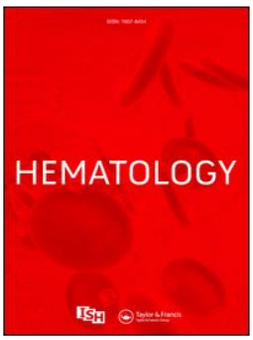


Hematology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/yhem20

A novel *EPB41* p.Trp704* mutation in a Korean patient with hereditary elliptocytosis: a case report

Soyoung Shin , Kyung-Ah Hwang , Kyuhyun Paik & Joonhong Park

To cite this article: Soyoung Shin , Kyung-Ah Hwang , Kyuhyun Paik & Joonhong Park (2020) A novel *EPB41* p.Trp704* mutation in a Korean patient with hereditary elliptocytosis: a case report, Hematology, 25:1, 321-326, DOI: 10.1080/16078454.2020.1807227

To link to this article: https://doi.org/10.1080/16078454.2020.1807227

Published online: 17 Aug 2020.

HEMATOLOGY
2020, VOL. 25, NO. 1, 321–326
https://doi.org/10.1080/16078454.2020.1807227

Taylor & Francis
Taylor & Francis Group

CASE REPORT

OPEN ACCESS Check for updates

A novel *EPB41* p.Trp704* mutation in a Korean patient with hereditary elliptocytosis: a case report

Soyoung Shin [a], Kyung-Ah Hwang[b], Kyuhyun Paik [c] and Joonhong Park [a,d]

[a]Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [b]Department of Research and Development, SML Genetree, Seoul, Korea; [c]Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [d]Department of Laboratory Medicine, Jeonbuk National University Medical School and Hospital, Jeonju, Korea

ABSTRACT

Objectives: Hereditary elliptocytosis (HE) is inherited in an autosomal dominant fashion, and the majority of HE-associated defects occur due to qualitative and quantitative defects in the RBC membrane skeleton proteins α-spectrin, β-spectrin, or protein 4.1R. The complex *EPB41* gene encodes a diverse family of protein 4.1R isoforms which are key components of the erythroid membrane skeleton that regulates red cell morphology and mechanical stability. The purpose of this study was to investigate the genome of a Korean patient with HE to discover the causative gene mutation using gene panel sequencing.

Methods: An 89-year-old female presented to the Emergency Department and was diagnosed with pancreatitis and gallstones. A peripheral blood smear revealed that approximately 60% of the RBCs were abnormally shaped and appeared oval or elongated, from slightly egg-shaped to rod or pencil forms (elliptocytes). Targeted gene panel sequencing consisting of 33 genes related to inherited RBC disorders and Sanger sequencing were performed.

Results: A heterozygous c.2112G > A of the *EPB41* gene leading to premature termination codon (NM_001166005.1:c.2112G > A, p.Trp704*) was identified. This variant, which had not been previously reported to be related to HE, was confirmed by Sanger sequencing. Thus, the patient's diagnosis of HE-1 was genetically confirmed.

Conclusion: The present study confirmed a novel mutation of the *EPB41* gene that plays an important role in expanding the mutational distribution in HE-1. It could also be helpful for understanding the correlation between the genotype and phenotype in HE.

KEYWORDS

Gene panel sequencing; *EPB41* mutation; hereditary elliptocytosis; pancreatitis; gallstone; protein 4.1R; SAB domain; RBC membrane

Introduction

Hereditary elliptocytosis (HE) is a hematologic disorder characterized by elliptically-shaped erythrocytes and a variable degree of hemolytic anemia. The clinical phenotype is usually mild with peripheral blood elliptocytes, but it can be moderately severe. In severe forms that achieve hereditary poikilocytosis, large red cell fragments are torn off, leaving erythrocytes with marked poikilocytosis. Usually inherited as an autosomal dominant trait, elliptocytosis results from mutation in any one of several genes encoding proteins of the red cell membrane skeleton [1]. HE-1 is caused by heterozygous or homozygous mutation in the gene encoding erythrocyte membrane protein 4.1 (EPB41) on chromosome 1p35 [2]. HE-2 is caused by mutation in the *SPTA1* gene [3]. HE-3 is caused by mutation in the *SPTB* gene [4]. HE-4, also known as Southeast Asian ovalocytosis, is caused by mutation in the *SLC4A1* gene [5]. Particularly, the complex *EPB41* gene encodes a diverse family of protein 4.1R isoforms which are key components of the erythroid membrane skeleton that regulates red cell morphology and mechanical stability [6].

