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ORIGINAL ARTICLE

Iron overload patients with unknown etiology from national survey in Japan

Katsuya Ikuta[1] · Mayumi Hatayama[1] · Lynda Addo[1] · Yasumichi Toki[1] · Katsunori Sasaki[2] · Yasuaki Tatsumi[3] · Ai Hattori[3,4] · Ayako Kato[3] · Koichi Kato[3] · Hisao Hayashi[3] · Takahiro Suzuki[5] · Masayoshi Kobune[6] · Miyuki Tsutsui[7] · Akihiko Gotoh[7] · Yasuo Aota[8] · Motoo Matsuura[9] · Yuzuru Hamada[10] · Takahiro Tokuda[11] · Norio Komatsu[7] · Yutaka Kohgo[1,12]

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Abstract Transfusion is believed to be the main cause of iron overload in Japan. A nationwide survey on post-transfusional iron overload subsequently led to the establishment of guidelines for iron chelation therapy in this country. To date, however, detailed clinical information on the entire iron overload population in Japan has not been fully investigated. In the present study, we obtained and studied detailed clinical information on the iron overload patient population in Japan. Of 1109 iron overload cases, 93.1% were considered to have occurred post-transfusion. There were, however, 76 cases of iron overload of unknown origin, which suggest that many clinicians in Japan may encounter some difficulty in correctly diagnosing and treating iron overload. Further clinical data were obtained for 32 cases of iron overload of unknown origin; median of serum ferritin was 1860.5 ng/mL. As occurs in post-transfusional iron overload, liver dysfunction was found to be as high as 95.7% when serum ferritin levels exceeded 1000 ng/mL in these patients. Gene mutation analysis of the iron metabolism-related genes in 27 cases of iron overload with unknown etiology revealed mutations in the gene coding hemojuvelin, transferrin receptor 2, and ferroportin; this indicates that although rare, hereditary hemochromatosis does occur in Japan.

Keywords Iron overload · Hemochromatosis · Post-transfusional iron overload · Hereditary hemochromatosis

Introduction

Iron is an essential metal for all living organisms; it is an important component of hemoglobin, which delivers oxygen to the whole body, and is also utilized in various important biological cellular reactions. However, in excess,

✉ Katsuya Ikuta
ikuta@asahikawa-med.ac.jp

1 Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University, 2-1-1-1, Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan

2 Department of Gastrointestinal Immunology and Regenerative Medicine, Asahikawa Medical University, 2-1-1-1, Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan

3 Laboratory of Medicine, Aichi Gakuin University School of Pharmacy, Nagoya, Aichi, Japan

4 Department of Hospital Pharmacy, Nagoya University, Nagoya, Aichi, Japan

5 Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan

6 Department of Medical Oncology and Hematology, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan

7 Division of Hematology, Department of Medicine, Juntendo University School of Medicine, Tokyo, Japan

8 Department of Internal Medicine, Kohsei Chuo General Hospital, Tokyo, Japan

9 Department of Nephrology, Metabology and Immunology, Sakai City Medical Center, Sakai, Osaka, Japan

10 Tazuke Kofukai Foundation, Medical Research Institute, Kitano Hospital, Osaka, Japan

11 Department of Cardiovascular Medicine, Saiseikai Yokohama City Eastern Hospital, Yokohama, Kanagawa, Japan

12 Department of Gastroenterology, International University of Health and Welfare, Nasushiobara, Tochigi, Japan

iron is harmful, whereby excess iron causes tissue and organ damage, particularly in the liver, heart, pancreas, and skin. Balancing systemic iron levels within narrow limits is, therefore, critical for health [1, 2].

