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CASE REPORT

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Novel mutation of transferrin receptor 2 causing hereditary hemochromatosis type 3 in a Japanese patient

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Abstract

Hereditary hemochromatosis (HH) is recognized as a progressive iron-storage disorder, and leading to severe organ impairments, including liver cirrhosis. Hereditary hemochromatosis type 3 arises from mutations in the transferrin receptor 2 (*TFR2*) gene. However, HH type 3 is rare in Asia, and information regarding genetic mutations and associated phenotypes remains limited. Here, we reported the case of a Japanese patient with HH type 3, with a novel homozygous mutation of the *TFR2* gene. A 69-year-old woman presented to our hospital with hand joint pain and was referred due to liver impairment. Viral hepatitis and autoimmune liver diseases were ruled out. However, the transferrin saturation was 92.2%, and the serum ferritin level was 1611.8 ng/mL. Additionally, abdominal computed tomography showed diffuse increased density of the liver parenchyma. Abdominal magnetic resonance imaging also suggested iron deposition. There is no history of prior treatments involving blood transfusions or iron agents. Her parents were involved in a consanguineous marriage, prompting genetic testing. She had a homozygous novel mutation, c.1337G>A (p.G446E), in the *TFR2* gene. Serum hepcidin-25 level was decreased to 2.9 ng/mL. According to the American Society of Medical Genetics and Genomics guideline, the mutation was classified as likely pathogenic, leading to the diagnosis of HH type 3. Following phlebotomy, her arthritis resolved, and serum transaminase levels were normalized. This case marks the first demonstration of homozygous mutation, c.1337G>A (p.G446E), in the *TFR2* gene in patients with HH type 3.

KEYWORDS

hemochromatosis, hereditary hemochromatosis, iron, transferrin receptor 2

Abbreviations: ACMG, American Society of Medical Genetics and Genomics; CADD, Combined Annotation-Dependent Depletion; CT, computed tomography; *HAMP*, human antimicrobial peptide; HH, hereditary hemochromatosis; *HJV*, hemojuvelin; MRI, magnetic resonance imaging; RBC, red blood cell; *SLC40A1*, solute carrier family 40 member 1; *TFR2*, transferrin receptor 2.

INTRODUCTION

Hereditary hemochromatosis is a genetic disorder characterized by disrupted iron regulation in the body. There are four types of HH based on mutated genes: type 1 involves mutations in the *HFE* gene, type 2 involves mutations in the *HJV* and *HAMP* genes, type 3 involves mutations in the *TFR2* gene, and type 4 involves mutations in the *SLC40A1* gene.[1] Mutation in the *TFR2* gene leads to a decrease in hepcidin expression and secretion, resulting in uncontrolled iron absorption from the duodenum and excessive iron accumulation in various organs, primarily in the liver.[2] Here, we reported the case of a Japanese patient of HH type 3 presenting a novel homozygous mutation of *TFR2* gene.

CASE PRESENTATION

The 69-year-old woman presented to the rheumatology department of our hospital with complaints of gradually worsening pain in the metacarpophalangeal joints of both hands over the past 15 years. Liver dysfunction was noted, prompting a referral to our department.

Blood tests revealed mild elevation of liver enzymes with alanine aminotransferase and aspartate aminotransferase (Table 1). Her habits included consuming 10 g alcohol per day and smoking 5 cigarettes per day. Tests for hepatitis B virus surface antigen, hepatitis C virus antibody, antinuclear antibodies, and antimitochondrial antibodies were negative. Serum iron levels were elevated at 261 μg/dL, while unsaturated iron binding capacity was 22 μg/dL, resulting in a significant increase in transferrin saturation to 92.2% and ferritin levels to 1611.8 ng/mL. Abdominal computed tomography scan showed diffuse increased density of the liver parenchyma (Figure 1a). Additionally, abdominal MRI revealed decreased signal intensity of the liver parenchyma on T1-weighted images in the in-phase compared to the opposed-phase and diffuse hypointensity of the liver parenchyma on T2 star-weighted images, suggestive of iron deposition, which led to a diagnosis of hemochromatosis (Figure 1b–d).

