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【 CASE REPORT 】

Late-onset Hemochromatosis: Co-inheritance of β-thalassemia and Hereditary Hemochromatosis in a Chinese Family: A Case Report and Epidemiological Analysis of Diverse Populations

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Abstract:
Hereditary hemochromatosis and β-thalassemia can both result in the inappropriately low production of the hormone hepcidin, which leads to an increase in intestinal absorption and excessive iron deposition in the parenchymal cells. To the best of our knowledge, there have been no reports on the coexistence of the two disorders in China. We herein report a case in a Chinese who presented with late-onset hepatic cirrhosis with hereditary hemochromatosis and β-thalassemia. We analyzed the pedigree of the two disorders and the iron status in his family members. Our case supports that a heterozygous H63D mutation can interact with β-thalassemia, leading to late-onset hemochromatosis.

Key words: hereditary hemochromatosis (HH), HFE gene, β-thalassemia, H63D heterozygous mutation, iron overload

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Introduction

Hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in Caucasians, affecting 1 in every 200-400 individuals (1). In contrast, it is relatively rare in Asians (2). HH is an inborn error of iron metabolism that is characterized by increased intestinal iron absorption, which leads to the progressive accumulation of iron in the body. Excess iron causes irreversible damage to various organs (3). The hemochromatosis (HFE) gene has been identified to be mainly responsible for HH. Thus far, there have been no precise epidemiological data on the incidence of HH-related genotypes, and symptomatic HH is very rare in China. In a retrospective analysis of the Peking Study, which was conducted from 2002 to 2012, there were only 39 confirmed cases of clinical HH (4). The β-thalassemias (β-thal) are a diverse group of disorders of hemoglobin synthesis, all of which result from the reduced output of the β chains of adult hemoglobin (5). It is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East, as well as in countries along the north coast of Africa and South America (6). The reported prevalence of β-thal in the Sichuan district of China is 3.2% (7). The coexistence of the two disorders is very rare in China. In this report, we describe the case of a Chinese patient who presented with late-onset hepatic cirrhosis with HH and β-thal. We analyzed the mutations associated with these two disorders and the iron status of the patient's family members. Six members of 3 generations of the family underwent hemoglobin electrophoresis, the evaluation of their iron metabolism parameters, HFE gene exon sequencing and β-thal genetic testing.

Case Report

The proband was a 68-year-old man whose family had lived for generations in Sichuan province of the People's Republic of China. The patient presented with a 2-month history of dizziness and weakness, accompanied by progres-

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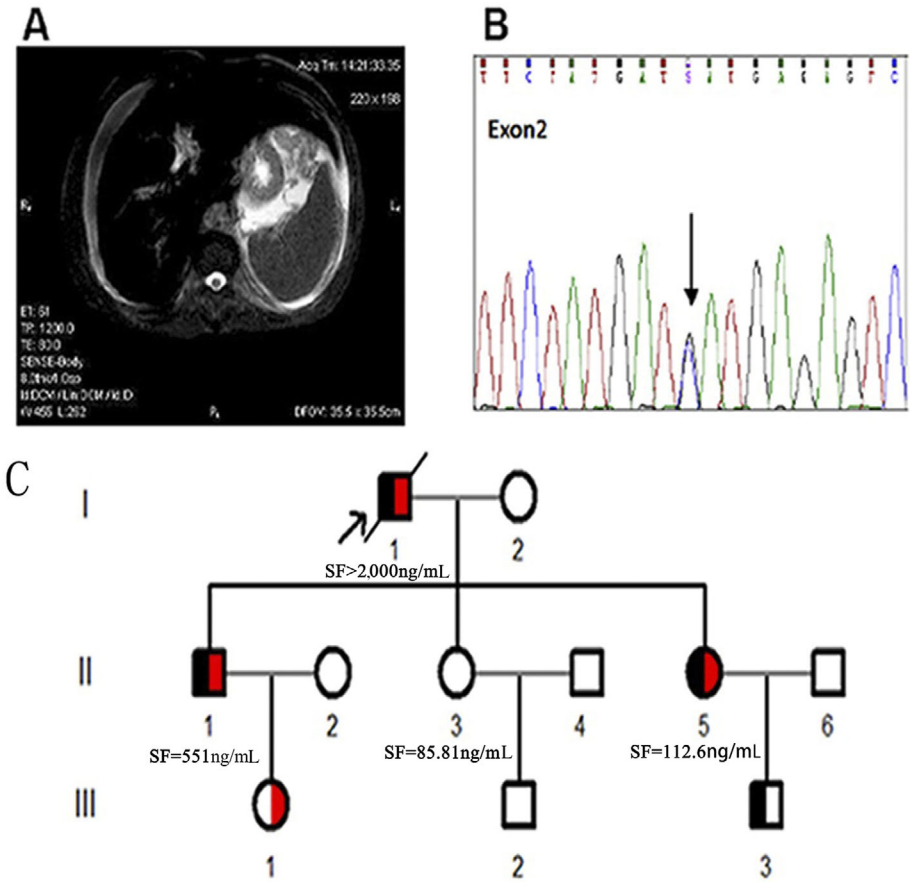