Hereditary hemochromatosis is a genetic disorder that the regulation of iron in the body is disturbed. In western countries, hereditary hemochromatosis is known to be the main cause of iron overload. Researchers have identified mutations in five main genes that cause hereditary hemochromatosis; these include *HFE*, *transferrin receptor 2 (TFR2)*, *hemojuvelin (HFE2)*, *HAMP*, and *ferroportin (SLC40A1)* [3, 4]. Mutations in *HFE*, *TFR2,* and *HFE2* have also been shown to regulate the production of hepcidin, the main iron regulatory hormone, which is upregulated in iron overload. Hepcidin is encoded by the *HAMP* gene and controls plasma iron concentration and tissue iron distribution by inhibiting intestinal iron absorption and iron recycling from the reticuloendothelial system [5, 6]. Hepcidin controls iron recycling from the reticuloendothelial system by internalizing and degrading ferroportin (FPN), the major iron export protein encoded by *SLC40A1* and located on the cell surfaces of enterocytes, macrophages, and hepatocytes [7]. Mutation in *HFE*, *TFR2,* and *HFE2* leads to inappropriately low hepcidin expression and mutation in *SLC40A1* alters the function of FPN; these mutations finally lead to iron accumulation in the body.

In addition to hereditary hemochromatosis, transfusions should also be an important cause of iron overload especially. The patients with severe congenital anemias, such as thalassemia and sickle cell anemia, must require prolonged and frequent red blood cell transfusions [8]. In Japan, repeated red blood cell transfusion is also believed to be the main cause of iron overload. Transfusion therapy in Japan is most commonly indicated in myelodysplastic syndrome (MDS) and aplastic anemia (AA). Previous nationwide survey on post-transfusional iron overload has reported high mortality rates among severe iron overload patients in Japan, and this led to the establishment of guidelines for iron chelation therapy in the country [9]. However, no comprehensive nationwide investigation on iron overload patients has been performed in the country. Consequently, neither the ratio of post-transfusional iron overload in the entire iron overload patient population nor the frequency of hereditary hemochromatosis-related genetic mutations in Japan has been precisely elucidated.

In the present study, we aimed to reveal the ratio of post-transfusional iron overload in the entire iron overload patient population in Japan. In addition, in the cases of iron overload of unknown etiology, mutation analysis of hereditary hemochromatosis-related genes was performed; accumulating analyzed data of mutations in such genes even in small population of iron overload should be helpful for clinicians to decide the necessity of mutation analysis in the future.

Materials and methods

Ethics

Approval for this study was obtained from the Ethics Review Committee of the Asahikawa Medical University. Informed consent was also obtained from patients who participated in the gene mutation analysis experiments.

Nationwide survey of iron overload in Japan

Questionnaires were first sent out to 379 hospitals around the country with hematology, hepatology, or endocrinology departments, from June to August 2010, to enquire about iron overload or suspected cases of iron overload. Iron overload was defined as serum ferritin levels greater than 500 ng/mL or increased liver iron content with computed tomography (CT). Patient red blood cell transfusion history was also investigated.

32 hospitals that reported having iron overload patients with no apparent history of transfusions were sent a second questionnaire to obtain further patient details regarding gender, age and underlying diseases. Patient information concerning comorbidities, previous clinical history, such as ineffective erythropoiesis, alcoholic liver damage, viral hepatitis, heart failure, arrhythmia, liver dysfunction, diabetes mellitus, thyroid dysfunction, dyspituitarism, and hypogonadism, was also investigated. In addition, patient clinical history regarding transfusion, iron administration, chemotherapy, and transplantation was investigated. Laboratory data, including serum ferritin, serum iron, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), hemoglobin A1c (HbA1c), and transaminases, were also collected.

Mutation analysis for the genes involved in hereditary hemochromatosis

Mutation analysis of hereditary hemochromatosis-related genes was performed in 27 of the cases of iron overload of unknown origin. After obtaining a written informed consent from each of the patients, blood samples were collected, followed by genomic DNA purification using the QIAamp DNA Blood Mini Kit (QIAGEN). To sequence all the coding regions of the five target genes (*HFE*, *HFE2*, *HAMP*, *TFR2,* and *SLC40A1*), long DNA fragments were amplified using the polymerase chain reaction (PCR); primers for each gene in the 1st PCR reaction are shown in Table 1. For the 1st PCR reaction, 25 ng of genomic DNA, forward and reverse primers (200 nM each), 0.4 mM dNTPs, and 1 unit KOD FX Neo (TOYOBO) were mixed (total reaction volume 25 μL) and amplified under the following conditions,

