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Original Article

A Revised Classification of Primary Iron Overload Syndromes

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Abstract

Background and Aims: The clinical introduction of hepcidin25 (Hep25) has led to a more detailed understanding of its relationship with ferroportin (FP) and divalent metal transporter1 in primary iron overload syndromes (PIOSs). In 2012, we proposed a classification of PIOSs based on the Hep25/FP system, which consists of prehepatic aceruloplasminemia, hepatic hemochromatosis (HC), and posthepatic FP disease (FP-D). However, in consideration of accumulated evidence on PIOSs, we aimed to renew the classification. ***Methods:*** We reviewed the 2012 classification and retrospectively renewed it according to new information on PIOSs. ***Results:*** Iron-loading anemia was included in PIOSs as a prehepatic form because of the newly discovered erythroferrone-induced suppression of Hep25, and the state of traditional FP-D was remodeled as the BIOIRON proposal. The key molecules responsible for prehepatic PIOSs are low transferrin saturation in aceruloplasminemia and increased erythroferrone production by erythroblasts in iron-loading anemia. Hepatic PIOSs comprise four genotypes of HC, in each of which the synthesis of Hep25 is inappropriately reduced in the liver. Hepatic Hep25 synthesis is adequate in posthepatic PIOSs; however, two mutant FP molecules may resist Hep25 differently, resulting in SLC40A1-HC and FP-D, respectively. PIOS phenotypes are diagnosed using laboratory tests, including circulating Hep25, followed by suitable treatments. Direct sequencing of the candidate genes may be outsourced to gene centers when needed. Laboratory kits for the prevalent mutations, such as C282Y, may be the first choice for a genetic analysis of HC in Caucasians. ***Conclusions:*** The revised classification may be useful worldwide.

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Introduction

C282Y and H63D mutations in the *HFE* gene were identified in patients with hemochromatosis (HC) in 1996.[1] One year later, the C282Y mutation responsible for autosomal recessive HC was shown to be prevalent in Caucasians but rare in non-Caucasians.[2] Subsequent clinical investigations confirmed that there were a large number of patients with the C282Y mutation and a small number of non-C282Y mutations in the *HFE* gene of Caucasian patients.[3] However, both genotypes were very rare in Japanese patients.[4] Nevertheless, a small number of patients were affected not only by autosomal recessive HC associated with mutations in *TFR2*,[5] *HJV*,[6] and *HAMP*,[7] but also by autosomal dominant ferroportin (FP) disease (FP-D) with mutant genes in *SLC40A1*,[8,9] regardless of ethnicity.

Aceruloplasminemia (ACP) was initially reported in a Japanese patient with the iron-induced middle-aged onset of diabetes mellitus (DM), dementia, and retinopathy.[10] It is now regarded as a worldwide core disease of neurodegeneration with brain iron accumulation (NBIA).[11] Biochemical tests are characterized by the lack of circulating ceruloplasmin (CP), namely ferroxidase, and resultant circulating transferrin (TF) with a low iron saturation (TSAT) under systemic iron overloading. DM in ACP complicated with NBIA is multifactorial because, in addition to the pancreas, most endocrine organs including the pituitary gland, are affected

Keywords: Ethnicity; Ferroportin; Hemochromatosis; Hepcidin25; Iron overload syndrome.

Abbreviations: ACP, aceruloplasminemia; BDL, below the detection limit; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CDA, congenital dyserythropoietic anemia; CNS, central nervous system; CP, ceruloplasmin; DM, diabetes mellitus; DHS, dihydrated hereditary stomatocytosis; DMT1, divalent metal transporter1; ERFE, erythroferrone; F, female; FP, ferroportin; FP-D, ferroportin disease; HAMP, human antimicrobial peptide; Hb, hemoglobin; HC, hemochromatosis; Hep25, hepcidin25; HFE, hemochromatosis protein; HJV, hemojuvelin; ILA, iron-loading anemia; M, male; nd, not determined; Ph, phlebotomy; PIOS, primary iron overload syndrome; Ref, reference; TF, transferrin; SLC40A1, gene name of ferroportin1; TFR, transferrin receptor; TSAT, transferrin saturation.

