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【 CASE REPORT 】

A 70-year-old Woman with Asymptomatic Ferroportin Disease

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Abstract:
A 59-year-old Japanese woman presented with hyperferritinemia. We decided against iron removal treatment because there were no symptoms or signs of iron-induced organ damage. A follow-up study revealed a gradual increase in transferrin saturation. The patient underwent a second examination at 66 years old. A liver biopsy showed substantial iron deposits in hepatocytes and Kupffer cells but no inflammation or fibrosis. Serum hepcidin-25 levels were highly parallel with hyperferritinemia. A genetic analysis revealed a *G80S* mutation in *SLC40A1*. These features are compatible with those of ferroportin disease. The patient remained asymptomatic at 70 years old, suggesting that the iron-loading condition may have been benign.

Key words: ferritin, ferroportin, hemochromatosis, hepcidin, iron

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Introduction

Iron is an essential nutrient for humans but is toxic when present in excess. Iron homeostasis is mainly maintained by divalent metal transporter 1 in enterocytes, the iron regulatory hormone hepatic hepcidin (Hep), and the iron exporter ferroportin (FPN) in enterocytes, macrophages, and hepatocytes. Hep binds to FPN on the cell surface, leading to its internalization and subsequent degradation (1).

The most prominent condition caused by disruption of iron homeostasis is autosomal recessive hemochromatosis (HC). HC is a genetically heterogeneous disorder with uncontrolled intestinal iron absorption, resulting in primary iron overload syndrome (PIOS) with life-threatening complications, such as diabetes mellitus, heart failure, liver cirrhosis, and hepatocellular carcinoma. HC with impairment of the intra-hepatocellular signals of Hep production consists of the following genotypes: high Fe (*HFE*)-, transferrin receptor 2 (*TFR2*)-, hemojuvelin (*HJV*)-, and hepcidin (*HAMP*)-HC (2, 3). In HC with these genotypes, hepcidin-25 (Hep-25), a polypeptide comprising 25 amino acids, is inappropriately suppressed in hepatic production and then is released into the circulation (2, 3).

It should be noted that the genetic background of HC differs between Japanese and Caucasian populations due to the absence of the *C282Y* mutation in *HFE*-HC in non-Caucasian ethnicities (4, 5). Nonetheless, the clinical significance of the disease entities of non-*C282Y-HFE*-HC, which shows a relatively low frequency among HC types, may be important regardless of ethnicity.

The other genotype that causes PIOS is ferroportin disease (FD), which is characterized by the dysregulation of FPN encoded by the *SLC40A1* gene. In FD, Hep-25 is normally produced in the liver and released into circulation. Unlike autosomal recessive HC, autosomal dominant FD shows an array of iron overloading conditions (IOCs) and is generally classified into classical and nonclassical phenotypes (6-8). The classical phenotype of FD is characterized by hyperferritinemia, normal-to-low transferrin saturation

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Table 1. New Classification of HC Proposed by BIOIRON Society.

Novel classification	Molecular pattern
HFE-related	*C282Y* homozygosity or compound heterozygosity of *C282Y* with other rare *HFE* pathogenic variants[106–109] or *HFE* deletion[110]
Non-*HFE*-related	Rare pathogenic variants in "non-*HFE*" genes: • *HJV*-related • *HAMP*-related • *TFR2*-related • *SLC40A1* (GOF)-related
Digenic	Double heterozygosity and/or double homozygosity/heterozygosity for mutations in 2 different genes involved in iron metabolism (*HFE* and/or non-*HFE)*
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)

An overview of the new HC classification proposed by BIOIRON Society (excerpt from the original paper).[3]
HFE: high Fe, *HJV*: hemojuvelin, *HAMP*: hepcidin, *TFR2*: transferrin receptor 2, *SLC40A1*: ferroportin, GOF: gain-of-function

(TSAT), and mild iron deposition in macrophages of the reticuloendothelial system (RES), including Kupffer cells in the liver. In contrast, the non-classical phenotype is characterized by hyperferritinemia, elevated TSAT, and substantial iron deposition in hepatocytes in addition to macrophages, including Kupffer cells. In both phenotypes, serum Hep-25 levels are normal or elevated, reflecting levels of circulating iron and ferritin (6-8).

