 10.1111/j.1365-2141.2008.07091.x.
3. Kocaağa A, Çakmak HM. Identification of Novel Mutations in Children with Hereditary Spherocytosis by Targeted Exome Sequencing: A Single Center Experience. Med J West Black Sea. 2022;6(3):296-301. doi: 10.29058/mjwbs.1200958.
4. Russo R, Andolfo I. Hereditary spherocytosis and allied disorders. Hemasphere. 2019;3(Suppl):157-159. doi: 10.1097/HS9.0000000000000198.
5. Shah S, Vega R. Hereditary spherocytosis. Pediatr Rev. 2004;25(5):168-172. doi: 10.1542/pir.25-5-168.
6. Huang TL, Sang BH, Lei QL, et al. A de novo ANK1 mutation associated to hereditary spherocytosis: a case report. BMC Pediatr. 2019;19(1):62. doi: 10.1186/s12887-019-1436-4.
7. Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet. 2008;372(9647):1411-1426. doi: 10.1016/S0140-6736(08)61588-3.
8. Mahajan V, Jain SK. Hereditary spherocytosis. NeoReviews. 2016;17(12):e697-e704. doi: 10.1542/neo.17-12-e697.
9. Xi B, Liu S, Zhu Y, Zhang D, Zhang Y, Liu A. Case report: Genetic analysis of a novel intronic inversion variant in the SPTB gene associated with hereditary spherocytosis. Front Genet. 2023;14:1309040. doi: 10.3389/fgene.2023.1309040.
10. Jang W, Kim SK, Nahm CH, Choi JW, Kim JJ, Moon Y. A Novel de novo Mutation in ANK Gene Identified through Targeted Next-Generation Sequencing in a Neonate with Hereditary Spherocytosis. Ann Clin Lab Sci. 2021;51(1):136-139.
11. Guan H, Liang X, Zhang R, et al. Identification of a de novo ANK1 mutation in a Chinese family with hereditary spherocytosis. Hematology. 2018;23(6):357-361. doi: 10.1080/10245332.2017.1398210.
12. Du Z, Luo G, Wang K, Bing Z, Pan S. Identification of a novel heterozygous SPTB mutation by whole genome sequencing in a Chinese patient with hereditary spherocytosis and atrial septal defect: a case report. BMC Pediatr. 2021;21(1):291. doi: 10.1186/s12887-021-02771-4.
13. Fu P, Jiao YY, Chen K, et al. Targeted next-generation sequencing identifies a novel nonsense mutation in ANK1 for hereditary spherocytosis: A case report. World J Clin Cases. 2022;10(15):4923-4928. doi: 10.12998/wjcc.v10.i15.4923.
14. Wu Y, Liao L, Lin F. The diagnostic protocol for hereditary spherocytosis-2021 update. J Clin Lab Anal. 2021;35(12):e24034. doi: 10.1002/jcla.24034.
15. Yamamoto KS, Utshigisawa T, Ogura H, et al. Clinical and genetic diagnosis of thirteen Japanese patients with hereditary spherocytosis. Hum Genome Var. 2022;9(1):1. doi: 10.1038/s41439-021-00179-1.
16. Christensen RD, Yaish HM, Gallagher PG. A pediatrician's practical guide to diagnosing and treating hereditary spherocytosis in neonates. Pediatrics. 2015;135(6):1107-1114. doi: 10.1542/peds.2014-3516.
17. Gallagher PG. Difficulty in Diagnosis of Hereditary Spherocytosis in the Neonate. Pediatrics. 2021;148(3):e2021051100.

doi: 10.1542/peds.2021-051100.
18. Aggarwal A, Jamwal M, Sharma P, et al. Deciphering molecular heterogeneity of Indian families with hereditary spherocytosis using targeted next-generation sequencing: First South Asian study. Br J Haematol. 2020;188(5):784-795. doi: 10.1111/bjh.16244.
19. Kager L, Jimenez−Heredia R, Zeitlhofer P, et al. A single−center cohort study of patients with hereditary spherocytosis in Central Europe reveals a high frequency of novel disease−causing genotypes. Hemasphere. 2024;8(1):e31. doi: 10.1002/hem3.31.
20. Bolton−Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ; General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis and management of hereditary spherocytosis–2011 update. Br J Haematol. 2012;156(1):37-49. doi: 10.1111/j.1365-2141.2011.08921.x.
21. Park J, Jeong DC, Yoo J, et al. Mutational characteristics of ANK1 and SPTB genes in hereditary spherocytosis. Clin Genet. 2016;90(1):69-78. doi: 10.1111/cge.12749.
22. Nakanishi H, Kanzaki A, Yawata A, Yamada O, Yawata Y. Ankyrin gene mutations in japanese patients with hereditary spherocytosis. Int J Hematol. 2001;73(1):54-63. doi: 10.1007/BF02981903.
23. He BJ, Liao L, Deng ZF, Tao YF, Xu YC, Lin FQ. Molecular Genetic Mechanisms of Hereditary Spherocytosis: Current Perspectives. Acta Haematol. 2018;139(1):60-66. doi: 10.1159/000486229.
24. Wang Y, Liu L, Liu D, Liu W. A de novo ANK1 mutation in a childhood hereditary spherocytosis: a case report. BMC Pediatr. 2023;23(1):267. doi: 10.1186/s12887-023-04092-0.