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Table 1. Patients with prehepatic ACP

Class	Disease	Genetic Study	Age/ Sex	Serum ferritin, ng/mL	Serum Hep25, ng/mL	TSAT, %, (Hb, g/dL), CP, mg/mL	Organ damage	Ref
Prehepatic	ACP-1	2679G>A, homozygous in *CP*	35/M	3,530	BDL	16.3 (9.5) BDL	Hepatocyte-dominant iron, DM, osteomyelitis	30
	ACP-2	1285-1286ins5, homozygous in *CP*	44/M	961	2.7	22.0 (12.3) BDL	Hepatocyte-dominant iron, DM, hypopituitarism	18
	ACP-3	2630G>A, homozygous in *CP*	55/F	1,035	13.1	14.6 (9.4) BDL	Mixed-cell iron, DM	16,18
	ACP-4	606-607insA, homozygous in *CP*	59/F	885	10.3	11.2 (10.2) BDL	Hepatocyte-dominant iron, DM, dementia	16,18
	ACP-5	1285-1286ins5, homozygous in *CP*	62/M	1,140	18.2	12.0 (8.9) BDL	Mixed-cell iron, DM, cardiac failure, dementia	16,18

Normal serum ferritin values were 26–310 ng/mL for men and 7–110 ng/mL for women. Normal serum Hep25 levels were 1.6–12.7 ng/mL. Normal values were 40–70% for TSAT, 13.5–17.0 g/dL for Hb in men and11.2–14.5 g/dL for women, and 21–37 mg/mL for CP. ACP, aceruloplasminemia; BDL, below the detection limit; CP, ceruloplasmin; DM, diabetes mellitus; F, female; Hb, hemoglobin; Hep25, hepcidin25; M, male; Ref, reference; TSAT, transferrin saturation.

by iron overloading.[12]

Evidence has accumulated for a molecule-based mechanism of iron absorption in the gut and internal iron recycling between the spleen and bone marrow. The main molecules involved in iron homeostasis are the iron regulatory hormone hepcidin25 (Hep25)[13,14] and the iron exporter FP.[15] The clinical introduction of Hep25 has allowed for the classification of primary iron overload syndromes (PIOSs) based on the Hep25/FP system.[16] Serum levels of Hep25 are low in ACP and HC, but are high in FP-D. ACP is always associated with low circulating Hep25 under low TSAT, while impaired hepatic synthesis of Hep25 has been identified as a pathogenic factor for hepatic HC.[13] The mutant FP molecules coded by the *SLC40A1* gene have been shown to resist circulating Hep25 in the gut and macrophages,[17] thereby disturbing iron homeostasis. Based on these findings, we proposed a classification of PIOSs in 2012 consisting of the following three categories: prehepatic ACP, hepatic HC, and posthepatic FP-D.[18]

The subsequent accumulation of evidence since the initial proposal in 2012 indicated that the classification of PIOSs needs to be renewed based on the Hep25/FP system: new evidence of the involvement of the erythroferrone (ERFE)/Hep25/FP system in iron-loading anemia (ILA),[19–21] the controversial state of traditional FP-D,[22,23] and the identification of the two genotypes of C282Y and non-C282Y HFE-HC in Japanese patients.[24,25] In addition, ACP has been introduced into the international monographs of PIOSs.[26,27] Therefore, the classification of PIOSs was revised as the classification of HC by the BIOIRON Society[23] and the guidelines of HC by the EASL.[28]

Methods

This study included Japanese patients enrolled in the first classification of PIOSs proposed in 2012.[18] Five patients had TFR2-HC, three had HJV-HC, and one had HAMP-HC. No patient with HFE-HC was enrolled in the first study. The first classification also included two subtypes of PIOSs other than HC. Four had ACP and three had FP-D. They were diagnosed by the standard criteria of ACP,[10] HC,[3] and FP-D.[3] Direct sequencing of *HFE*, *TFR2*, *HJV*, *HAMP*, and *SLC40A1* was performed for patients with HC and FP-D. *CP* was analyzed in patients with ACP. Serum levels of Hep25 were assessed by LC-MS/MS.[29]