There was no history of hematologic conditions or treatments involving blood transfusion or iron agents. During the investigation of family history, it was discovered that her parents were involved in a consanguineous marriage (Figure 2a). Therefore, genetic testing for iron metabolism-related genes, including *HFE*, *TFR2*, *HJV*, *HAMP*, and *SLC40A1*, was carried out after genetic counseling. These genetic testing was analyzed using targeted next-generation sequencing analysis by hybrid capture method, covering boundary regions between exons and introns, including intronic regions up to 10 base pairs. The obtained nucleotide sequences were compared to GRCh38 to analyze low-frequency nucleotide substitutions, as well as the presence of nucleotide deletions or insertions within the gene sequences. The results showed that this patient had a new homozygous mutation, c.1337G>A (p.G446E), in the *TFR2* gene. Serum hepcidin-25 level measured by a hepcidin-25 ELISA kit (BMA Biomedicals) was decreased to 2.9 ng/mL.

The mutation at the same position, p.G446R (c.1336G>A), in the *TFR2* gene is a pathogenic mutation that has been described by gnomAD. However, this variant has not been previously reported in databases including ClinVar, dbSNP, and gnomAD. Therefore, we predicted its pathogenicity by utilizing four prediction software tools: PolyPhen-2,[3] SIFT,[4] PROVEAN,[5] and CADD.[6] All software programs showed that the p.G446E (c.1337G>A) mutation was damaging (Table S1). A CADD score of >20 is widely used as a cut-off for predicting pathogenic variants.[7] This variant was predicted to be deleterious with a CADD score of 25.8. Moreover, the novel mutation was diagnosed as likely pathogenic according to the ACMG guideline.[8]

Based on these results, we have diagnosed HH type 3, and regular phlebotomy therapy was carried out along with dietary changes, resulting in decline of serum ferritin levels and improvement in liver function and arthritis.

DISCUSSION

The patient showed very high plasma transferrin saturation and deposition of iron in the liver, strongly suggesting hemochromatosis. Hemochromatosis encompasses HH and secondary hemochromatosis, which develops as a result of iatrogenic iron administration, hematologic conditions leading to ineffective erythropoiesis, or repeated packed RBC transfusions.[9] The main cause of iron overload is RBC transfusions in Japan[10]; however, there is no record of prior treatments involving blood transfusions or iron agents in this case. Considering HH, genetic testing was undertaken to assess the possibility of HH. The patient was found to have a new homozygous mutation, c.1337G>A (p.G446E), in the *TFR2* gene. This novel mutation was identified as likely pathogenic according to the ACMG guideline based on the following criteria: PM1, the variant is located in the functional domain (Figure 2b); PM5, it is a novel missense change at amino acid residue where a different missense variant is pathogenic; PP3, multiple lines of computational evidence support a deleterious effect; and PP4, the phenotype of the patient is highly consistent with HH type 3. The genomic sequencing results confirm the diagnosis of HH type 3. Serum hepcidin-25 level was very low, suggesting an influence of mutations in the *TFR2* gene.

In recent years, responsible genes have been identified, leading to an increase in reports of HH type3 worldwide.[11,12] However, the frequency of HH type 3 with mutations in the *TFR2* gene in Asian patients is very rare.[12] There were no international marriages in the family of this case. The cases of HH type 3 associated with the *TFR2* gene mutation in Japan are described in Table 2. Hereditary hemochromatosis type 3 is inherited in an autosomal recessive

TABLE 1 Laboratory data of a 69-year-old woman with type 3 hereditary hemochromatosis.

