



# 다중유전자패널검사로 새로운 ANK1 변이가 발견된 유전구형적혈구증 환자의 1예 보고

## Identification of a *de novo* ANK1 Variant in a Patient with Hereditary Spherocytosis on Multi-gene Panel Testing

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Hereditary spherocytosis (HS) is caused by defects in red blood cell membrane components encoded by *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, and *EPB42*. The low sensitivity and specificity of conventional tests such as peripheral blood smear and osmotic fragility test (OFT) limit the diagnosis of HS, especially in patients with mild phenotypes and no family history of the condition. Mutations in *ANK1*, which encodes ankyrin-1 protein, are present in more than half of HS cases and is known as the most common cause of HS in Korea. Mutations in *ANK1* are mostly inherited in an autosomal dominant manner. Here, we report the case of a Korean patient with HS carrying a *de novo* mutation in *ANK1*. The patient was suspected of having HS due to neonatal jaundice and clinical manifestations of hemolysis, but the OFT results were unclear. At the age of 20, the patient was definitively diagnosed with HS, caused by a *de novo* heterozygous *ANK1* variant c.856C>T (p.Arg286\*), using next-generation sequencing-based multi-gene panel testing and Sanger sequencing analysis.

**Key Words:** *ANK1*, Hereditary spherocytosis, Multi-gene panel test, Next-generation sequencing

### INTRODUCTION

Hereditary spherocytosis (HS) is a common cause of congenital hemolytic anemia with an incidence rate of 1 in 2,000 Caucasians

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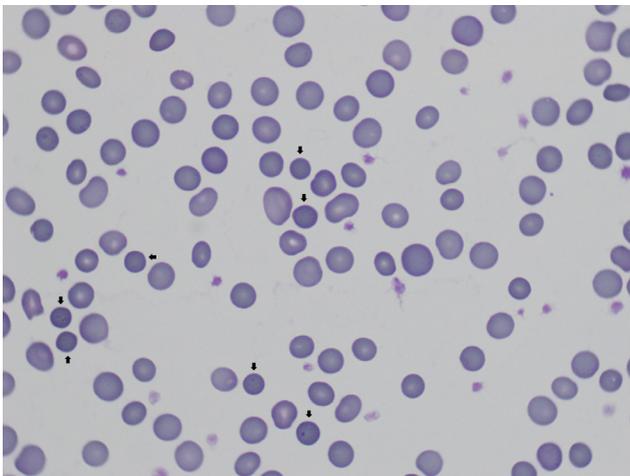
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and 1 in 5,000 Koreans [1]. HS is caused by defects in red blood cell (RBC) membrane components, such as  $\alpha$ -spectrin,  $\beta$ -spectrin, ankyrin, band 3, and protein 4.2, which are encoded by *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, and *EPB42*, respectively [2]. Spherocytosis, type 1 (OMIM #182900) caused by ankyrin defects is most common in Korea and is mostly inherited in an autosomal-dominant manner [1, 3].

HS is often suspected from hemolytic anemia with spherocytes on a peripheral blood smear (PBS) and the presence of a family history. However, its diagnosis is very difficult if the phenotype is mild and if there is no family history. Indeed, some cases have remained undiagnosed for decades [4, 5]. In addition, the low sensitivity and specificity of conventional tests such as PBS and osmotic fragility test (OFT) limit the diagnosis of HS [6]. Molecular diagnostic tests can overcome these limitations, which are especially useful in patients with a mild phenotype and no family history. Here, we report a case of HS that presented uncertain results from OFT but was diagnosed by a *de novo* *ANK1* variant using multi-gene panel testing.