In the decade after the publication of the first study in 2012, 17 Japanese patients with PIOSs were retrospectively identified and newly enrolled in the revised classification. ILA has been added as a disease entity of PIOSs based on confirmation of the ERFE-induced suppression of Hep25 in a wide range of genetic anemias associated with hemolysis and reticuloclocytosis.[19–21] The inclusion criteria of ILA are anemias associated with ineffective erythropoiesis and hemolysis, low levels of circulating Hep25, and iron-induced multiorgan damage.[19,20] The same criteria as those used in the first study, such as hyperferritinemia, iron-induced organ damage, and the detection of disease-causing mutations, have been employed to diagnose ACP, HC, and SLC40A1-PIOSs.[18] Serum Hep25 levels were used for differential diagnosis of the prehepatic and hepatic PIOSs associated with pathologically low Hep25 and posthepatic SLC40A1-PIOSs with adequate Hep25. As the state of traditional FP-D is controversial.[22,23] we examined the phenotypes of SLC40A1-PIOSs using the additional criterion of long-term follow-up.[22] The clinical study included an assessment of serum Hep25 levels and a genetic analysis of PIOSs, which were performed after obtaining informed consent from each patient. Instead of informed consent, OPTOUT was permitted for patients. There were two exceptional cases, a patient with ILA diagnosed after autopsy and an aged patient in a PIEZO1-induced dihydrated hereditary stomatocytosis (DHS) family. They missed the opportunity for a Hep25 assessment and gene analysis. The publication of anonymized results in an international journal was approved by the Ethics Committees of each institute.

Results

The number of patients increased from 16 in the original study to 34 in the revised version, 11 with prehepatic PIOSs (5 ACP and 6 ILA), 15 with hepatic PIOSs (3 HFE, 5 TFR2, 6 HJV, and 1 HAMP), and eight with posthepatic PIOSs (5 SLC40A1-HC and 3 FP-D). Anonymized information of the patients with the prehepatic class of ACP and ILA is summarized in Tables 1 and 2,[16,18,30–34] and that of HC and SLC40A1-PIOSs is shown in Table 3.[16–20,22,24,25,35–40] The clinical features of new patients in each disease category are described briefly, and the characteristics of each category are summarized in Table 4.[18–21,23]

One of the five patients was newly enrolled in ACP of prehepatic PIOSs (Table 1).[30] A 35-year-old male patient had a large discrepancy between serum ferritin and Hep25: 3,530 ng/mL vs. below the detection limit (BDL). TSAT was low at 16.3%. As summarized in Table 4, the clinical features of ACP were deficient levels of serum CP coded by the *CP* gene

Table 2. Patients with prehepatic ILA

Class	Disease	Genetic study	Age/ Sex	Serum ferritin, ng/mL	Serum Hep25, ng/mL	TSAT, %, (Hb, g/mL), reticulo-C, ‰	Organ damage	Ref
Prehepatic	ILA-1	6008C>A, heterozygous in *PIEZO1*	23/F	815	4.2	85.2 (9.9) 84	Liver iron accumulation in imaging, anemia, neither DM nor endocrine disorders	32
	(DHS)							
	ILA-2	3503C>T, heterozygous in *CDAN1*	38/M	4,058	nd	95.3 (7.5) 27	HC-liver, anemia, DM, hypogonadism	33
	(CDA-type 1)		48/M	186	0.8	nd (8.5) nd		
			Post-Ph					
	ILA-3	7488-7489insACTGGA, heterozygous in *PIEZO1*	41/M	4,350	3.2	120 (6.6) 49	HC-liver, anemia, MD, gallstones	31
	(DHS)							
	ILA-4	7488-7489insACTGGA, heterozygous in *PIEZO1*	50/F	4,315	nd	nd (7.9) 39	HC-liver, anemia, MD, hypothyroidism, hypogonadism	31
	(DHS)							
	ILA-5	nd	61/F	1,960	nd	96.0 (10.7) nd	HC-liver, anemia, DM, cardiac failure	34
	ILA-6	nd	82/M	5,141	nd	nd (10.2) 100	HC-liver, DM, hypothyroidism	31
	(DHS)	(A member of the DHS family)						