Table 1 Primers used for the 1st PCR

	Amplified region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified length (bp)
HFE	Exon 1 to Exon 6	GGCTGTGGAAGGTGTTTCAGTAGGA	AAATGAATCATGTAAGTCCCCCA	7632
HFE2	Exon 1 to Exon 4	TCAGTAGCCACCTCCCTCCCTGCT	CCATCTTCCCATCTGAATGTACCATA	4563
HAMP	5′UTR to Exon 3	CCCATCTGAGGCCATCTTTATTCAT	TGCTTGCAAGGCAGGGTCAGGACAA	4685
TFR2	Exon 1 to Exon 6	GTGGTGAGGAGCAGCCTTGGTTCAG	AGCACCCTGAACGATTCTCACTGGC	8717
	Exon 4 to Exon 17	TTCCTAAACTCAGGAACCCCTCGCC	GGTCCTCCAGCCTGACCGATCTATG	7097
	Exon 16 to Exon 18	CCCCAGCGTCCACCCTGTCCTGGC	GGAAGAAGCATGAAGGCGCTTATCAA	7145
SLC40A1	Exon 1 to Exon 5	GGCGCGCAAGGTTGACGGGA	TTACAGCCTCATTTATCACCACCGA	9287
	Exon 5 to Exon 8	CTAGATGATACAGGTTAGGACATTA	AATGCTGCCTTGTCTTGTAGTTC	11,821

one cycle of 94 °C for 2 min and 30 cycles of 98 °C for 10 s and 68 °C for 6 min 30 s.

Using the forward and reverse primers shown in Table 2, a 2nd PCR reaction was performed to amplify exon 1–6 of *HFE* as 6 fractions, exon 1–4 of *HFE2* as 7 fractions, 5′ untranslated region (5′UTR) and exon 1–3 of *HAMP* as 5 fractions, exon 1–18 of *TFR2* as 13 fractions, and exon 1–8 of *SLC40A1* as 9 fractions. All primers for the 2nd PCR reaction included M13 sequences for sequencing (M13 forward: 5′-GTAAAACGACGGCCAG-3′, M13 reverse: 5′-CAGGAAACAGCTATGAC-3′). Products of the 1st PCR reaction (diluted 20 times) were used as templates in the 2nd PCR reaction; 1 μL template, forward and reverse primers (500 nM each), 0.2 mM dNTPs, and 0.2 μL Phusion High-Fidelity DNA polymerase (Fenzyme) were mixed (total reaction volume 20 μL) and amplified under the following conditions, one cycle of 98 °C for 30 s, 25 cycles of 98 °C for 5 s, 50 °C for 10 s, and 72 °C for 15 s.

Products of the 2nd PCR reaction (diluted 50 times) were then used as templates in sequencing reactions performed using the BigDye Terminator v3.1 cycle Sequencing Kit (Applied Biosystems) as the reaction solution, following the manufacturer's instructions. Unreacted labeled nucleotides were removed using the CleanSEQ Kit (Beckman Coulter), after which the purified solutions were sequenced using 3130 Genetic Analysis (Applied Biosystems). Genetic mutation was then evaluated by comparing the sequencing results to open access genetic information on the NCBI website.

Results

Nationwide survey of iron overload in Japan

184 (48.5%) of the 379 hospitals responded to the questionnaires sent. These responses came from 84 hematology, 80 hepatology, and 16 endocrinology departments, as well as 4 other departments (internal medicine) with physicians familiar with iron metabolism. Of the 184 responses, 119 hospitals (65%) reported a total of 1467 patients with or suspected of having iron overload. 65 hospitals (35%) reported that they did not see any iron overload patient.

Proof or suspected causes of iron overload were further analyzed in 1109 of the 1467 cases, of which 1033 (93.1%) were thought to have occurred post-transfusion; this indicates that transfusion is the main cause of iron overload in Japan (Fig. 1). No answer was obtained concerning proof or suspected cause of iron overload in 358 of the 1467 cases, so that those cases were excluded.

From the second questionnaire, further clinical data were obtained for 32 of the patients with iron overload of unknown origin. Except for one male patient, whose age was not reported, average age of 31 patients was 59.0 years (13–97 years), which included 21 males (average of 20 males: 57.3 years) and 11 females (average: 62.1 years). There was no statistical difference in the average age between 20 males and 11 females ($p = 0.446$).

