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A Case Report and Analysis: Hereditary Hemochromatosis Caused by a Splice Site Mutation in the HFE Gene

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Abstract: Hereditary hemochromatosis (HH) is defined as an autosomal recessive iron metabolism disorder, typically characterized by excessive iron absorption leading to iron overload in multiple organs, particularly the liver, heart, and endocrine glands. Clinical manifestations commonly include diabetes, skin pigmentation, cirrhosis, abdominal pain, arthralgia, and fatigue. Mutations in the HFEgene, located on chromosome 6p22, have been identified as the primary genetic basis of HH. This gene encodes a protein critical for iron absorption and metabolism. Studies indicate that HH is most prevalent in European populations but rare in Asians. Due to reduced iron accumulation from menstrual blood loss in females, the disease predominantly affects adult males, with females often developing symptoms post-menopause. This article reports a rare case of HHin a premenopausal Chinese female caused by a homozygous HFE gene mutation (c.340 + 4T > C). The patient presented with chronic fatigue, abdominal pain, and lower limb petechiae. Diagnosis was confirmed via clinical evaluation, laboratory tests, and genetic analysis. This study identifies a novel pathogenic mutation in the Chinese population, contributing to early diagnosis and treatment of HH.

Keywords: Hereditary Hemochromatosis (HH); HFE gene; Chinese female

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1. Introduction

Hereditary hemochromatosis (HH) is an autosomal recessive disorder characterized by excessive intestinal iron absorption and systemic iron overload due to defective iron excretion regulation [1,2]. Iron accumulation in organs such as the liver, heart, pancreas, and skin leads to oxidative stress via reactive oxygen species (ROS), causing tissue damage [2].

The disease exhibits ethnic disparities, with higher prevalence in Europeans than Asians, Africans, or Oceanians [3,4]. Historically, HH is classified into four types based on mutations in iron-regulatory genes (HFE,

HAMP, HJV, TFR2, SLC40A1). However, recent classifications simplify this into HFE-related, non-HFE related, digenic, and molecularly undefined HH. HFE-related HH is the most common subtype [5,6].

The HFE gene is closely associated with hemochromatosis. This gene is located in the major histocompatibility complex (MHC) region on chromosome 6 and encodes HFE, a protein structurally similar to MHC class I molecules. Although small amounts of HFE are expressed in nearly all normal tissues and cells, its primary localization occurs in liver and intestinal cells [1,3,7]. HFE plays a crucial role in regulating iron metabolism through its interaction with the transferrin receptor (TfR). Cell surface TfR binds to the Fe^{2+}-transferrin (Tf) complex and facilitates iron uptake via endocytosis. HFE modulates this process by competitively binding to TfR, which both reduces the number of available Tf-binding sites and inhibits TfR-Tf endocytosis, thereby decreasing cellular iron uptake [1,8,9]. Furthermore, the HFE-TfR complex suppresses hepcidin production, a peptide hormone synthesized in hepatocytes that regulates iron homeostasis by inhibiting ferroportin (FPN), the primary cellular iron exporter in mammals. This dual mechanism downregulates both dietary iron absorption into circulation and iron release from macrophage storage pools [10–12]. However, in most hereditary hemochromatosis (HH) patients, HFE gene mutations impair this regulatory function, ultimately leading to systemic iron overload. The two most prevalent HFE-related HH mutations are C282Y and H63D. The C282Y mutation involves substitution of tyrosine for cysteine at position 282 in the HFE protein sequence. This alteration disrupts disulfide bond formation, impairing HFE's ability to bind β2-microglobulin. Consequently, the mutated HFE cannot reach the cell surface, preventing interactions with both hepcidin and TfR [1,2,12,13]. The H63D mutation refers to replacement of histidine with aspartic acid at position 63. While this variant may moderately affect iron homeostasis, it predominantly contributes to iron accumulation when co-occurring with the C282Y mutation [2,12].

Based on current research, early diagnosis and treatment of hereditary hemochromatosis (HH) can effectively delay the onset of clinical symptoms and reduce mortality rates. The two primary approaches for reducing iron overload are blood removal and iron chelation therapy. The former includes phlebotomy, venesection, and erythrocytapheresis established as first-line therapy for HH, which has been proven to significantly reduce cardiovascular disease and hepatocellular carcinoma mortality [1,3,14,15]. The latter is primarily reserved for patients contraindicated for phlebotomy, such as those with severe anemia or heart failure, where iron chelators have demonstrated safety and efficacy in reducing ferritin levels, particularly in C282Y homozygotes [14]. Emerging evidence also suggests proton pump inhibitors (PPIs) may effectively reduce phlebotomy requirements in HH patients [16].

In this study, we report a female patient presenting with recurrent abdominal distension, pain, fatigue, and weight loss. Laboratory investigations revealed markedly elevated serum ferritin (SF) levels, with liver biopsy and MRI confirming hepatic iron overload. Genetic analysis through Sanger sequencing identified a novel splice-site mutation (c.340 + 4T > C) in the HFE gene. This discovery expands the spectrum of pathogenic HFE mutations and provides new insights for early diagnosis and therapeutic strategies in HH.