## CASE

The patient was a 20-year-old woman without a family history of HS. She was born at 38 weeks of gestation and had a history of neonatal jaundice. At 3 months of age, she was transferred from a local clinic for bronchiolitis, sinusitis, and anemia. The initial complete blood count was as follows: Hb 8.4 g/dL (reference range: 11.0–15.0 g/dL); Hct 24.1% (34.3–49.9%); mean corpuscular volume 67.2 fL (80.0–97.7 fL); mean corpuscular hemoglobin concentration 34.7 g/dL (31.6–35.8 g/dL); reticulocyte 5.3% (0.5–1.5%). The corrected reticulocyte count was 2.8%. Total bilirubin was within normal range, but lactate dehydrogenase was elevated to 634 IU/L (100–225 IU/L), and haptoglobin was decreased to 36.2 mg/dL (55–324 mg/dL). The direct and indirect antiglobulin test results were negative. Hepatosplenomegaly was absent, and spherocytes were not observed in PBS. Two months later, the patient's Hb level was 8.4 g/dL, with an increased reticulocyte count of 6.6% and urinalysis result of 1+ for urobilinogen. At the age of 18 months, her Hb level was 11.8 g/dL, with an increased reticulocyte count of 5.0%. The OFT was performed without RBC transfusions, and the results were within the normal range [hemolysis began 0.5%; completed 0.36% NaCl (0.5% and 0.35%)]. She visited our hospital again, at the age of nine, when she received pneumococcal and meningococcal vaccination. Hb was 9.7 g/dL, and the results of the OFT indicated increased fragility (began 0.64%; completed 0.48% NaCl). At the age of 16 years, the patient underwent a cho-



**Fig. 1.** Peripheral blood smear (PBS) of the 20-year-old patient. Wright-Giemsa-stained PBS at 1,000× magnification field (oil immersion field) shows spherocytes with moderate/2+ grade (black arrows).

lecystectomy for cholecystitis. In PBS, microcytic hypochromic anemia with spherocytosis was observed with a moderate/2+ grade (Fig. 1) [7]. Hb was 10.2 g/dL, and the OFT was within the normal range (began 0.52%; completed 0.36%). We did not perform an eosin-5'-maleimide (EMA) binding flow cytometry test.

When the patient was 20 years old, next-generation sequencing (NGS)-based multi-gene panel testing was conducted with the patient's consent. The multi-gene panel included more than 2,155 probes targeting 85 genes associated with hematopathies. NGS was performed using the Illumina NextSeq 550Dx (Illumina, San Diego, CA, USA) with 150 bp paired-end sequencing. Alignment of sequence reads to the reference genome hg19, variant calling, and annotation were also conducted [8, 9].

We identified a heterozygous variant, NM\_000037.3(*ANK1*): c.856C>T (p.Arg286\*), in exon 9 of *ANK1*. This variant was validated using Sanger sequencing and was classified as a pathogenic variant (PVS1+PM2+PP5) according to the 2015 American College of Medical Genetics and Genomics–Association for Molecular Pathology guidelines [10]. The patient's parents did not carry this variant (Fig. 2). This study was approved by the institutional review board of our institute (IRB 2020-12-011). The patient has been prescribed folic acid and is being examined biannually at an anemia clinic without regular RBC transfusion or splenectomy.

## DISCUSSION

HS is characterized by spherocytes in PBS. The defects in the RBC membrane components induce a reduction in the surface area, resulting in the formation of spherocytes. Spherocytes show increased osmotic fragility and are trapped and destroyed in the spleen. Destruction of spherocytes in the spleen leads to hemolytic anemia, jaundice, and splenomegaly [11]. Clinical manifestations vary from asymptomatic to severe. Undiagnosed HS may lead to complications such as gallstones and aplastic anemia. Complications may be an initial manifestation in patients with asymptomatic or mild phenotypes [12]. Therefore, early diagnosis is important to reduce the risk of complications [13].

HS is often misdiagnosed because conventional tests, such as PBS and OFT, have low sensitivity and specificity. Approximately 10% of HS patients have very few or no spherocytes in PBS [14]. The OFT results can also be normal in 10–20% of HS patients [15]. In addition, these tests are not specific because secondary spher-