Median of serum ferritin was 1860.5 ng/mL (22.8–8930 ng/mL); serum ferritin levels exceeded 1000 ng/mL in 23 patients, and were less than 1000 ng/mL in nine patients. Only one patient showing that serum ferritin level of 22.8 ng/mL was diagnosed as iron overload by CT.

Organ damage was common among patients with iron overload of unknown origin, with the rate of liver dysfunction found to be as high as 84.4% (27 of 32 patients). Diabetes mellitus was also frequent 37.5% (12 of 32 patients), but cardiac dysfunction, heart failure, or arrhythmia was only present in 9.4% of the patients (3 of 32 patients). The coexistence of liver dysfunction and diabetes mellitus was observed in 37.5% (12 of 32 patients), but there was no statistical significance for the relationship between them (Fisher's exact test: $p = 0.130$) (Table 3).

Serum ferritin analysis revealed the rate of liver dysfunction to be as high as 95.7% (22 of 23 patients) when

Table 2 Primers used for the 2nd PCR

	Amplified region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified length (bp)
HFE	Exon 1	M13F-AGATCAGAACATTGCGAAGC	M13R-TTGCGGATAGGGTTGAGCAG	515
	Exon 2	M13F-ACAAAATGAGGACCAGACAC	M13R-GCTCCCACAAGACCTCAGAC	511
	Exon 3	M13F-TGCTTCCTGAGATCATTTGG	M13R-AGAATTTGGAGAGGCACACA	518
	Exon 4	M13F-GGGTATTTCCTTCCTCCAAC	M13R-ACTGCCATAATTACCTCCTC	491
	Exon 5	M13F-CCTGAGGAGGTAATTATGGC	M13R-CTTTCATTCTGGGGAGAACC	418
	Exon 6	M13F-AATTGAGATGGGTGAATGAG	M13R-GTATGTCTCTGAGGTGACGG	470
HFE2	Exon 1	M13F-TCAGTAGCCACCTCCCTCC	M13R-ATGGAGATTGGGGCACCTT	398
	Exon 2	M13F-CCCCAAATTCCAGTCTGTTC	M13R-TCTCAGCGCCTATCTCTCCT	437
	Exon 3a	M13F-GAGCAAACTACACTCCGAT	M13R-GCCTTCATAGTCACAAGGGT	461
	Exon 3b	M13F-GTACATGGCATCGAAGA	M13R-GAGGTTGAGGAAGAAAAGGG	490
	Exon 4a	M13F-GCCATAGTAGTCCTGCATCT	M13R-GCCGTCTGGCAGTATCAAT	526
	Exon 4b	M13F-CAGCTCTCCTTCTCCATCAA	M13R-AGAGGAACCCCAGCATC	371
	Exon 4c	M13F-ATCGTCGGGGAGCTATAA	M13R-TCCTAGGCCCTGCTTCCTTT	414
HAMP	5'UTR 1	M13F-AGAGGCAAAATGTGCAAGGG	M13R-GCAGCACTTACTGCCCCACC	638
	5′UTR 2	M13F-GTGAAGGAAATGAGTGTCCG	M13R-CTTTTGGCCATAAATGACAG	609
	5′UTR 3	M13F-AGATGGGGTCTCCCTATGT	M13R-CACACTGCTCACCAGCCATC	560
	5′UTR 4 to Exon 1	M13F-GCTTAACCGCTGAAGCAAAA	M13R-TCTCCCATCCCTGCTGCCCT	531
	Exon 2 to Exon 3	M13F-CCACTTGGAGAGGAGCAGGT	M13R-CTCGGCAGAGAGAAAGGACA	510
TFR2	Exon 1	M13F-GAGGAGCAGCCTTGGTTCAG	M13R-AAGAAGCGAGGTCAGGACAC	310
	Exon 2	M13F-TCACTGACCTCATTATTGCC	M13R-CAGTAGGAAGGCTGGCGGGT	480
	Exon 3	M13F-CCCCTCCCCAGAAGTGAA	M13R-GGCAGATGGGAGGACTCAGG	437
	Exon 4 to Exon 5	M13F-CTTTTCCTAAACTCAGGAACCC	M13R-TTCGAGACCCAGGAAAGG	547
	Exon 5 to Exon 6	M13F-CTACGTGGGGCTGCAAT	M13R-CCTGAACGATTCTCACTGGC	550
	Exon 7 to Exon 8	M13F-CGTGGGATGGACAGTTGC	M13R-GTTCACCCACAATCACCCTG	617
	Exon 9	M13F-AGCGTCCAGAGGCAGCGA	M13R-TGCACCAGCCCCCTATCTTG	408
	Exon 10	M13F-CTGAGAGACACAGGCAGA	M13R-GGATGCCGAGGTCCAA	
	Exon 11 to Exon 13	M13F-CTGGGGGACCAGGACAGAA	M13R-GTTGGGGAAGGGCACTGA	597
	Exon 14 to Exon 15	M13F-GCAAGAGCACCCCAGGAATA	M13R-CATAAGTGTCCTCCTTTGTG	494
	Exon 16	M13F-CTCCTTTATGGAGGTGAGAC	M13R-TGGGCTGGATTGCCAGAGAG	479
	Exon 17	M13F-TCATCCTGCCTCCAGCAC	M13R-GGACTGGGAAGAGAGCATC	473
	Exon 18	M13F-AAAAAGACTGGCTGGCGG-GAA	M13R-CCGTGGAGAGATGTGTAGG	534
SLC40A1	Exon 1	M13F-GCGCAAGGTTGACGGGAG	M13R-TTCCTTAACGTCCACCAAA	
	Exon 2	M13F-GGTTTGTCCTGCAAAGTAGT	M13R-CTAACACTCATGGGGAAAGA	404
	Exon 3	M13F-CATAATGTAGCCAGGAAGTG	M13R-CCTCAAGTGTGGCATGCAGA	424
	Exon 4	M13F-ATTGAGAGTAGTTGAGGCA	M13R-AATAGCTTCAAAGCAT	
	Exon 5	M13F-GGACATTATGCCCATTGACT	M13R-GTTAACTTCGTAACAGTGAG	542
	Exon 6	M13F-GGGACTTGACCCAAACAAC	M13R-ATATTTAACCTCATCTGGC	
	Exon 7a	M13F-GGAAGGGGAATAGAAGGAAA	M13R-TCACACACAAGATCAAACAG	532
	Exon 7b	M13F-ACTCAGGGACTGAGTGGTT	M13R-AATTTCGTAAGAGTGGAT	
	Exon 8	M13F-AAGGCAAGGCTATGGTAT	M13R-AAACAGAGCAAAACACCCAG	595