The purpose of this study was to investigate the genome of a Korean patient with HE suffering from pancreatitis and gallstones in order to discover the causative gene mutation using gene panel sequencing.

Case presentation

An 89-year-old female with diabetes mellitus and hypertension was admitted to our hospital for evaluation of abdominal pain. Physical examination revealed mild dehydration and right lower quadrant tenderness. Abdominal computed tomography showed pancreatitis with peri-pancreatic fat infiltration, abdominal edematous enlargement of the pancreas with calcification on the body, and multiple small gallstones without gallbladder dilatation. Interestingly, a peripheral blood smear showed normocytic normochromic anemia, and approximately 60% of the RBCs had an abnormal shape and appeared oval or elongated, from slightly egg-shaped to rod or pencil forms (elliptocytes) (Figure 1). Besides elliptocytosis, the complete blood count showed low hemoglobin and high reticulocytes. Results of laboratory blood analyses were

CONTACT Joonhong Park miziro@catholic.ac.kr
Supplemental data for this article can be accessed at https://doi.org/10.1080/16078454.2020.1807227.

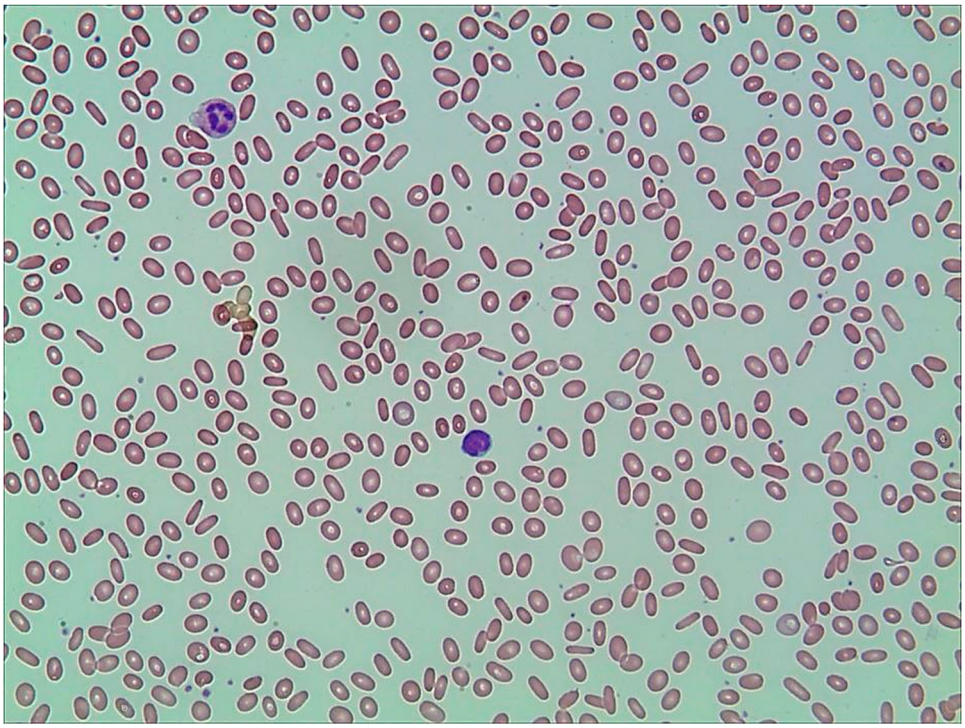


Figure 1. Peripheral blood smear revealed elliptocytes that appeared oval or elongated, from slightly egg-shaped to rod or pencil forms (Wright-Giemsa stained; 400× magnification).

summarized in Table 1. To resolve acute pancreatitis, the patient received intravenous fluids, antibiotics, and analgesics during the hospital admission and was discharged without subjective problems after seven days. The hemoglobin level raised from 8.9 to 11.2 g/dL after the acute episode of pancreatitis. Although the patient's family history was negative, genetic counseling and segregation analysis were recommended for her family members. However, because they did not experience any symptoms associated with HE in their daily lives, the other family members denied laboratory testing as well as genetic testing.

Table 1. Results of laboratory blood analyses in a Korean patient with hereditary elliptocytosis.