Figure. **(A) An MRI of the patient's abdomen showing hepatic hemosiderosis complicated by cirrhosis (dark liver sign). (B) A sequencing analysis of the proband's HFE gene. The " (arrow) " indicates heterozygous for the C>G substitution at position 187 in exon 2 of the HFE gene. (C) The family pedigree. The " (arrow) " shows the proband. H63D mutations are shown in black, and β-thal 654 mutations are in red. The proband (I-1) showed both mutations with severe iron overload. His son (II-1) presented with elevated ferritin (551 ng/mL) but no manifestations of HH. The other family members had no manifestations of HH, and their iron metabolism parameters were normal. SF: Serum ferritin**

sive skin pigmentation without any predisposing factors. He denied any history of blood transfusions, alcoholic liver disease, hepatitis B, fatty liver disease or autoimmune disease. He also denied a dietary basis for his symptoms and hemorrhoid hemorrhage. Ultrasonography, which was performed in a local hospital, showed an irregular hepatic surface, hepatomegaly and ascites. A physical examination revealed an anemic appearance, but no signs of hemolysis or hemorrhage, such as fever, jaundice or purpura. Mild hepatosplenomegaly and pigmentation of the skin were observed. Laboratory studies revealed the following findings: hemoglobin (Hb), 6.1 g/dL; mean corpuscular volume (MCV), 77.3 fL; mean corpuscular Hb (MCH), 23.6 pg; platelet (PLT) count, 137×10^9/L; white blood cell (WBC) count, 2.71×10^9/L; conjugated bilirubin, 6.0 μmol/L; unconjugated bilirubin, 3.8 μmol/L; alanine aminotransferase (ALT), 85 IU/L; aspartate transaminase (AST), 99 IU/L; alkaline phosphatase (ALP), 116 IU/L; lactate dehydrogenase (LDH), 207 IU/L; albumin (ALB), 31.7 g/L; prothrombin time (PT), 15.1 s; international normalized ratio (INR), 1.21; activated partial prothrombin time (APTT), 35.4 seconds; fibrinogen, 2.85 g/L; serum ferritin, >2,000 ng/mL; serum iron, 34.30 μmol/L; transferrin saturation, 72.8%; and total iron binding capacity, 47.10 μmol/L. The patient was negative for hepatitis B surface antigen (HBsAg), hepatitis C-virus (HCV)-Ab, antinuclear antibodies and positive for HBsAb. Capillary electrophoresis (SEBIA) indicated Hb A, 96.2%; Hb A_2, 3.8%; and Hb F, 0%. Computed tomography (CT) revealed an uneven density of the liver parenchyma, a widened hepatic fissure, and a dilated portal vein (diameter: 1.4 cm). Nuclear magnetic resonance imaging (MRI) of the abdomen showed hepatic hemosiderosis complicated by cirrhosis (dark liver sign) and mild enlargement of the liver and spleen, ascites (Figure A). His iron overload was not solely explained by thalassemia, as he had been healthy during the prior 6 decades without transfusion or obvious hepatosplenomegaly. We therefore performed genetic testing for both HH and thalassemia. β-thal genetic testing using a polymerase chain reaction (PCR)-Reverse dot-blot assay showed a heterozygous 654 mutation. HFE genetic testing revealed a c.187C>G (p.

Table 1. Frequency and Iron Status of Subclinical HH with H63D Mutation in β-thal.