M13F sequence: 3′-GTAAAACGACGGCCAG-5′, M13R sequence: 3′-CAGGAAACAGCTATGAC-5′

serum ferritin levels exceeded 1000 ng/mL, whereas the percentage of liver dysfunction reduced to 55.6% (5 of 9 patients) when serum ferritin levels were below 1000 ng/mL (Fig. 2a). In addition, diabetes mellitus was as high as 47.8% (11 of 23 patients) when serum ferritin levels exceeded 1000 ng/mL, whereas this percentage dropped

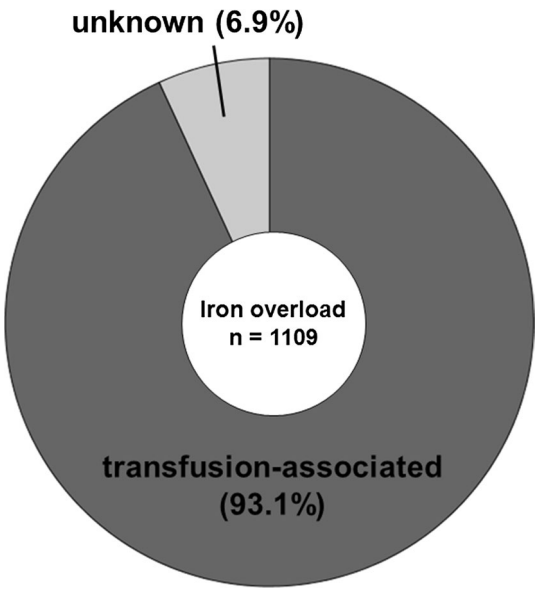

Fig. 1 Causes of iron overload in 1109 patients. In 1033 patients (93.1%), the cause of iron overload was considered to be transfusion

Table 3 Contingency table for liver dysfunction and diabetes mellitus observed in patients of iron overload with unknown etiology

		Glucose tolerance	
		Diabetes mellitus	Normal
Liver function test	Dysfunction	12	15
	Normal	0	5

Fisher's exact test: $p = 0.130$

to 11.1% (1 of 9 patients) when serum ferritin levels were lower than 1000 ng/mL (Fig. 2b).