Peripheral blood			Blood chemistry			Iron parameters		
WBC	7230	/μL	TP	6.7	g/dL	Fe	**261**	μg/dL
RBC	386	$\times 10^4$/μL	Albumin	3.9	g/dL	UIBC	**22**	μg/dL
Hb	10.5	g/dL	T-Bil	0.9	mg/dL	Transferrin saturation	**92.2**	%
Ht	31.7	%	AST	**33**	U/L	Ferritin	**1611.8**	ng/mL
Plt	28	$\times 10^4$/μL	ALT	**26**	U/L	Hepcidin-25	**2.9**	ng/mL
			ALP	53	U/L			
Coagulation			γGTP	24	U/L	Viral markers		
PT (%)	110.7		BUN	14.2	mg/dL	HBsAg	<0.01	IU/mL
PT-INR	0.94		Creatinine	0.57	mg/dL	HCV-Ab	0.04	S/CO
			Glucose	100	mg/dL			
			HbA1c	5.9	%			
			ANA	(–)				
			AMA	(–)				

Note: Abnormal values are presented in bold.

Abbreviations: ALP, alkaline phosphate; ALT, alanine aminotransferase; AMA, antimitochondrial antibody; ANA, antinuclear antibody; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Hb, hemoglobin; HbA1c, hemoglobin A1c; HBsAg, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; Ht, hematocrit; Plt, platelets; PT, prothrombin time; PT-INR, prothrombin time international normalized ratio; RBC, red blood cell; T-Bil, total bilirubin; TP, total protein; UIBC, unsaturated iron binding capacity; WBC, white blood cell; γGTP, γ-glutamyltransferase.

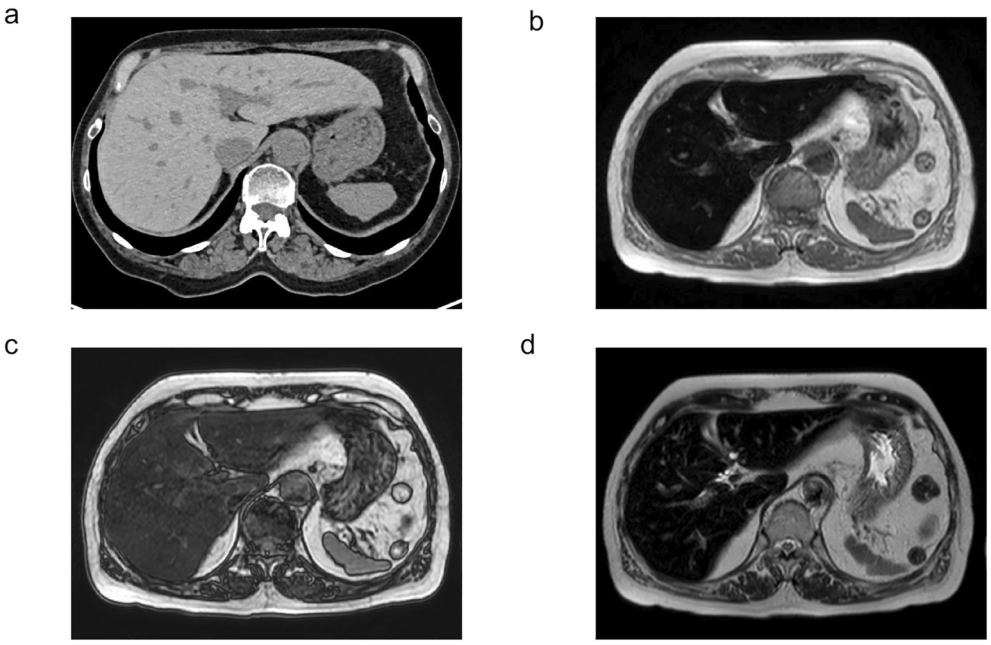

FIGURE 1 Computed tomography (CT) and magnetic resonance imaging of the patient, a 69-year-old woman with type 3 hereditary hemochromatosis. (a) CT showed hyperattenuation of the liver parenchyma values. T1-weighted image showed a decrease in signal intensity in the in-phase (b) compared to the opposed-phase (c), indicating iron overload. (d) T2 star-weighted images showed diffuse hypointensity of the liver parenchyma.

manner and manifests with homozygous mutations. Three families with a total of five patients with homozygous mutations have been reported in Japan.[13,14] Three cases of onset with heterozygous mutations have also been reported.[15–17] Single nucleotide polymorphisms in the *TFR2* gene affect serum iron levels, and individuals with heterozygous mutations in the *TFR2* gene may develop hemochromatosis when exposed to environmental factors that can lead to iron overload.[18] Nishio et al. reported that a high