2. Case presentation

A 49-year-old female patient presented to the Department of Hematology at Southwest Medical University with a 3-year history of abdominal pain, distension, and fatigue. During the disease course, she developed intermittent acid reflux, heartburn, and pain in the right hypochondrium and back, along with multiple ecchymoses and petechiae on both hands. Recent treatment included hepatoprotective therapy with ursodeoxycholic acid and bicyclol. The patient denied recent infections, liver injury, alcohol consumption, or prior blood transfusion history.

Physical examination revealed numerous ecchymoses and petechiae of varying sizes on both lower limbs, with no other significant abnormalities.

Previous laboratory tests showed elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol (TC). Serum ferritin (SF) levels were markedly increased, while transferrin saturation (TSAT) was not assessed. Tests for viral hepatitis, autoimmune hepatitis, and disseminated intravascular coagulation (DIC) were unremarkable. Gastrointestinal endoscopy identified a hyperplastic polyp in the cardia and six small intestinal polyps with mild glandular atypia, all of which were excised. Doppler ultrasound of the liver demonstrated coarse and heterogeneous parenchymal echogenicity, normal diameters of the portal and splenic veins, but increased blood flow velocity and elevated liver stiffness measurements, suggestive of hepatic fibrosis (specific values in **Table 1**). Liver MRI revealed transient arterial-phase enhancing lesions in segments V/VI, indicating possible perfusion abnormalities, along with features of chronic liver disease and hepatosplenic iron overload.

Table 1. Clinical laboratory and examination results for the patient

Auxiliary examination	Value	Reference range
Examination item		
ALT (U/L)	99	7–40
AST (U/L)	45	13–35
TC (mmol/L)	6.2	2.9–5.18
GLU (mmol/L)	6.4	3.9–6.1
Serum iron index		
SF (ng/mL)	431	3–150
Liver ultrasound		
PVE (cm/s)	91.41	10–20
SVF (cm/s)	43.74	10-20
STE (kPA)	11.51	< 6.5

SF: Serum Ferritin, PVF: Portal Venous Flow Velocity, SVE: Splenic Venous Flow Velocity, STE: Liver Stiffness Measurement

Following hepatoprotective therapy with ursodeoxycholic acid and bicyclol, the patient's symptoms showed no significant improvement, prompting further investigations via liver biopsy and genetic testing. Histopathological analysis revealed preserved hepatic lobular architecture with focal steatosis (mixed type), scattered spotty and focal necrosis within the lobules, and brownish granular deposits in hepatocytes (distributed across lobules, predominantly around central veins). Mild piecemeal necrosis was observed at the limiting plates. Portal tracts exhibited mild lymphomonocytic infiltration without significant plasma cell involvement. CK7 staining showed no notable bile duct proliferation, with native bile ducts intact. Foot and Masson stains demonstrated mild fibrotic expansion and focal fibrous septa formation. Additional immunohistochemistry confirmed HBsAg (-), HBcAg (-), CD38 (rare plasma cells +), and IgG1(-). Prussian blue staining identified iron granule deposition in hepatocytes.

Genetic testing revealed a homozygous HFE splice-site mutation (c.340 + 4T > C) (**Figure 1**). The

patient was lost to follow-up, and parental genetic testing was unavailable. Based on clinical manifestations, laboratory/imaging findings, and molecular evidence, a definitive diagnosis of hereditary hemochromatosis (HH) was established. Current evidence indicates that early diagnosis and interventionare critical for HH patients to achieve normal life expectancy. Therapeutic recommendations include iron chelation therapy or proton pump inhibitors (PPIs), coupled with dietary restrictions on iron-rich foods such as alcohol and shellfish [14].

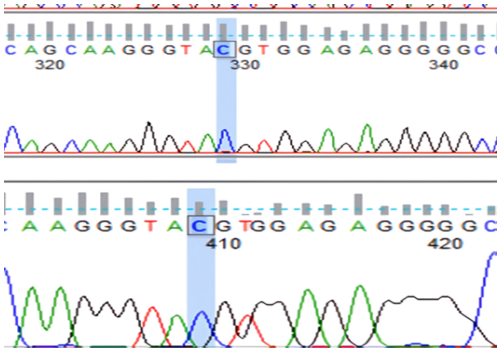

Figure 1. Sanger sequencing of the patient

3. Methods

Polymerase chain reaction (PCR) and Sanger sequencing were performed to confirm the identified mutation in genomic DNA extracted from the proband's peripheral blood. Reference Sequence Retrieval: The HFE gene sequence (NG_008720.1) was obtained from the NCBI Genome Browser (https://www.ncbi.nlm.nih.gov/).Neural Network Site (NNSplice) software (https://www.fruitfly.org/seq_tools/splice.html) and MaxEntScan(http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html) were employed to calculate splice site scores and identify canonical versus cryptic splice sites between exons 2 and 3 of the HFE gene. SpliceAid2 (http://193.206.120.249/splicing_tissue.html) was utilized to analyze splicing regulatory elements, including exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), and dynamic changes in canonical/cryptic splice site profiles within the mutated region.