Mutational analysis for the genes involved in hereditary hemochromatosis

Gene mutation analysis of hereditary hemochromatosis-related genes in 27 Japanese patients with iron overload of unknown origin revealed mutations in some genes involved in the pathogenesis of hereditary hemochromatosis in 3 of these patients (Table 4).

In the first patient (Case 1), compound heterozygous mutations at 745G>C (D249H) and 934C>T (Q312X) in *HFE2,* as well as heterozygous mutation at 224C>T (A75V) in *TFR2* were found. In the second (Case 2) and third patients (Case 3), however, heterozygous mutation at 485_487delTTG in *SLC40A1* was found. Heterozygous mutation at 714C>G (I238M) in *TFR2* was also found in Case 3. No mutation in *HFE* or *HAMP* was found in any of the patients studied.

Discussion

This study reveals that there are several iron overload patients in Japan. Although previous studies have reported on hereditary hemochromatosis-related genetic mutations in Japan, there seems to be no obvious data regarding the frequency of these mutations among the entire iron overload patient population in the country [10–14]. Our data showed that 93.1% of 1109 iron overloaded patients were considered to become iron overload by red blood cell transfusions. To the best of our knowledge, this is the first report to show that red blood cell transfusion is the main cause of iron overload in the iron overload patient population in Japan. However, we also found cases of iron overload of unknown etiology, which implies that clinicians may find difficulty in diagnosing and treating iron overload in Japan. From the data of 32 cases with iron overload of unknown etiology, iron overload was observed to occur in twice as many males than females. Disease onset of iron overload has usually been considered to begin earlier in males than in females because of the monthly menstrual blood loss in females; however, there was no statistical difference in the average age between males and females of iron overload patients with unknown etiology in the present study; small numbers of iron overload patients with unknown etiology might influence on this result and further accumulation of patients should be desired.

The wide age range (13–97 years) of iron overload onset also implies that there are several possible etiologies of iron overload, and it will be important for clinicians to keep this in mind even with pediatric and geriatric patients.

Liver dysfunction, found in 84.4% of patients (27 out of 32), and diabetes mellitus were all common among the patients studied. In addition, the frequencies of liver dysfunction and diabetes mellitus were higher among patients, whose serum ferritin levels exceeded 1000 ng/mL. Takatoku et al. had investigated 292 patients with transfusion-dependent MDS and AA, and reported 75 deaths; the cardiac failure and liver failure were noted in 24.0 and 6.7%, respectively, as causes of death. Of these patients, 97% had serum ferritin levels exceeded 1000 ng/mL. They also reported that abnormal cardiac and liver dysfunctions were observed in 21.9 and 84.6% of all assessed patients, respectively. Fasting blood sugar (FBS) abnormality was correlated with transfusion frequency but not with serum ferritin, and HbA1c was not changed by transfusion [9]. There was a difference of analyzed populations between Takatoku's report and the present study; Takatoku et al. analyzed iron overload caused by transfusion and we analyzed iron overload with unknown etiology. However, it might be important that similar complications were observed in both studies. We found that liver dysfunction and diabetes mellitus were observed with high