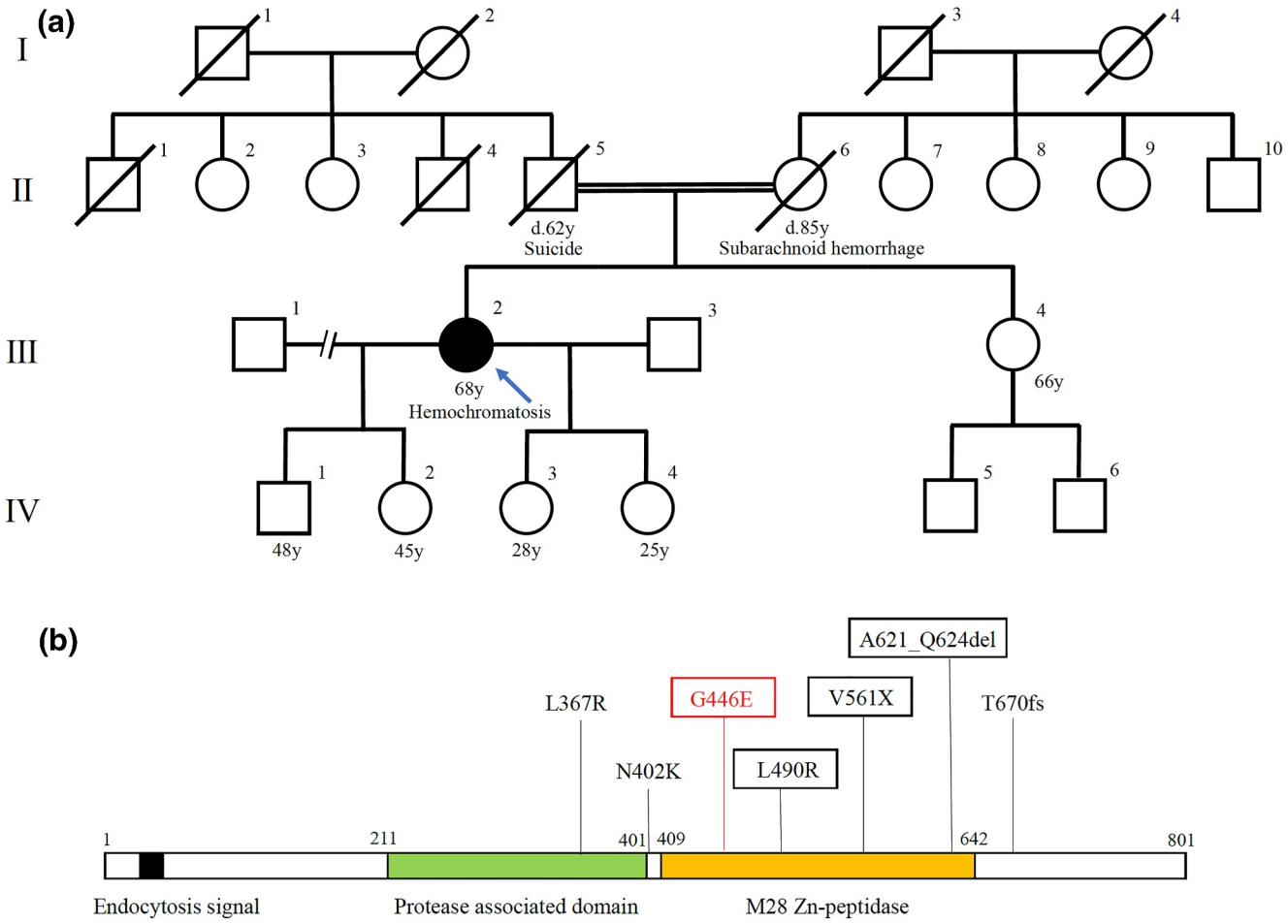

FIGURE 2 Pedigree and localization of the reported mutations in the *TFR2* gene. (a) Pedigree of the patient, a 69-year-old woman with type 3 hereditary hemochromatosis (indicated with an arrow). (b) Localization of mutations reported in Japanese individuals. Homogenic mutations are boxed. The new reported mutation is shown in red. y, years.