A functional analysis of mutated FPN (*SLC40A*1) in FD patients has revealed the existence of two distinctive genotypes: gain-of-function (GOF) and loss-of-function (LOF) mutations (9). Among the GOF variants, mutated FPN is believed to develop resistance to Hep, indicating that the iron-exporting capability of FPN is unchecked by Hep. Patients harboring these variants exhibit IOCs that are phenotypically and biochemically indistinguishable from Hep-deficient HC with heightened TSAT (6, 9, 10). In contrast, LOF variants of FPNs are believed to exhibit decreased iron export efficiency or decreased cell surface expression. Consequently, iron is trapped and accumulates in iron-recycling macrophages, including Kupffer cells. This in turn leads to a reduction in circulating iron levels and a tendency toward iron-restricted erythropoiesis. The hallmark clinical features are normal-to-low TSAT (6, 9, 11) and poor tolerance to phlebotomy.

Substantial differences in liver damage and disease phenotypes between the two FD genotypes may be explained by the lower cytotoxicity of iron accumulation toward macrophages than toward hepatocytes (6, 9, 12).

Based on these findings, the latest proposal of the International Society for the Study of Iron in Biology and Medicine (BIOIRON) has divided conventional FD into two distinctive entities: *SLC40A1*-HC with GOF mutations, and the newly defined FD with LOF mutations in *SLC40A1* (Table 1) (3). *SLC40A1*-associated FD with LOF mutations has thus been removed from the HC category and is instead considered to be a redefined FD. A substantial portion of *SLC40A1*-HC cases with GOF mutations are considered to exhibit non-classical phenotypes marked by IOCs. However, while FD cases with LOF mutations in SLC40A1 are anticipated to present classical phenotypes, previous reports have suggested that they exhibit a wide spectrum of IOCs, ranging from classical to non-classical phenotypes (8).

Whether or not FD plays a role in human health and hygiene remains unclear (6). The degree of iron toxicity in FD cannot be determined without any real-life evidence from untreated cases. It is important to clarify whether or not the IOC of FD progresses with aging and if iron-induced organ damage, such as cirrhosis, diabetes mellitus, and heart failure, occurs in the later stages of life. We previously reported a 90-year-old man with FD who remained free from any signs of IOC, except for sustained high serum ferritin (SF) levels (13).

We herein report the results of an 11-year follow-up study of a 59-year-old woman with asymptomatic FD.

Case Report

A 59-year-old Japanese woman was suspected of having IOC based on the computed tomography (CT) findings which showed a high number of Hounsfield units (HU) in the liver (91.7±0.9 Hounsfield units (HU)) during an annual health checkup (Fig. 1). There was no history of repeated transfusions or long-term iron supplementation, nor any signs of chronic liver disease or diabetes mellitus or skin pigmentation. Blood tests also ruled out liver disease and diabetes mellitus. The serum alanine aminotransferase (ALT) level was 12 IU/L, hemoglobin A1c (HbA1c) was 4.8%, serum iron was 125 μg/dL, SF was 1,781 ng/mL, total iron-binding capacity (TIBC) was 225 μg/dL, and TSAT was 55.5% (Table 2).

Follow-up revealed persistently elevated levels of SF (1,715 ng/mL) and a gradual rise in TSAT (63.4%); therefore, a liver biopsy was performed to confirm structural changes in the liver at 66 years old. Iron was found to be heavily deposited in the hepatocytes and Kupffer cells; however, fibrosis and inflammation were not observed in the portal tracts (Fig. 2). The serum level of Hep-25 measured