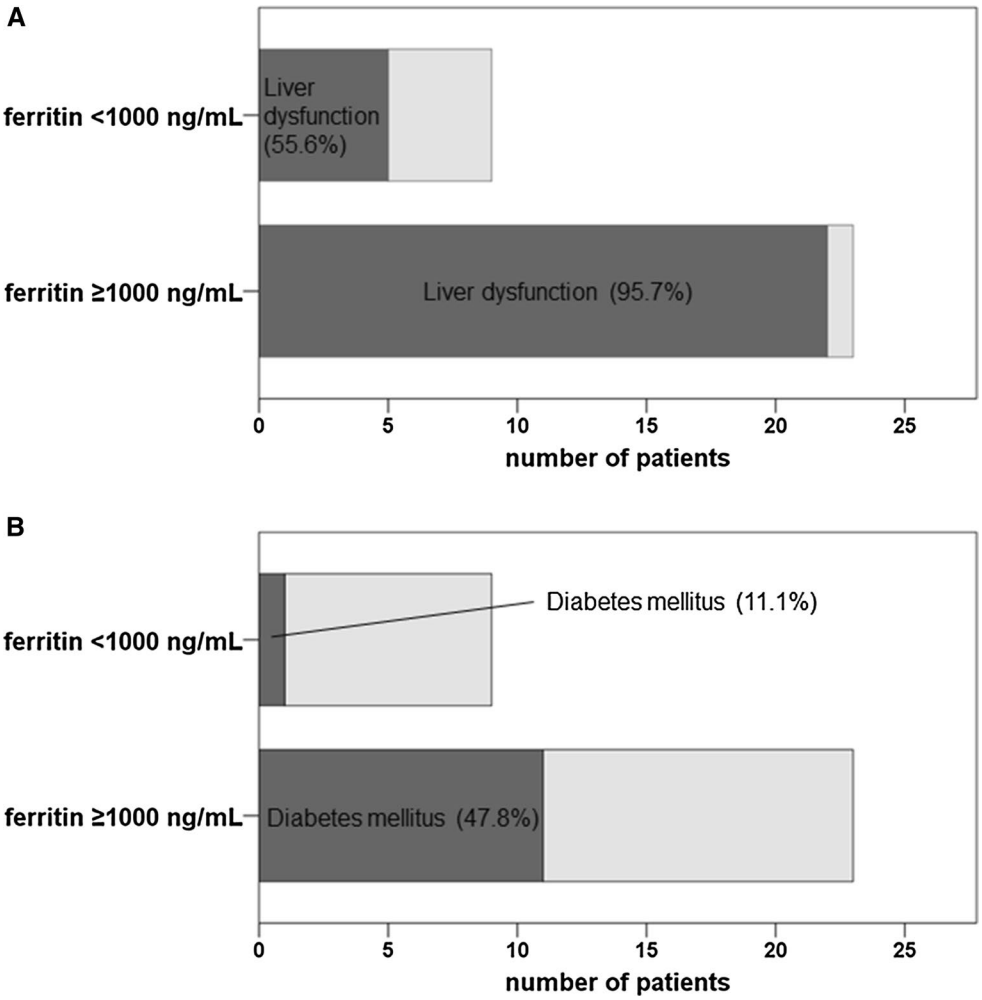

Fig. 2 Liver dysfunction was observed in 95.7% of patients (22 of 23 patients) when serum ferritin levels exceeded 1000 ng/mL, whereas the percentage of liver dysfunction reduced to 55.6% (5 of 9 patients) when serum ferritin levels were below 1000 ng/mL (**a**). Diabetes mellitus was observed in 47.8% of patients (11 of 23 patients) when serum ferritin levels exceeded 1000 ng/mL, whereas this percentage dropped to 11.1% (1 of 9 patients) when serum ferritin levels were lower than 1000 ng/mL (**b**)

frequencies in the present study; these should indicate the importance of checking liver function and glucose tolerance when we encounter iron overload patients. In addition, because the frequencies of liver dysfunction and diabetes mellitus were increased when serum ferritin levels exceeded 1000 ng/mL in both Takatoku's study and our study (Fig. 2a, b), iron chelation therapy should be considered when serum ferritin levels exceed 1000 ng/mL, even if the etiology of iron overload cannot be fully specified.