4. Discussion

In this case report, the patient presented to multiple hospitals with recurrent symptoms including abdominal pain, distension, fatigue, weight loss, and ecchymoses/petechiae on both lower limbs. During the disease course, laboratory investigations revealed elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum ferritin (SF), accompanied by hepatosplenic iron overload and hepatic fibrosis. Genetic analysis via Sanger sequencing identified a homozygous splice-site mutation (c.340 + 4T > C) in the HFE gene (**Figure 2**).

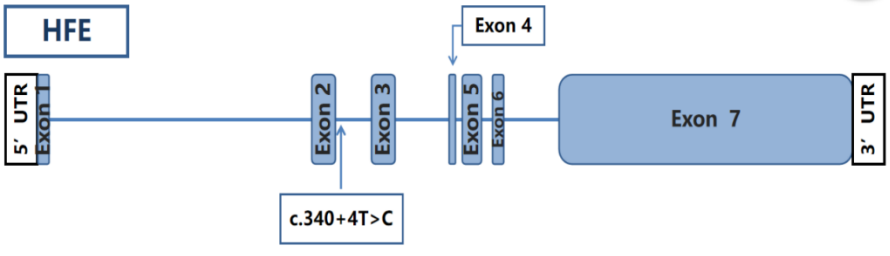

Figure 2. Denotes the precise location of the c.340 + 4T > C mutation within the HFE gene.

Bioinformatic analysis using Neural Network Site software (**Table 2**) indicated that the c.340 + 4T > C mutation did not alter exon-intron boundary sequences. Both the wild-type and mutant sequences exhibited potential splice sites at this locus with strong signal scores. However, MaxEntScan analysis (**Table 2**) revealed elevated splice site strength metrics in the mutant, suggesting a risk of aberrant splice activation, exon skipping, or intron retention.Subsequent analysis using SpliceAid2 (**Table 3**) identified six exonic splicing enhancer (ESE) binding sites in the wild-type sequence, all scoring the maximum (5 points). Notably, abundant ESEs—particularly ETR-3-enriched motifs—implied synergistic activation of splicing in the normal state. In contrast, the mutant sequence retained only two factors: SF2/ASF (a universal splicing activator) and hnRNP C2 (a known splicing suppressor). While SF2/ASF alone may insufficiently sustain efficient splicing, the co-occurrence of hnRNP C2 likely disrupts spliceosome assembly through competitive binding or direct inhibition.

Table 2. Analysis results and scores from neural network site software and MaxEntScan software

Neural network site				
Scour	**Start**	**End**	**Score**	**Exon Intron**
Normal	189	203	0.92	agcaagggtatgtgg
Patient	189	203	0.98	agcaagggtatgtgg
MaxEntScan				
Scour	MAXENT	MDD	MM	WMM
Normal	8.34	11.48	7.84	7.48
Patient	9.15	12.28	9.25	7.44

MAXENT: Maximum entropy model score, MDD: Minimum distance to the splice site, MM score: Polymorphism score of the splice site, WMM: Weighted maximum entropy model

Table 3. SpliceAid2 Site analysis results and corresponding scores

SpliceAid2				
Normal	**Position**	**Protein name**	**Recognized sequence**	**Score**
	262-268	SC35	AGGGUAU	5
	265-270	ETR-3	GUAUGU	5
	266-270	ETR-3	UAUGU	5
	266-271	ETR-3	UAUGUG	5
	267-271	ETR-3	AUGUG	5
	268-273	SRp55	UGUGGA	5
Patient	263-268	SF2/ASF	GGGUAC	5
	263-268	hnRNP C2	GGGUAC	-5

This mutation likely disrupts splicing through a dual mechanism:

1. Enhanced cryptic splice signal
   Increased splice site strength may promote non-canonical splicing, though adaptive compensation cannot be excluded.

2. Regulatory network collapse
   Loss of critical ESEs (e.g., ETR-3) coupled with gain of ESSs (hnRNP C2) significantly elevates splicing dysregulation risk, ultimately contributing to HH pathogenesis.

The above conclusions are derived from computational predictions. Due to loss of follow-up, further experimental validation (e.g., qPCR for aberrant transcripts or Western blot for HFE protein truncation) was unattainable. Future studies should prioritize longitudinal tracking of such patients and functional validation using serum or biopsy specimens.

5. Conclusion

Hereditary hemochromatosis (HH) is extremely rare in the Asian population. We report a case of a 49-year-old Chinese woman with HH caused by a homozygous mutation of c.340 + 4T > C in the HFE gene, and explain how this specific mutation contributes to the disease. Given that HH is a progressively developing condition, early diagnosis and treatment significantly impact the patient's prognosis. However, a lack of awareness among many physicians about this disease may lead to missed or incorrect diagnoses. This article provides information on a rare mutation site responsible for hereditary hemochromatosis in the Chinese population, which can help enhance physicians' understanding of the disease and its early detection and diagnosis.

Disclosure statement

The authors declare no conflict of interest.

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