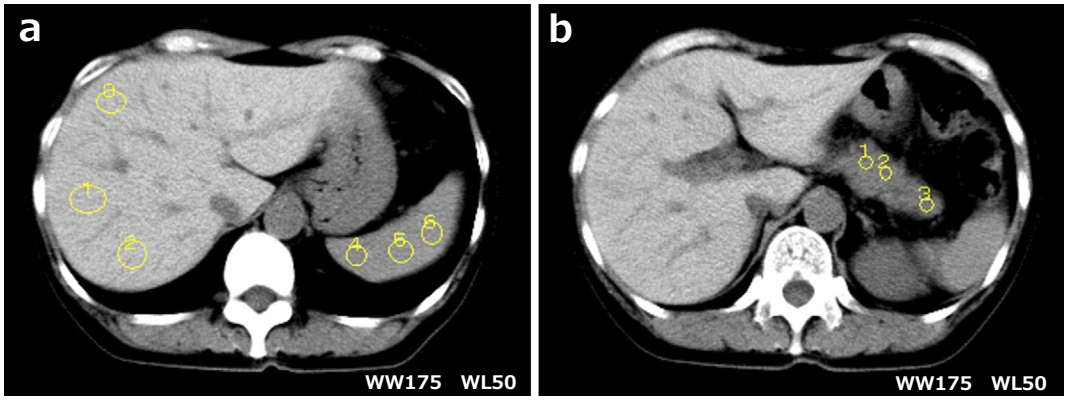

Figure 1. **Computed tomography (CT) findings at referral visit. CT images of the liver, spleen, and pancreas are shown. The CT findings for these organs were evaluated using a window width (WW) of 175 and window level (WL) of 50 at 3 areas for each organ. The CT findings are presented as the mean±standard deviation. The liver morphology was normal, but the CT findings of the liver was diffusely elevated to 91.7±0.9 Hounsfield units (HU). There were no notable findings in the biliary system, pancreas, spleen, adrenal glands, or kidneys except for a slightly elevated number of Hounsfield units (HU) in the liver. in the spleen (64.2±3.4 HU).**

Table 2. **Changes in Parameters during the 11-year Follow-up.**[*1]

Parameters[*2]	Jan. 2012 (First test)	Feb. 2023 (Latest test)	Reference range
Age	59	70	
ALT	12	14	6-27 U/L
Albumin	4.2	4.2	4.0-5.0 g/dL
Glucose	106	95	70-109 mg/dL
HbA1c	4.8	5.3	4.3-5.8%
Fe	125	133	43-172 μg/dL
TIBC	225	257	258-441 μg/dL
TSAT	55.5	51.8	20-50%
SF	1,781	2,119	5-204 ng/mL

SF: serum ferritin, TIBC: total iron-binding capacity, TSAT: transferrin saturation.
*1: In Apr. 2019, the iron overload condition was further evaluated by serum hepcidin25, liver biopsy, and a genetic analysis (refer to the text).
*2: Parameters are presented as serum levels, except for age (years), glucose, and albumin. Glucose levels were measured using plasma and HbA1c levels using whole blood.

by liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) (14) was 79.0 ng/mL (reference value: ≤20 ng/mL in healthy adult), in parallel with elevated levels of SF and TSAT. A genetic analysis revealed the heterozygous mutation *G80S* in the *SLC40A1* gene (15-17) and the heterozygous polymorphism *E3D* in the *HJV* gene.

The clinical features were consistent with FD, and therefore extensive iron removal treatment, which is normally required in cases with the appearance of organ damage, was not performed. She had two sons who did not exhibit any signs of IOC. IOC was sustained during the 11-year follow-up period, and the patient remained asymptomatic at 70 years old. The biochemical parameters remained within the normal ranges for 11 years, except for a gradual increase in SF (Table 2).

Discussion

Classical HC consists of four genotypes and the *SLC40A 1*-related phenotype of HC (FD), which differ in inheritance patterns (autosomal recessive vs. dominant), histological iron-loaded cell types (parenchymal vs. both parenchymal and RES), and circulating levels of Hep-25 (inappropriately low vs. appropriately high when measured by LC/ESI-MS/MS) (11).

Most patients with FD traits are asymptomatic and do not experience acute episodes, such as a fever, diarrhea, or ab-