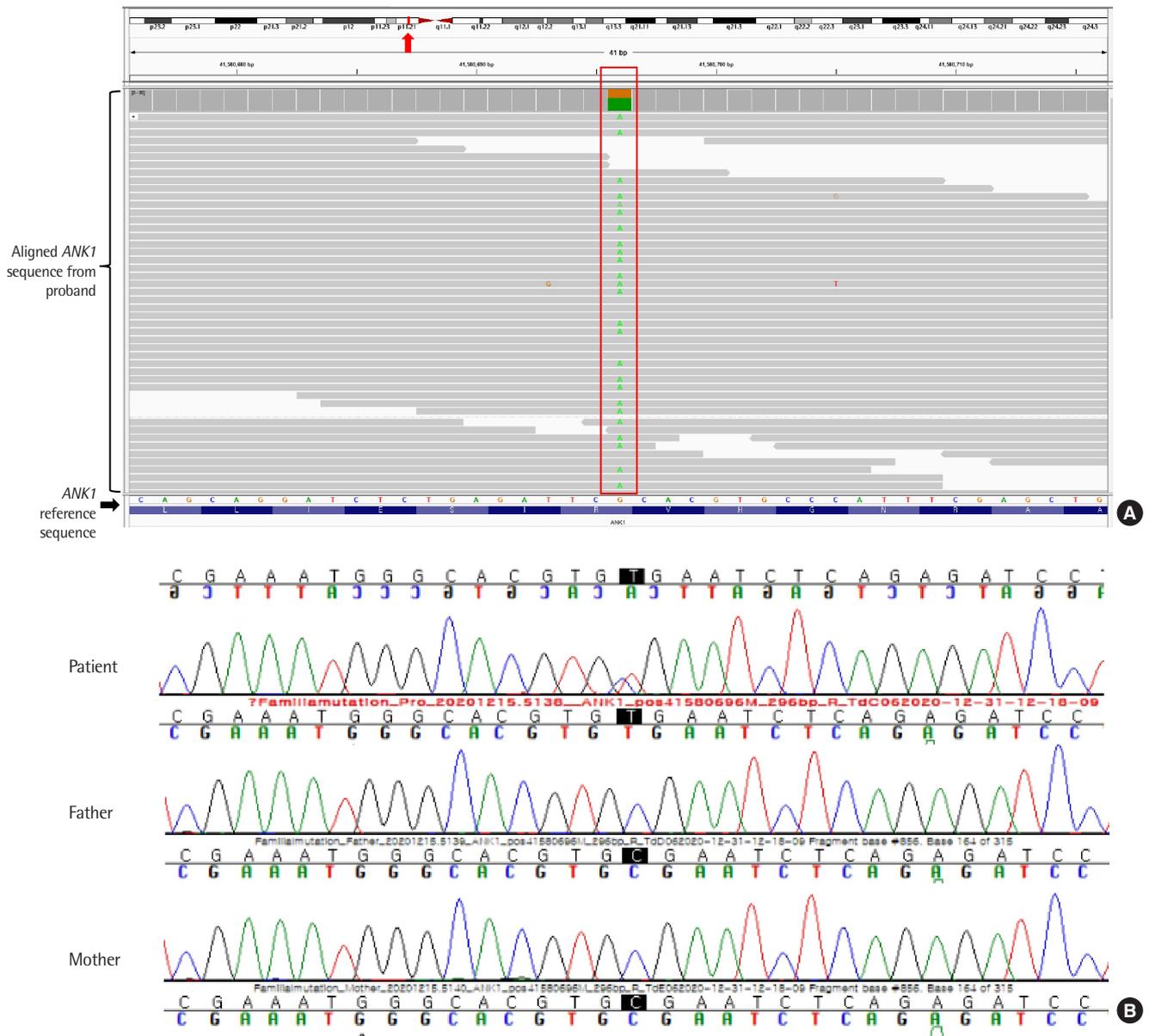


Fig. 2. ANK1 variant identified in the patient. (A) A heterozygous variant in ANK1 (c.856C>T; p.Arg286\*) of the proband visualized on Integrative Genomics Viewer. (B) Sanger sequencing revealed that her parents did not carry this variant.

rocytosis cannot be distinguished. While the flow cytometry-based EMA binding test is the test of choice and current guidelines recommend its use for HS screening [15], it is not available in our laboratory, and we did not use it for our patient. Therefore, molecular genetic testing is very useful for diagnosis when the results of conventional tests are uncertain. According to a previous study, the results of PBS and OFT were normal in 8% and 13.2% of patients with genetic variants, respectively [1]. In our patient, spherocytes and splenomegaly were first observed at the age of 16 years.

