Idiopathic Hemochromatosis with the Mutation of Ala176Val Heterozygous for HFE Gene

Hiroyasu IMANISHI, Weidong LIU*, Jidong CHENG**, Naoto IKEDA, Yoshiki AMURO and Toshikazu HADA

Abstract

Most patients with hereditary hemochromatosis are homozygous for C282Y in the HFE gene in populations of Celtic origin, but the genetic cause of this disease is unknown in Japan because of its rarity. A 48-year-old Japanese patient was recently diagnosed with idiopathic hemochromatosis. Analysis of the entire coding region of the patient's HFE by RT-PCR showed a heterozygous nucleotide substitution at nucleotide 527 from C to T, which resulted in A176V amino acid substitution. Another mutation at nucleotide 942 from T to C was observed, but this was a nonsense mutation. C282Y and another mutation, H63D, were not found in the patient. The mutation may have a possible role on the cause of hemochromatosis in this Japanese case.

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Key words: C282Y, RT-PCR, point mutation

Introduction

Hemochromatosis is characterized by inappropriately high iron absorption that leads to iron overload of many organs. Hereditary hemochromatosis is one of the most common inherited diseases in the northern Europe population with a Celtic origin with a prevalence of approximately 1: 300 and a carrier rate of 1: 12.

In 1996 the gene hereditary hemochromatosis (HFE) was identified (1). Two mutations, Cys282 to Tyr: C282Y (G to A at nucleotide 845) and His63 to Asp: H63D (C to G at nucleotide 187) have been described in this gene. Especially, many studies confirmed that the C282Y substitution was the main mutation in the development of hereditary hemochromatosis (2–4). However, hereditary hemochromatosis can occur in adults who do not have C282Y or H63D mutations. The C282Y substitution is absent in Asian people (5, 6) including Japanese

(7). Actually more than 10% of European and Asian patients with hemochromatosis do not have the mutations (8–10). These findings suggest that Japanese hemochromatosis has the possibility of other mutations which have not yet been reported in the HFE gene. Recently, a Japanese man suffering from idiopathic hemochromatosis was found and treated by venesection at regular intervals, and the entire coding region of the patient's HFE gene was analyzed.

Case Report

Patient

A 48-year-old Japanese man was referred to our hospital because of liver dysfunction. There was no history of alcohol abuse, transfusions or excessive iron intake. There was no known family history of hereditary hemochromatosis or hereditary anemia. Physical examination showed mild hepatomegaly and gray-slaty pigmentation of the skin. There were no ascites or hepatic encephalopathy. Liver function tests (Table 1) revealed a mild elevation of aspartate aminotransferase 80 IU/l, alanine aminotransferase 96 IU/l and alkaline phosphatase 3.5 BLU. Total bilirubin was 0.8 mg/dl, albumin 3.8 g/dl and prothrombin time 69%. The patient had elevated serum iron 151 µg/dl (normal: 80–150), serum ferritin 6,487 ng/ml (normal: 29–181) and transferrin saturation 86% (normal: 20–45; transferrin saturation was calculated by dividing the serum iron concentration by the total serum iron-binding capacity). Results of serological tests for hepatitis B and C were negative. An oral glucose tolerance test showed a diabetic pattern. Computed tomographic scans of the abdomen showed increased hepatic CT density (Fig. 1A). A liver biopsy was performed for diagnostic purposes and staging of his disease. The liver biopsy specimen was prepared with hematoxylin/eosin and berline blue staining. Histological examination of the liver biopsy specimen showed cirrhosis with massive hepatocellular siderosis: grade 3 (according to the classification of Scheuer) (11) as well as iron in bile duct cells and macrophages (Fig. 2).

The patient has been treated with phlebotomy of 300 ml of blood twice a month for approximately 2 years. Hepatic CT

From the Third Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya and *the Department of Infectious Disease and **Tumor Institute, Shantou University Medical College, Shantou, Guangdong 515031, China

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Reprint requests should be addressed to Dr. Toshikazu Hada, the Third Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501

Table 1. Laboratory Data on Admission

Hematology	
Red blood cells	395×10 ⁴ /μl
Hemoglobin	12.1 g/dl
Hematocrit	36.2%
Platelets	13.4×10 ⁴ /μl
White blood cells	5,500/μl
Prothrombin time	69%
Blood chemistry	
Total protein	7.2 g/dl
Albumin	3.8 g/dl
Total bilirubin	0.8 mg/dl
Direct bilirubin	0.2 mg/dl
Aspartate aminotransferase	80 IU//
Alanine aminotransferase	96 IU//
Alkaline phosphatase	3.5 BLU
γ-glutamyltranspeptidase	30 IU//
Ferritin	
Ferritin	6,487 ng/ml
TIBC	174 μg/dl
UIBC	26 μg/dl
Serum iron	151 μg/dl
Transferrin saturation	86%
Virus markers	
Hepatitis B surface antigen	(-)
Anti-hepatitis B surface antibody	(+)
Anti-hepatitis C virus antibody	(-)
Tumor marker	
α-fetoprotein	4.5 ng/ml

TIBC: total serum iron-binding capacity, UIBC: unsaturated serum iron-binding capacity.

density (Fig. 1B) and iron status improved after the treatment (ferritin: 6,487 ng/ml to 877 ng/ml; transferrin saturation: 86% to 60%).

Methods

Informed consent was obtained before taking the blood sample for HFE analysis.