WBC, $\times 10^9$/L (reference range, 4-10)	7.3	Amylase, IU/L (41–134)	248
RBC, $\times 10^{12}$/L (4.5-5.9)	3.19	Lipase, U/L (13–60)	144
Hemoglobin, g/dL (13-17)	8.9	BUN, mg/dL (6–20)	15.2
MCV, fL (85–100)	92.1	Creatinine, mg/dL (0.5–1.2)	1.26
MCH, pg (28–34)	31.0	AST, IU/L (8–32)	21
MCHC, % (32–36)	33.6	ALT, IU/L (5–33)	8
RDW, % (11.5–14.5)	16.7	ALP, IU/L (35–105)	64
Reticulocyte, % (0.5–2)	3.0	GGT, IU/L (6–42)	11
Iron, ug/dL (33–194)	58	Total bilirubin, mg/dL (0.2–1.2)	0.5
TIBC, ug/dL (228–428)	210	Direct bilirubin, mg/dL (0–0.3)	0.3
Transferrin saturation, % (20–50)	27.6	HbA1c, % (4.5–5.6)	4.3
G-6-PDH, U/gHb (4.6–13.5)	6.7	Irregular Antibody	Negative
Osmotic fragility	Normal	Coombs (Direct/Indirect)	All negative

Notes: WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; TIBC, total iron binding capacity; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; G-6-PDH, glucose-6-phosphate dehydrogenase; GGT, gamma glutamyl transpeptidase.

Methods

Gene panel sequencing

The study protocol was approved by the Institutional Review Board of the Catholic University of Korea. To identify the underlying genetic cause of the patient's abnormal laboratory examinations, her genomic DNA was analyzed with AmpliSeq on-demand panel that was customized for RBC membrane disorders, RBC enzyme deficiency disorders, and hemoglobin variant disorders using the Ion AmpliSeq Designer online tool (www.ampliseq.com). A total of 33 targeted genes were selected after their associations with inherited RBC disorders were reported in published molecular studies [7,8]. A list of the 33 selected genes is provided in Supplementary Table S1. The libraries were prepared with the on-demand panel primer pools using the Ion AmpliSeq library kit plus (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Briefly, 20 ng of the genomic DNA isolates were used in two target amplification reactions, which were then combined. The prepared

libraries for the custom panel were partially digested and phosphorylated using the FuPa reagent, ligated to different barcode adapters, and then purified. The purified libraries were quantified using the Ion library TaqMan quantitation kit (ThermoFisher). A bacterial DNA standard provided by the kit was used as the standard for the quantification of the libraries. The template of the pooled libraries of one sample was generated using the Ion PGM Hi-Q Chef kit (Thermo Fisher Scientific) on an Ion Chef system (Thermo Fisher Scientific) for Ion 318 v2 chip loading according to the manufacturer's instructions. The final library molecules were 50 pM in concentration, which is appropriate for downstream template preparation. Semiconductor sequencing was performed using an Ion 318 v2 chip (Thermo Fisher Scientific) with the Ion PGM Hi-Q sequencing kit (Thermo Fisher Scientific) on the Ion Torrent PGM Dx sequencer (Thermo Fisher Scientific) according to the manufacturer's instructions. The sequences of each individual gene were generated as average 200 bp read lengths.

Integrative analysis and in-silico *prediction*

Automated analysis of sequencing raw data was performed sequentially by Torrent Suite 5.10 and Ion Reporter 5.10 (https://ionreporter.thermofisher.com/ir/). Sequencing results were analyzed for all modes of inheritance and variants, and the results were initially filtered for rare variants (Minor Allele Frequency, MAF <0.01) from the large-scale public sequence databases (gnomAD, https://gnomad.broadinstitute.org; 1000Genomes, https://www.internationalgenome.org). The remaining SNVs and small indels located on inherited RBC disorder-associated genes were chosen according to web-based genetic databases (ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/; Human Gene Mutation Database, https://www.hgmd.cf.ac.uk/ac/index.php; OMIM, https://www.omim.org/). The filtered missense variants were predicted to be damaging or pathogenic by *in-silico* analysis for prediction of functional effect (PolyPhen-2, https://genetics.bwh.harvard.edu/pph2/; MutationTaster, https://www.mutationtaster.org/; SIFT, https://sift.bii.a-star.edu.sg/) as well as for conservation level across the different vertebrate species (SiPhy, https://omictools.com/siphy-tool; phastCons, https://varianttools.sourceforge.net/Annotation/PhastCons).