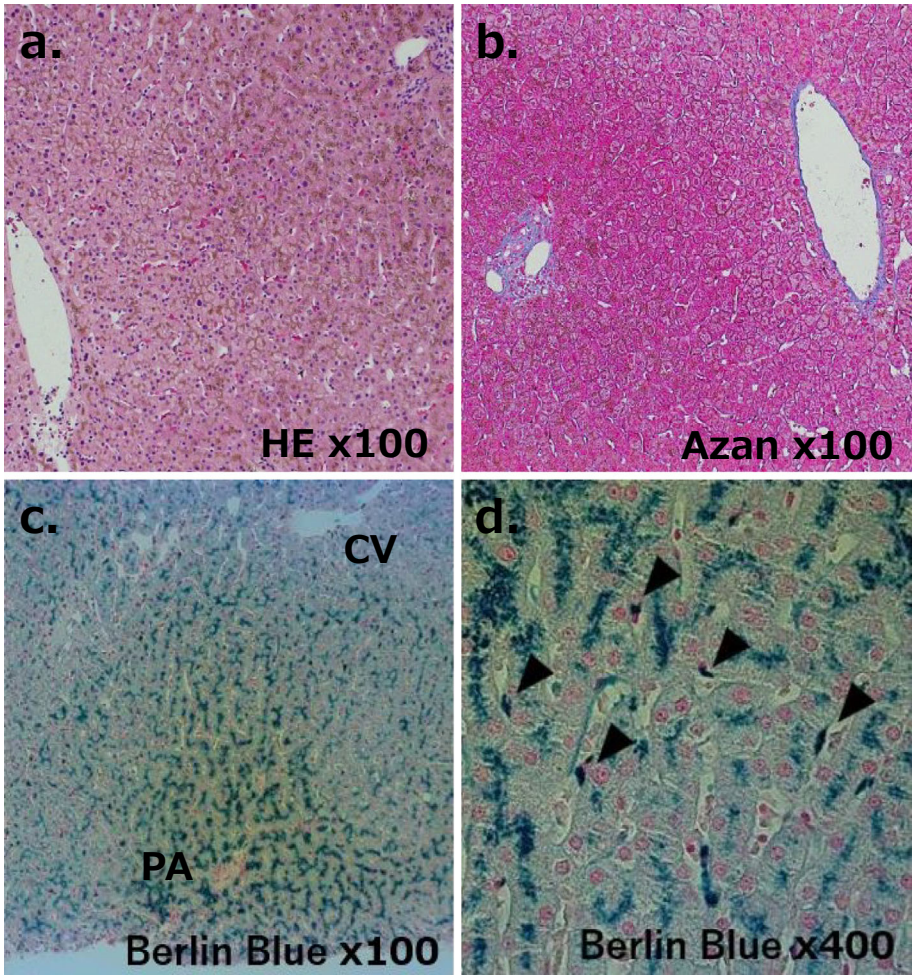

Figure 2. Findings of a histological examination of liver biopsy specimens. a. Hematoxylin and Eosin staining (×100): No inflammatory cell infiltration was observed in the liver, but deposition of lipofuscin-like brown granules was observed in the cytoplasm of hepatocytes. b. Azan staining (×100): No fibrosis was observed in the liver. c. Berlin blue staining (low magnification, ×100): Despite heavy iron loading, the portal area (PA) and central vein (CV) were well arranged in the liver. Hepatocellular iron loading was more intense in zones 1 (periportal zone) and 2 (midlobular zone) than in zone 3 (peri-central vein zone). d. Berlin blue staining (high magnification, ×400): Kupffer cells, designated by arrowheads, were heavily loaded with a larger amount of iron than hepatocytes.

dominal pain. Incidental evaluations of SF or abdominal CT and MRI may reveal the existence of IOCs, as in the current case. The absence of iron-induced organ damage and the presence of high Hep-25 levels in parallel with hyperferritinemia suggested that the pathophysiology in the current case was consistent with FD (8, 9). The detection of the heterozygous mutation *G80S* in *SLC40A1* strongly supported the clinical diagnosis of FD in the present case. This mutation was previously identified in different ethnicities worldwide (15-17). A functional study indicated that *G80S* is the LOF mutation in *SLC40A1* (17).

The IOC of the present case was relatively severe and consistent with that of FD with a non-classical phenotype, but did not appear to fit within the *SLC40A1*-related HC categorization in the latest classification of HC established by the BIOIRON working group (3). The patient did not show the classic triad of HC (liver cirrhosis, diabetes mellitus, and skin pigmentation) at 70 years old. Her liver was neither biochemically nor histochemically damaged except for iron accumulation. The presence of the heterozygous mutant *E3D* in the *HJV* gene does not contribute to IOC because *HJV*-HC is inherited in an autosomal recessive manner.

The present case is the second oldest among the patients we experienced with FD. We previously reported a 90-year-old man who did not exhibit any signs of IOC except for hyperferritinemia (13). In the present case, IOC has slowly progressed in the later period of the patient's life. Nevertheless, she has remained mostly free from iron-induced organ damage. It thus appears that mild or moderate iron deposition does not induce organ damage in FD patients. Further studies in a larger number of patients are needed to confirm that FD is not an actual disease but rather a constitutional property for which iron removal treatment may not be indi-

cated in clinical practice.

Anti-iron regimens generally consist of venesection, oral iron chelators, and a low-iron diet or the restriction of hemoprotein-rich meat and fish (2). Venesection is not recommended for patients with FD due to delayed recovery from post-iron removal anemia. Furthermore, daily intake of an iron chelator and an iron-low diet may not be tolerated by patients. If FD is a constitutional trait, then the intake of hemoprotein-rich foods should be permitted for such patients.

Conclusion

Iron toxicity did not damage the liver or endocrine system in a 70-year-old Japanese woman with FD. FD may not be a disease but rather a constitutional property for which extensive iron removal may not be necessary during the lifespan of patients.

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

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