Reference	Country	Subjects	Type	Serum ferritin (ng/mL)	"interacting" effect
13	India	110	H63D/H63D: 1(1%)	504**	Yes
		β-thal	H63D/wt: 16 (16%)	143.16±80.3**	
		carriers	wt/wt: 83(83%)	88.64±92.43**	
19	Italy	152	H63D/H63D: 4 (2.6%)	389±75*	Yes
		β-thal	H63D/wt: 45 (29.6%)	295±186	
		carriers	wt/wt: 103 (67.8%)	250±138*	
20	Portugal	101	H63D/H63D: 0 (0%)	-	Yes
		β-thal	H63D/wt: 29 (28.7%)	99.2	
		carriers	wt/wt: 67 (66.3%)	73.7	
21	India	308	H63D/H63D: 3 (1.0%)	2,500±236.2**	Yes
		β-thal	H63D/wt: 43 (14.0%)	675.0±146.9**	
		majors	wt/wt: 262(85%)	NA	
22	Egypt	41	H63D/H63D: 1 (2.4%)	NA	Yes
		β-thal	H63D/wt: 13 (31.7%)	NA	
		carriers	wt/wt: 17 (41.5%)	NA	
23	Egypt	50	H63D/H63D: 0 (0%)	-	Yes
		β-thal	H63D/wt: 5 (10%)	6,778±581**	
		majors	wt/wt: 45 (90%)	3,121.8±1,600**	
24	Italy	216	H63D/H63D: 5 (2.3%)	1,884	No
		β-thal	H63D/wt: 59 (27.3%)	1,884	
		majors	wt/wt: 144 (66.6)	1,484	
25	Italy	71	H63D/H63D: 1 (1.4%)	3,462**	No
		β-thal	H63D/wt: 15 (21.1%)	138.2**	
		majors	wt/wt: 53 (74.6%)	NA	
26	India	215	H63D/H63D: 3 (1.4%)	48.3	No
		β-thal	H63D/wt: 32(14.9%)	64	
		traits	wt/wt: 180(83.7%)	52.2	
28	Thailand	94	H63D/H63D: 0 (0%)	-	No
		β-thal	H63D/wt: 9 (9.6 %)	139.2	
		carriers	wt/wt: 85 (90.4%)	132.3	
29	Brazil	138	H63D/H63D: 4 (2.9%)	202.1	No
		β-thal	H63D/wt: 28(20.3%)	202.1	
		carriers	wt/wt: 96(69.6%)	174.9	
27	Espana	142	H63D/H63D: 4(3%)	NA	No
		β-thal	H63D/wt: 49 (35%)	NA	
		carriers	wt/wt: 87 (61%)	NA	

NA: not available, wt: wild type, *: there was statistically difference between the groups, p<0.05. **: p<0.01

H63D) missense heterozygous mutation by direct nucleotide sequencing (Figure B). Thus, the patient was diagnosed with β-thal minor and clinical HH, complicating decompensated cirrhosis. His symptoms were gradually relieved by iron chelation with deferoxamine mesylate (2,000 mg, daily) for three months, and his serum ferritin level decreased to 317 ng/mL. An additional five family members were studied after obtaining their written informed consent. The pattern of inheritance is shown in Figure C. The proband's son and younger daughter were both β-thal carriers with a heterozygous 654 mutation and a heterozygous H63D mutation, whereas the older daughter remained unaffected. We also checked the family for another 700 genes associated with hereditary hematological and immune deficiency disorders (including hepcidin (HAMP), transferrin receptor 2, and ferroportin) using next generation sequencing. We did not find any other known pathogenic abnormalities. Furthermore, the son had already developed a high ferritin level (551 ng/mL) but no manifestation of HH. None of his grandsons or granddaughters carried both mutations; moreover, they did not have symptoms or indices of an iron metabolism abnormality.

Discussion

HFE-hemochromatosis results from the mutations of 2 genes, C282Y and H63D (8). Homozygous C282Y mutations are mainly responsible for clinical HH in Caucasians. However, this alone cannot account for iron overload in non-Caucasians because of the low prevalence (9). Similarly to reports from Korea (10), Japan (11) and India (12, 13), a study of 1,615 healthy volunteers in the Chinese population

Table 2. Reported Cases of Coexistence Clinical HH with β-thal.

Reference	Age/ Gender	Race	Hb (g/dL)	Hb A_2 (%)	TS (%)	Serum ferritin (ng/mL)	HFE	β-thal	Organ damages
30	63 male	NA	7.8	5.6	100	3,000	C282Y/ wt	Cod 41/42-TCTT	Liver Spleen Skin
32	27 female	Italian	9.9	NA	63	621	C282Y/ wt	Cod 39/IVS1-6	Liver
25	6 female	Italian	NA	NA	NA	3,462	H63D/ H63D	Lepore thalassemia	Liver fibrosis
31	38 female	Brazilian	11.9	5.3	88	2,162	C282Y/ C282Y	Heterozygosity mutation	Arthralgia
33	18 male	Italian	NA	NA	NA	NA	H63D/ H63D	IVS1-110/ IVS1-110	Cardiomyopathy Hypogonadism
33	37 male	Italian	NA	NA	NA	NA	H63D/ H63D	IVS1-110/ codon 39 (C>T)	Cardiomyopathy Hypogonadism
34	They reported 16 Italian patients with β-thal trait and a classical HH phenotype, including seven C282Y homozygotes, one C282Y heterozygotes, three compound heterozygotes, four H63D heterozygotes and one H63D homozygotes. Most of them had elevated serum ferritin and organ damages.								