mRNA expression analysis for *EPB41* mutation

To determine the pathogenicity of candidate *EPB41* mutation, quantitative reverse transcription polymerase chain reaction (RT–PCR) was performed on a CFX96™ Real Time PCR Detection System (BioRad, Hercules, CA, USA) using the 5′ reporter FAM™ dye-labeled and 3′ quencher MGB-labeled probes (Applied Biosystems,

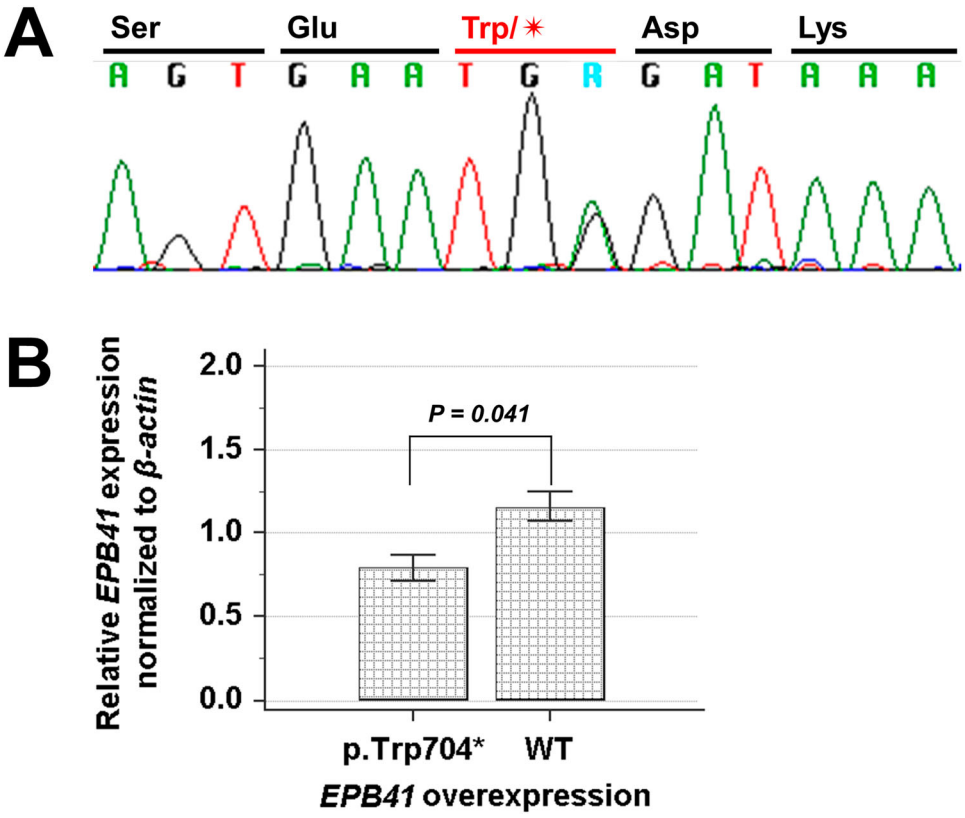


Figure 2. Sanger sequencing and mRNA expression analysis for *EPB41* mutation. (A) Sanger sequencing confirmed heterozygous c.2112G > A (p.Trp704*) in the *EPB41* gene. (B) Quantitative reverse transcription polymerase chain reaction indicates that the nonsense mutation (p.Trp704*) affect the mRNA expression level of the *EPB41*.

Foster City, CA, USA): 5′-CCTAGTGAATGAGATAA-3′ for mutant *EPT41* and 5′-TAGTGAATGGGATAAACG-3′ for wild type *EPB41*. Gene expression level was normalized to *β-actin* endogenous control and analyzed according to the relative quantification method ($2^{-\Delta\Delta Ct}$). Three independent experiments were performed.