intake of meat and lifestyle habits may have contributed to hemochromatosis with heterozygous mutations in the *TFR2* gene.[17] Hereditary hemochromatosis type 3 with heterozygous mutations has been reported in Africa, suggesting a possible association with supplement intake.[19] Alcohol exposure can increase iron absorption itself, potentially through an inhibitory effect on hepcidin production.[20] In this case, alcohol intake in addition to genetic mutations might influence the iron overload. As for symptoms, all cases presented with liver impairment, and some cases showed diabetes, pigmentation, heart failure, and/or pituitary dysfunction. Sites of iron-mediated toxicity in hemochromatosis include the brain, heart, liver, pancreas, and bones.[21] Arthritis often occurs before other symptoms, and the participation of the second and third metacarpophalangeal joints is a distinguishing characteristic of HH.[22] Arthritis related to hemochromatosis was observed for the past 15 years in this case. Treatment for patients with HH involves phlebotomy therapy, which can lead to improvement in liver fibrosis, reduction in the risk of developing cirrhosis and diabetes, and decreased mortality rates.[23] Following phlebotomy, this patient's arthritis resolved, and serum transaminase levels were normalized.

Despite our important findings, there are a few limitations. First, a liver biopsy has not been carried out. In the past, the measurement of iron levels in liver tissue samples has been used for diagnosing iron overload, but is no longer commonly used for this purpose.[21] Instead, MRI techniques for assessing liver iron content has been validated and proven to provide accurate estimations of liver iron concentrations.[24] Second, we could not test for mutations in the *TFR2* gene for her family members. The patient's parents have passed away, and genetic testing was not possible. Although the patient has a living sister, she declined testing.

In conclusion, this is the first report of a novel compound homozygous mutation, c.1337G>A (p.G446E), in the *TFR2* gene in a Japanese HH type 3 patient. This case report highlights several important clinical considerations. When encountering hemochromatosis without a history of blood transfusion or iron supplementation, it is crucial to consider HH and to conduct appropriate diagnosis including genetic testing.

TABLE 2 Hereditary hemochromatosis type 3 patients with *TFR2* mutations in Japan.

Number	Sex	Age (years)	Homogenic/ heterogenic	Codon change	Amino acid change	Phenotypes				Reference
						Liver	Diabetes	Pigmentation	Other	
1[a]	Male	50	Homogenic	1861-1872del12	A621_Q624del	(+)	(−)	(−)	-	15
2[a]	Male	47	Homogenic	1861-1872del12	A621_Q624del	(+)	(−)	(+)	-	15
3[a]	Female	53	Homogenic	1861-1872del12	A621_Q624del	(+)	(−)	(−)	-	15
4	Male	41	Homogenic	1469T>G	L490R	(+)	(+)	(−)	-	16
5	Male	58	Homogenic	1665delC	V561X	(+)	(+)	(+)	Heart failure	16
6	Male	40	Heterogenic	1100T>G/WT	L367R	(+)	(+)	(−)	Hypopituitarism	18
				2008-9delAC/WT	T670fs					
7	Female	79	Heterogenic	1206C>A/WT	N402K	(+)	(−)	(+)	-	17
8	Male	38	Heterogenic	1206C>A/WT	N402K	(+)	(−)	(−)	-	19
9	Female	69	Homogenic	1337G>A	G446E	(+)	(−)	(−)	Arthritis	This case

Abbreviation: WT, wild type.

[a]Case numbers 1, 2, and 3 are siblings.

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CONFLICT OF INTEREST STATEMENT

Hayato Nakagawa is an Editorial Board member of *Hepatology Research*. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: This case report did not require the approval of the research protocol in accordance with Japanese regulations.

Informed consent: The patient provided their written informed consent to participate in this study.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Research involving recombinant DNA: N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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