TS: transferrin saturation, NA: not available, wt: wild type

found no C282Y mutations (14, 15). This was in accordance with the report by Tsui et al. from Hong Kong in which the C282Y mutation was not found in 49 Chinese patients who had been diagnosed with hemochromatosis based on liver biopsy or autopsy findings (16). However, in a retrospective analysis at Peking Union Medical College Hospital, 24 clinical HH patients (61.5%) had homozygous C282Y mutations, 10 (25.6%) had homozygous H63D mutations, and 4 (10.3%) had heterozygous mutations (4). According to the largest epidemiological dataset, the incidence of H63D heterozygotes and homozygotes in 1,615 Chinese individuals was 10.2% and 0.24%, respectively; which is much lower in comparison to Caucasians (24%), Native Americans (20%), and Hispanics (18%) (9, 14). Thus, the effect of the H63D mutation on iron overload in Chinese individuals remains controversial.

However, the coexistence of a H63D mutation with other disorders that predispose a person to iron absorption may have an interacting effect. The ferritin levels of patients with myelodysplastic syndrome (MDS) and aplastic anemia (AA), were higher among patients with H63D mutations (14, 17). Similarly to MDS and AA, β-thal can also cause acquired iron overload through repeated transfusion and increased intestinal iron absorption. β-thal can be classified as thalassemia major, thalassemia intermedia, or thalassemia minor. Transfusion is usually unnecessary for thalassemia intermedia and thalassemia minor. Although these patients are at risk of iron overload secondary to increased intestinal iron absorption, severe iron overload and target organ damage are not common (18). The role of H63D mutation polymorphism in β-thal major or carrier conditions has been studied in regions with a high incidence of H63D, such as in Southern Europe and Asia. Various studies have yielded conflicting conclusions (shown in Table 1): some studies from Italy, Portugal, India and Egypt suggested that iron overload might arise from the interacting effect of β-thal with homozygous or even heterozygous H 63 D mutations (13, 19-23); other reports from Italy, India, Thailand, Brazil and Spain indicated that the iron status was not related to the H63D mutation status (24-29). The discrepancy may be due to the sample size, hereditary background variations in different racial populations, the sex ratio and the severity of thalassemia. In these studies, patients with β-thal major seemed to have elevated ferritin levels if a H63D mutation was present.

Interestingly, Rees et al. published a study on a prototype family in which mutations associated with HFE and thalassemia had a co-effect on iron overload. The proband and his daughter had heterozygous C282Y mutations, and his mother had a homozygous C282Y mutation. Only the proband had β-thal and developed clinical hemochromatosis. His mother carried a homozygous C282Y mutation, without a β-thal mutation, and did not develop iron overload (30). This finding strongly demonstrated the effect of the coexistence of HFE mutations and other diseases on the iron status. The coexistence of β-thal and clinical HH is very rare. There are only twenty-one reported cases involving the coexistence of β-thal and clinical HH, including eight C282 Y homozygotes, three C282Y heterozygotes, three compound heterozygotes, four H63D heterozygotes and three H63D homozygotes (25, 30-34). Their clinical and laboratory data are shown in Table 2. All of the patients developed severe iron overload or target organ damage. None of these individuals were from Chinese families.

In our report, the proband was diagnosed with β-thal minor. During the previous 60 years, he had only been a carrier of the β-thal mutation and had no symptoms of anemia or history of repeated blood transfusions. However, when he presented to our department with symptoms of anemia, hemochromatosis was confirmed based on an increased fer-

ritin level (>2,000 ng/mL) and severe target organ damage (dark liver sign). This hemochromatosis might not have been completely secondary to β-thal. It is suggested that the co-existence of HFE mutations may be the genetic basis for severe iron overload and cirrhosis. "Compound" heterozygous involving different genes might have an interacting effect on iron overload and ultimately lead to a phenotypic abnormality. The patient's 44-year-old son and younger daughter presented with a similar mutation pattern, and his son had an elevated ferritin (551 ng/mL) level but no manifestations of HH. His daughter had no symptoms or indices of iron metabolism abnormality, possibly due to menometrorrhagia. The follow-up of the patient's children will be very important.

Thus, if a patient with thalassemia presents with iron overload that is inconsistent with their transfusion history, genetic screening should be performed to detect thalassemia-related genes and a concomitant mutation in a second gene, either within the HFE gene or elsewhere, such as the genes encoding hepcidin, transferrin receptor 2, and ferroportin. Further epidemiological studies would help to differentiate the power of the interaction between HFE gene mutations and β-thal in Chinese populations and to establish preventive and therapeutic strategies for iron overload.

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

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