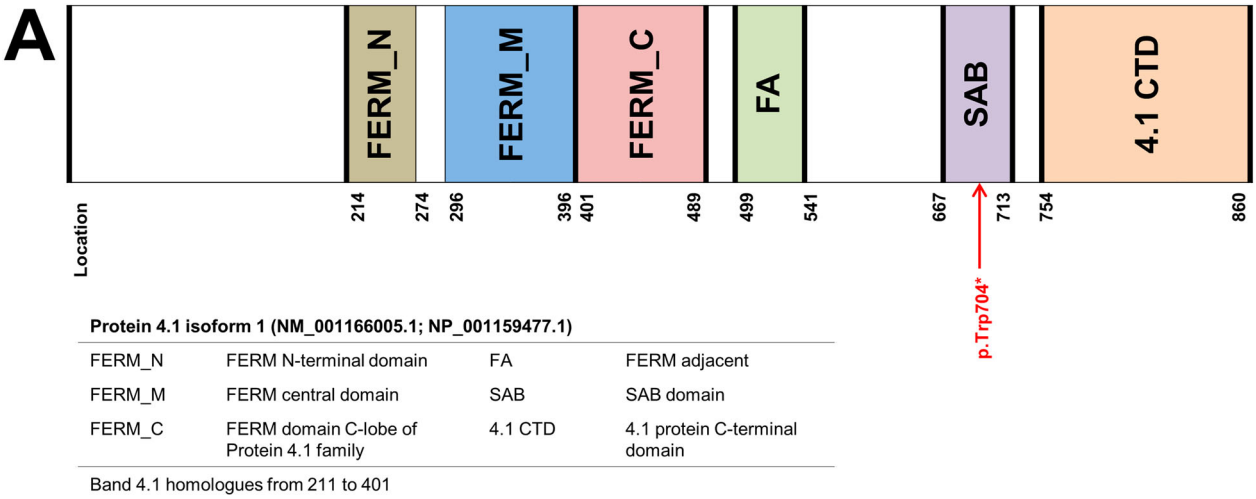
Results

By estimating the sequence quality along all sequences, an average yield on a target of 3.2 million reads was generated from the patient's sample. The median of on-target reads was 91%, the median read length was 188 bp, the median depth of on-target regions was 1,071x, and the median for uniformity of base coverage was 89%. Overall, the performance exceeded the manufacturer's specifications (>95% of amplicons should have a read depth >500×). Integrative analysis of the gene panel sequencing revealed a heterozygous c.2112G > A of the *EPB41* gene as a best candidate cause of HE. This variant, leading to premature termination codon (NM_001166005.1:c.2112G > A, *p.Trp704**), was confirmed by Sanger sequencing (Figure 2A), and the variant had not been previously reported as related to HE. Thus, the patient's diagnosis of HE-1 was genetically confirmed. In addition, quantitative RT–PCR revealed that the expression of *EPB41* mutant was lower than that of wild-type ($P = 0.041$) (Figure 2B).

Discussion

HE occurs in 0.3–0.5 per 1000 newborns, and patients are asymptomatic in about 90% of cases [9]. Approximately 95% of patients with HE have a mutation in genes responsible for α- and β-spectrin expression, i.e. polypeptides which in tetrameric form compose the basis of the cell cytoskeleton [6]. Mutations associated with the protein 4.1 and glycophorin C are rare [6]. Patients with a mutation on only one allele are asymptomatic, while in cases when it is bilateral suffer moderate or more severe hemolytic anemia [6,9]. In addition, the hereditary nature of the disorder is also supported by the absence of elements indicating other conditions that are associated with the presence of elliptocytes, such as deficiencies in iron, folic acid, or vitamin B12 [9].

A questionnaire survey in Korea revealed that the overall frequency of hereditary hemolytic anemia had an increasing tendency in adults but not in children from 1997 to 2006, and the most common cause was