Normal serum ferritin values were 26–310 ng/mL for men and 7–110 ng/mL for women. Normal serum Hep25 levels were 1.6–12.7 ng/mL. Normal values were 40–70% for of TSAT, 13.5–17.0 for Hb in men and 11.2–14.5 g/dL for women, and < 10‰ for reticulocytes. CDA, congenital dyserythropoietic anemia; DM, diabetes mellitus; DHS, dihydrated hereditary stomatocytosis; F, female; Hb, hemoglobin; HC, hemochromatosis; Hep25, hepcidin25; ILA, iron-loading anemia; M, male; nd, not determined; Ph, phlebotomy; Ref, reference; TSAT, transferrin saturation.

and resultant low TSAT values and serum Hep25 levels. Serum ferritin levels were high and a large amount of iron was deposited in hepatocytes; however, the liver showed neither inflammation nor fibrosis. All patients had DM, and central nervous system (CNS) disorders, including cognitive failure, were late complications.

In the last decade, we identified six patients affected by PIOS compatible with ILA (Table 1).[31–34] The etiologies of genetic anemias associated with ineffective erythropoiesis and hemolysis varied. Two patients with DHS in one family[30] and one patient with the same disease in another family[31] had PIEZO1-induced hemolytic anemia associated with hypohepcidinemia. An 82-year-old male patient with PIOS in the first family[30] missed the opportunity for a gene analysis for autosomal dominant disease and an assessment of serum Hep25 levels. A 48-year-old male patient with congenital dyserythropoietic anemia (CDA) type 1[33] was affected by ILA associated with a heterozygous mutation in the *CDAN1* gene. A 61-year-old female patient[34] died from congestive heart failure associated with the HC triad of cirrhosis, MD, and pigmentation. She had a history of severe anemia since adolescence but did not have a previous history of iron supplementation or transfusion. An autopsy confirmed iron-induced multiorgan damage, but opportunities for an assessment of Hep25 levels and a genetic analysis were missed. All six patients with ILA had severe anemia with hemoglobin (Hb) between 6.6 and 10.7 g/mL, and varied reticulocytosis. In contrast, serum ferritin levels were high, ranging from 1960 to 4,350 ng/mL. Hep25 levels in three patients were < 5.0 ng/mL. All six patients had hepatocellular iron-positive fibrosis or cirrhosis, five had DM, and three had endocrine disorders. As summarized in Table 4, the clinical features of ILA were characterized by anemia associated with ineffective erythropoiesis and hemolysis, hyperferritinemia, and hypohepcidinemia. In addition, iron-induced multiorgan damage of chronic liver diseases, cardiac failure, and endocrine diseases, including DM, were reported in ILA.

Three Japanese patients with HFE-CH were newly enrolled in the revised version (Table 2).[24,25] A 65-year-old female patient with HC associated with the homozygous mutation of C282Y in HFE was identified in 2001.[24] A second family of two patients with HFE-HC associated with homozygous Y231del was identified in 2011.[25] A 43-year-old male patient had the typical phenotype of HC with the triad, and responded fairly well to standard phlebotomy. His iron biomarkers measured after treatment were 150 ng/mL for ferritin and 4.0 ng/mL for Hep25. His younger sister with the same genetic background also had DM. However, iron overloading was mild. No