Furthermore, mutations in some hemochromatosis-related genes were found in three patients with iron overload of unknown origin. A compound heterozygous mutation of c.745G>C (D249H)/c.934C>T (Q312X) in the *HFE2* gene, which has also been reported in other Japanese patients [15, 16], was found in one patient (Case 1) in this study. Hemojuvelin (HJV) encoded by *HFE2* has been shown to be involved in the regulation of hepcidin production, and thus mutated HJV results in inappropriately low hepcidin expression, which also leads to iron overload [17–19]. A75V mutation in TfR2 protein, which has been shown to be expressed on the cell surface of hepatocytes and to regulate hepcidin expression, was also found in this same patient. While this mutation has been reported in other studies [20, 21], this is the first reported case among Japanese patients. Hereditary hemochromatosis caused by the mutations of *HFE2* and *TFR2* has been reported to be inherited in an autosomal recessive pattern, and the all genetic mutations observed in this study were found to occur in the heterozygous form. However, mutations in *HFE2* could be considered as compound heterozygous form, and may thus explain its likely contribution to the pathogenesis of iron overload in this patient.

c.485_487delTTG (V162del) mutation in exon 5 of *SLC40A1* which was found in the other two patients (Cases 2 and 3) has also been reported previously [22, 23]. Functional analysis of this mutation in *SLC40A1*, which is believed to have contributed to iron overload in the two patients, revealed an autosomal dominant inheritance pattern [24]. c.714C>G (I238M) mutation in exon 5 in *TFR2* was also found in Case 3; because it was observed in the heterozygous form, its contribution to iron overload in this patient is uncertain.

Table 4 Characteristics of three patients with mutations in *HFE2*, *TFR2*, and *SLC40A1* genes

Case	Gender	Age (year)	Hemoglobin (g/dL)	Serum ferritin (ng/mL)	Transferrin saturation (%)	Organ dysfunction	Identified mutations
1	Male	26	11.8	4354	75.9	Liver dysfunction (ALT 67 IU/L) Diabetes mellitus (HbA1c 9.9%)	Heterozygous mutation at 745G>C (D249H) in *HFE2* Heterozygous mutation at 934C>T (Q312X) in *HFE2* Heterozygous mutation at 224C>T (A75 V) in *TFR2*
2	Male	63	13.2	18695	31.5	Liver dysfunction (ALT 74 IU/L)	Heterozygous mutation at 485_487delTTG (V162del) in *SLC40A1*
3	Male	54	15.3	7038	62.3	Liver dysfunction (ALT 85 IU/L)	Heterozygous mutation at 485_487delTTG (V162del) in *SLC40A1* Heterozygous mutation at 714C>G (I238M) in *TFR2*

ALT alanine aminotransferase, *HbA1c* hemoglobin A1c

It is also important to note that the C282Y or H63D *HFE* mutation [25, 26], which is responsible for most hereditary hemochromatosis cases in Caucasians, was not found in this study. This suggests a significant genetic difference between Japanese and Caucasians, especially regarding the iron metabolism-related genes.

Although the entire frequency of hereditary hemochromatosis-related genetic mutations in Japan cannot be determined in the present study, the results of this study show that mutations in the hereditary hemochromatosis-related genes may contribute to the pathogenesis of iron overload in some patients and further indicates that gene mutational analysis of the hemochromatosis-related genes may be valuable even in Japan.

Finally, mutations in other iron homeostasis-related genes, such as *ferritin* and *DMT1*, have also been shown to contribute to iron dysregulation [27, 28]; however, these genes were not analyzed in this study. Further analysis of these genes may, therefore, help explain the pathogenesis of iron overload of unknown etiology.

Conclusion

To the best of our knowledge, this study is the first to confirm that the main cause of iron overload in Japan is red blood cell transfusion. In the cases of iron overload of unknown etiology, mutations in some of the hereditary hemochromatosis-related genes were found, which indicate that mutational analysis of the hemochromatosis-related genes would be valuable even in Japan.

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Compliance with ethical standards

Conflict of interest This study was partly supported by the Grants-in-Aid for Scientific Research, Ministry of Health, Labor and Welfare of Japan; Asahikawa Medical University (Yutaka Kohgo, Katsuya Ikuta, Katsunori Sasaki, Mayumi Hatayama, Lynda Addo, Yasumichi Toki), Aichi Gakuin University School of Pharmacy (Yasuaki Tatsumi, Ai Hattori, Ayako Kato, Koichi Kato, Hisao Hayashi), Jichi Medical University (Takahiro Suzuki), Sapporo Medical University School of Medicine (Masayoshi Kobune) received this Grant. Katsuya Ikuta, Mayumi Hatayama, Lynda Addo, Yasumichi Toki, Yutaka Kohgo

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