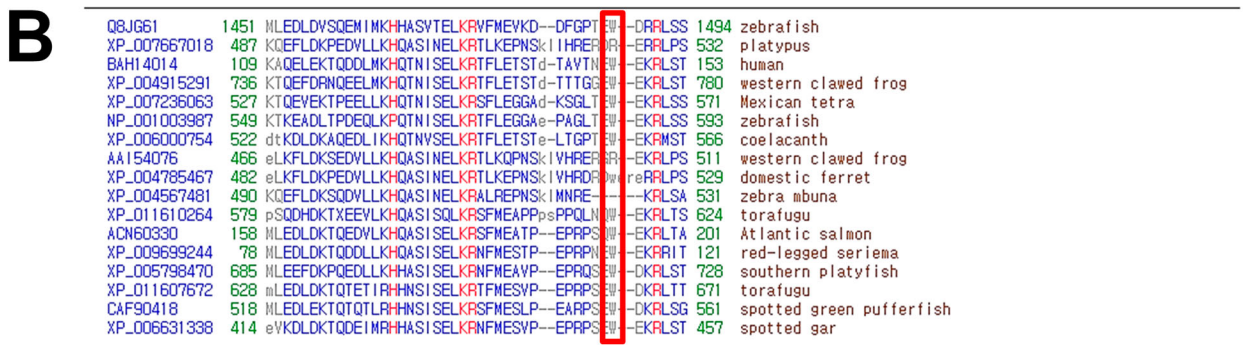


Figure 3. Schematic diagram of domain structures and local alignment in the EPB41 protein. (A) The Trp704 residue is located on the SAB (Spectrin and Actin Binding) domain of the EPB41 protein. This domain is found to bind to both spectrin and actin, hence the name SAB domain. (B) Sequence alignment of the conserved SAB domain of the EPB41 protein in multiple vertebral species. The protein sequence of the Trp704 residue, highlighted in the red box, is highly conserved across compared species.

an RBC membrane disorder (89%, 383/431) including hereditary spherocytosis (HS) (87%, 376/431) and HE (1%, 6/431) [10]. To the best of our knowledge, the present report describes the first Korean case of HE-1 with pancreatitis and gallstones in a patient carrying the *EPB41* mutation, since two Korean HE with *SPTA1* mutation have been reported previously [11,12]. Although HE is usually inherited in an autosomal dominant manner, rare instances of *de novo* mutation have been reported [9]. Thus, the genetic trait, inheritance mode (autosomal dominant or sporadic), and the characteristics of the mutation origin could not be determined in this family. In addition, one Korean patient with HS who harbored mutations in *SPTB* and *EPB41* (p.T283I on FERM N-terminal domain of EPB41 protein) was reported previously [13]. However, the *SPTB* mutation is sufficient to explain the HS phenotype, p.T283I of the *EPB41* would be classified as a private mutation or a variant of uncertain significance.

The *EPB41* gene encodes protein 4.1R which is the prototypical member of a protein family that includes 4.1G, 4.1B, and 4.1N. Among them, protein 4.1R plays a crucial role in maintaining membrane mechanical integrity by binding cooperatively to spectrin and actin through its spectrin-actin-binding (SAB) domain [14]. In the present study, the nonsense p.Trp704* mutation located on the SAB domain was found to bind to both spectrin and actin (Figure 3A). The protein sequence of the Trp704 residue in the SAB domain of the *EPB41* protein is highly conserved across compared species (Figure 3B). The 4.1R-spectrin-actin ternary complex may serve as a general paradigm for the regulation of spectrin-based cytoskeleton interaction in various cell types [14]. Weakening of lateral linkages among different skeletal proteins leads to membrane fragmentation and decreased surface area in HE. In contrast, weakening of vertical linkage between the lipid bilayer and spectrin-based membrane skeleton leads to membrane loss in HS [7]. Even if the erythrocyte paradigm indicates that spectrin–actin binding is critical in certain cell or developmental situations, the common activity of 4.1 proteins is their interaction with multiple membrane proteins and their assembly into macromolecular complexes [15].

Establishing an exact genetic diagnosis is crucial for patients' optimal clinical management and genetic counseling for family members. Massively parallel sequencing (MPS), a kind of high-throughput approach to DNA sequencing, provides a fast and accurate method to identify the causative mutation among several different genes in patients with hereditary RBC disorders. Particularly, targeted gene panel sequencing or whole exome sequencing using a MPS platform were efficiently applied to identify or confirm the diagnosis of HE, especially in cases with atypical clinical manifestation, significant overlap in the typical clinical presentations of these disorders, and in severe, transfusion-dependent cases where the RBC phenotype could be not evaluated [16–18]. Moreover, causative molecular diagnosis allows genotype-phenotype correlations in these heterogeneous disorders and may assist in prognosis discussions [19–21]. An approach that integrates medical history, clinical and molecular testing, and pedigree analysis is beneficial for these patients and families [22].

In summary, the present study confirmed a novel mutation of the *EPB41* gene and plays an important role in expanding the mutational distribution in HE-1. This evidence can help overcome inconclusive and less accurate biochemical analyses of HE-1 and be helpful for understanding the correlation between genotype and phenotype in HE.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) [grant number 2020R1F1A1077316].