The total RNA of the patient's peripheral white blood cells was extracted using an IsoQuick nucleic acid extraction kit (ORCA Research, Bothell, WA, USA). Then mRNA was purified from the total RNA using Oligotex-dT30<super> (Takara, Otsu, Japan). Nested RT-PCR was performed with newly designed primers which covered the entire coding region of the HFE gene using a RNA PCR kit (AMV) Ver 2.1 (Takara, Otsu, Japan) (Fig. 3). The DNA sequences of the PCR primers were F1 (forward, external): 5'-AGCGGAGATTAAACGGGGACGT-3'; R1 (reverse, external): 5'-TTGTCTCCTTCCCACAGTGA-3'; F1a (=F1, forward, internal): 5'-AGCGGAGATTAAACGGGGACGT-3'; R1a (reverse, internal): 5'-ACTTGTGGTCCAAAACACCTCT-3'; F1b (forward, internal): 5'-AGCTGAGTCAGAGTCTGA-3'; R1b (reverse, internal): 5'-TAGTTCAAGGCCCCGACACCG-3'; F1c (forward, internal): 5'-AACCACAGCAAGGAGTCCCACA-3'; R1c (reverse, internal): 5'-ACTCCAATGACTAGGGTGCCAG-3'; F2 (=F1b, forward, external): 5'-AGCTGAGTCAGAGTCTGA-3'; R2 (reverse, external): 5'-GAGAGGTCACAGCTAGGGATCA-3'; F2a (forward, internal): 5'-GCCTCCTTTGGTGAAGGTGACA-3'; R2a (=R1c, reverse, internal): 5'-ACTCCAATGACTAGGGTGCCAG-3'; F2b (forward, internal): 5'-GAGCAGAGATATACGTGCCAGG-3'; R2b (=R1, reverse, internal): 5'-TTGTCTCCTTCCCACAGTGA-3'; F2c (forward, internal): 5'-ATGGGGCACTACGTCTTAG-3' and R2c (=R2, reverse, internal): 5'-GAGAGGTCACAGCTAGGG-ATCA-3'.

Figure 1. Abdominal CT shows increased hepatic CT density which was decreased after repeated phlebotomy. (A) before treatment; (B) after treatment.

Figure 2. Berlin blue stain of the liver. The liver specimen shows cirrhosis. Iron deposits in the hepatocytes (grade 3), macrophages and bile ducts (A: $\times 40$, B: $\times 100$).

Figure 3. Strategy for nested RT-PCR. Bold dotted lines indicate PCR products produced by external primers. Other dotted lines indicate PCR products produced by internal primers. We use the numbering system which begins at initial methionine: nucleotide number (amino acid number).

After confirming the sizes and homogeneity of the PCR products by agarose gel electrophoresis, direct sequencing of the entire coding region of the patient's HFE gene was performed using PCR primers and a Dye terminator cycle sequencing core kit (Perkin-Elmer, Branchburg, NJ, USA) and ABI 373 DNA sequencer. Sequences were determined for both sense and antisense strands. The data were analyzed using DNASIS-MAC v3.6. Blood samples from four volunteers were also used as normal controls after informed consent.

Results

Two point mutations were observed in comparison with the sequence of the reported HFE gene (GenBank accession number U60319) (Fig. 4). The first point mutation was C to T transition at nucleotide 527 resulting in the substitution of valine for alanine at amino acid 176. [We used the numbering system which began at initial methionine.] This Ala176Val (A176V) mutation was a heterozygote.

The second mutation, a homozygous mutation, was T to C transition at nucleotide 942, but this mutation did not result in

Figure 4. A) DNA sequence from the patient representing heterozygous mutation at nucleotide 527C→C+T. B) DNA sequence from the patient representing homozygous mutation at nucleotide 942T→C.

amino acid change. Normal control subjects did not have any nucleotide change in the HFE gene. The C282Y and H63D mutations were not observed in the patient's HFE.

Discussion

Most patients with hereditary hemochromatosis are homozygous mutation (C282Y/C282Y) or compound heterozygous mutation (C282Y/H63D) of the HFE gene in populations of northern European origin. However, more than 10% of European patients with hemochromatosis and most Asian patients with this disease do not have the C282Y mutation. The possibility of a new mutation in the HFE gene has been raised to explain the disease without C282Y mutation.

Here, we report heterozygous A176V mutation in a Japanese patient with idiopathic hemochromatosis. The first discussion point is that the mutation may represent only a polymorphic variant. Several studies for systemic sequencing of the HFE gene showed several mutations of the HFE gene in addition to C282Y and H63D (12, 13). These are predominantly localized in intron sequences except for Ser65Cys (S65C) in exon 2. Although S65C is initially thought to represent only a polymorphic variant, Mura et al (14) suggested that S65C substitution is associated with the mild form of hemochromatosis. [S65C mutation was not found in our patient.] Therefore, we suspect that A176V substitution could be a contributing factor to iron overload in hemochromatosis patients like S65C.

The second point is that the A176V is heterozygous in our patient. As for the C282Y mutation, serum markers of iron status (ferritin and transferrin saturation) were shown to be higher in people heterozygous for C282Y than in people homozygous for wild type (15). However, the levels of serum ferritin and transferrin saturation were not as high as those in people ho-

mozygous for C282Y. The mutation found in our patient was heterozygous for the HFE gene, but the levels of serum markers of iron status were as high as those of the patients whose mutation is homozygous for C282Y. It is unclear whether the A176V heterozygous mutation contributed to iron overload. Although a heterozygote of A176V substitution may possibly increase the risk of developing hemochromatosis, a genetic mutation other than the HFE gene must be studied in order to clarify the meaning of A176V mutation. The present case may present only a private mutation. However, it is worth reporting because of the rarity of idiopathic hemochromatosis in Japan. This is the first report of a Japanese patient with idiopathic hemochromatosis whose entire coding region of the HFE gene was analyzed.

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