In addition, OFT was performed three times, but the results differed each time. Therefore, achieving a definite diagnosis is difficult with these traditional tests.

Approximately 75% of HS cases are inherited in an autosomal dominant manner, whereas both parents of the remaining HS cases do not display clinical symptoms and are often normal in hematological tests [16]. In these remaining patients, HS could be inherited in an autosomal recessive manner or developed due to a *de novo* mutation [16]. Autosomal dominant HS is associated with

variants in *ANK1*, *SLC4A1*, and *SPTB*. Autosomal recessive HS is mostly associated with variants of *SPTA1* and *EPB42*. Variants in *ANK1* and *SPTB* are also associated with autosomal recessive HS. *De novo* HS is mostly associated with variants of *ANK1* and *SPTB* [17]. In *ANK1*, approximately 15–20% of variants have been reported as *de novo* [18]. Identifying the inheritance pattern of HS is important for family planning and proper genetic counseling. However, in the absence of a family history, the inheritance pattern of HS cannot be identified without genetic testing. Specifically, when both parents are clinically and hematologically normal, it is impossible to distinguish whether HS is inherited in an autosomal recessive manner or occurs *de novo*, without genetic testing. Therefore, HS diagnosis with genetic testing is useful when conventional methods cannot confirm the disease.

In Korean patients with HS, *ANK1* is the most common causative gene, followed by *SPTB* [3]. Between HS patients with *ANK1* and those with *SPTB* variants, there were no significant differences in clinical characteristics except for the frequency of splenectomy. Splenectomy was more frequent in patients with *ANK1* variants than in those with *SPTB* variants [3]. In patients with *ANK1* variants, there were differences in Hb levels depending on the location of the variants. Patients harboring variants in the spectrin-binding domain exhibit more severe anemia than those harboring variants in the membrane protein binding domain or regulatory domain [3, 19]. The variant found in our patient was located in the membrane protein-binding domain. She did not have severe anemia, with an Hb level of  $9.2 \pm 2.0$  g/dL. Another case with an *ANK1* p.Arg286\* variant has also been reported to show a mild phenotype [19].

In our patient, findings of spherocytosis in PBS with an increased reticulocyte count, decreased haptoglobin levels, negative antiglobulin tests, and uncertain results of OFT were detected, but a definitive HS diagnosis was not reached without molecular genetic testing. Therefore, multi-gene panel testing, including HS-related genes, is a useful diagnostic tool in *de novo* cases. In patients with unexplained hemolytic anemia, molecular genetic testing should be considered for early diagnosis and proper management.

## 요 약

유전구형적혈구증은 적혈구막 구성요소들 중 *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, *EPB42* 유전자의 돌연변이에 의해 발생한다. 말초

혈액 도말검사 및 삼투압취약성검사와 같은 전통적인 검사법은 민감도와 특이도가 낮아 유전구형적혈구증을 진단하는 데 제한이 있고 특히 임상 증상이 심하지 않고 가족력이 없는 환자의 경우 진단에 어려움이 있다. Ankyrin-1 단백질 발현에 관여하는 *ANK1* 유전자에서의 변이는 유전구형적혈구증 원인의 절반 이상 빈도를 보이는 것으로 알려져 있으며, 한국인 유전구형적혈구증의 가장 흔한 원인으로 알려져 있다. *ANK1* 변이는 대부분 상염색체 우성 방식으로 유전된다. 본 연구에서는 *ANK1* 유전자의 *de novo* 변이에 의해 발생한 유전구형적혈구증 환자 증례를 보고하고자 한다. 환자는 신생아 황달 및 용혈 증상들을 고려하여 유전구형적혈구증으로 추정되었지만 몇 차례의 삼투압취약성검사에서는 명확한 결과를 확인할 수 없었다. 환자의 나이 20세에 다중유전자패널검사와 염기서열분석을 통해 *ANK1* 유전자의 이형접합 *de novo* 변이 c.856C>T (p.Arg286\*)가 발견되어 분자유전학적으로 유전구형적혈구증을 확인할 수 있었다.

## Conflicts of Interest

None declared.

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