ORCID

Soyoung Shin http://orcid.org/0000-0002-8422-441X
Kyuhyun Paik http://orcid.org/0000-0002-3145-3497
Joonhong Park http://orcid.org/0000-0001-7354-4234

References

[1] Da Costa L, Galimand J, Fenneteau O, et al. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev. 2013;27:167–178.
[2] McGuire M, Smith BL, Agre P. Distinct variants of erythrocyte protein 4.1 inherited in linkage with elliptocytosis and Rh type in three white families. Blood. 1988;72:287–293.
[3] Lecomte MC, Garbarz M, Gautero H, et al. Molecular basis of clinical and morphological heterogeneity in hereditary elliptocytosis (HE) with spectrin alpha I variants. Br J Haematol. 1993;85:584–595.
[4] Dhermy D, Lecomte MC, Garbarz M, et al. Spectrin beta-chain variant associated with hereditary elliptocytosis. J Clin Invest. 1982;70:707–715.
[5] Wilder JA, Stone JA, Preston EG, et al. Molecular population genetics of SLC4A1 and Southeast Asian ovalocytosis. J Hum Genet. 2009;54:182–187.
[6] Gallagher PG. Hereditary elliptocytosis: spectrin and protein 4.1R. Semin Hematol. 2004;41:142–164.
[7] Narla J, Mohandas N. Red cell membrane disorders. Int J Lab Hematol. 2017;39(Suppl. 1):47–52.
[8] Grace RF, Glader B. Red blood cell enzyme disorders. Pediatr Clin North Am. 2018;65:579–595.
[9] Gallagher PG. Hereditary elliptocytosis: spectrin and protein 4.1R. Semin Hematol. 2004;41:142–164.

[10] Cho HS, Hah JO, Kang IJ, et al. Hereditary hemolytic anemia in Korea: a retrospective study from 1997 to 2006. Korean J Hematol. 2007;42:197–205.
[11] Liu C, Eun HS, Nah H, et al. Newborn hereditary elliptocytosis confirmed by familial genetic testing. Int J Lab Hematol. 2020;42:20–22.
[12] Han E, Kim A, Park J, et al. Spectrin Tunis (Sp alpha (I/78)) in a Korean family with hereditary elliptocytosis. Ann Lab Med. 2013;33:386–389.
[13] Choi HS, Choi Q, Kim JA, et al. Molecular diagnosis of hereditary spherocytosis by multi-gene target sequencing in Korea: matching with osmotic fragility test and presence of spherocyte. Orphanet J Rare Dis. 2019;14:114.
[14] Gimm JA, An X, Nunomura W, et al. Functional characterization of spectrin-actin-binding domains in 4.1 family of proteins. Biochemistry. 2002;41:7275–7282.
[15] Baines AJ, Lu HC, Bennett PM. The Protein 4.1 family: hub proteins in animals for organizing membrane proteins. Biochim Biophys Acta. 2014;1838:605–619.
[16] Khurana M, Edwards D, Rescorla F, et al. Whole-exome sequencing enables correct diagnosis and surgical management of rare inherited childhood anemia. Cold Spring Harb Mol Case Stud. 2018;4:a003152.
[17] Wang X, Liu A, Huang M, et al. Hereditary elliptocytosis with variable expression and incomplete penetrance in a Chinese family. Br J Haematol. 2019;186:e159–e162.
[18] Xi Y, Wang L, Zhang P, et al. A novel mutation in SPTA1 identified by whole exome sequencing in a Chinese family for hereditary elliptocytosis presenting with hyperbilirubinemia: A case report. Medicine (Baltimore). 2019;98:e15800.
[19] Niss O, Chonat S, Dagaonkar N, et al. Genotype-phenotype correlations in hereditary elliptocytosis and hereditary pyropoikilocytosis. Blood Cells Mol Dis. 2016;61:4–9.
[20] He Y, Jia S, Dewan RK, et al. Novel mutations in patients with hereditary red blood cell membrane disorders using next-generation sequencing. Gene. 2017;627:556–562.
[21] Lin PC, Chiou SS, Lin CY, et al. Whole-exome sequencing for the genetic diagnosis of congenital red blood cell membrane disorders in Taiwan. Clin Chim Acta. 2018;487:311–317.
[22] Iolascon A, Andolfo I, Russo R. Advances in understanding the pathogenesis of red cell membrane disorders. Br J Haematol. 2019;187:13–24.