Table 3. Patients with hepatic HC and posthepatic SLC40A1-PIOS

Class	Disease	Genetic study	Age/ Sex	Serum ferritin, ng/mL	Serum Hep25, ng/mL	TSAT, 40–70%	Organ damage	Ref
Hepatic	HFE-HC-1	691-693del (Y231del), homozygous in *HFE*	42/F	423	nd	85.7	Iron deposits in the liver and pancreas on abdominal imaging, liver dysfunction, DM, oligomenorrhea	25
			Post-Ph	192	19.6	71		
	HFE-HC-2	691-693del (Y231del), homozygous in *HFE*	43/M	1,698	nd	120	Liver dysfunction, DM	25
			Post-Ph	150	4	88.7		
	HFE-HC-3	845G>A (C282Y), homozygous in *HFE*	65/F	5,660	nd	89.8	HC-liver, DM, pigmentation	24
	TFR2-HC-1	1199T>G/2008-2009delAC in *TFR2*	40/M	10,191	12.2	94.2	HC-liver, DM, hypogonadism	16,18
	TFR2-HC-2	1861-1862del12, homozygous in *TFR2*	47/M	4,400	BDL	90.6	HC-liver, pigmentation	18
	TFR2-HC-3	1469T>G, homozygous in *TFR2*	49/M	1,057	2.8	93.5	HC-liver, DM	16,18
	TFR2-HC-4	1861-1862del12, homozygous in *TFR2*	50/M	2,485	BDL	94.5	No liver biopsy, liver dysfunction	18
	TFR2-HC-5	1861-1862del12, homozygous in *TFR2*	53/F	1,470	BDL	93.2	HC-liver	18
	HJV-HC-1	515-516insC, homozygous in *HJV*	13/M	16,000	nd	90	Liver fibrosis, cardiac disease	18
			29M	6,624	BDL	94.4	HC-liver, cardiac failure	
	HJV-HC-2	515-516insC, homozygous in *HJV*	17/M	2,500	nd	92	DM	18
			33/M	2,222	2	94	No liver biopsy, DM	
	HJV-HC-3	842T>C, homozygous in *HJV*	37/F	2,274	BDL	93	HC-liver, DM, hypogonadism	35
	HJV-HC-4	449A>G/820G>A, compound heterozygous in *HJV*	39/M	6,421	7.4	98.4	Iron overload on abdominal imaging, liver dysfunction	36
	HJV-HC-5	745G>C, homozygous in *HJV*	48/M	6,115	BDL	94.8	HC-liver, DM, cardiac failure, pigmentation	18
	HJV-HC-6	842T>C, homozygous in *HJV*	52/F	4,340	BDL	92	Liver dysfunction, DM, hypogonadism, cardiac failure	35
	HAMP-HC-1	223C>T, homozygous in *HAMP*	45/M	3,000	nd	94.4	HC-liver, DM, hypogonadism, pigmentation	18
			58/M Post-Ph	16.3	BDL	11.6		
Post-Hepatic	SLC40A1-HC-1	1520A>G, heterozygous in *SLC40A1*	42/M	3,751	132.5	93	Liver dysfunction, cataracts, DM	37

(continued)

Table 3. *(continued)*

Class	Disease	Genetic study	Age/ Sex	Serum ferritin, ng/mL	Serum Hep25, ng/mL	TSAT, 40–70%	Organ damage	Ref
	SLC40A1-HC-2	1520A>G, heterozygous in *SLC40A1*	46/M	1,722	139.6	91.3	HC-liver, DM	38
	SLC40A1-HC-3	470A>C, heterozygous in *SLC40A1*	66/M	7,980	156.7	89.3	FP-liver, DM, pigmentation	16,18,40
	SLC40A1-HC-4	No mutation detected in *SLC40A1*	79/F	6,403	185.6	88.3	FP-liver, DM, pigmentation	40
	SLC40A1-HC-5	470A>C, heterozygous in *SLC40A1*	79/M	18,610	nd	95.8	FP-liver, hypopituitarism	39
	FP-D-1	1467A>C, heterozygous in *SLC40A1*	49/M	696	42.5	28.6	Mild mixed iron, no liver dysfunction, no organ damage	16,18
	FP-D-2	238G>A, heterozygous in *SLC40A1*	66/F	1,832	79	43.4	Severe mixed iron, no liver dysfunction, no organ damage	unpublished
	FP-D-3	1467A>C, heterozygous in *SLC40A1*	81/M	2,639	155	88.3	No liver dysfunction, no organ damage	16,18

Normal serum ferritin values were 26–310 ng/mL for men and 7–110 ng/mL for women. Normal serum Hep25 levels were 1.6–12 ng/mL. Normal values were 40–70% for TSAT, 13.5–17.0 g/dL for Hb in men and 11.2–14.5 g/dL for women. The classification of PIOSs was revised from the original classification[17] and includes a new disease entity, ILA.[18–20] The terminology of SLC40A1-PIOSs was adjusted to the new proposal of HC by the BIOIRON Society.[22] HC-liver was hepatocyte-dominant iron-loading fibrosis or cirrhosis and FP-liver was fibrosis or cirrhosis with mixed iron loading in hepatocytes and Kupffer cells. BDL, below the detection limit; DM, diabetes mellitus; F, female; FP, ferroportin; FP-D, ferroportin disease; Hb, hemoglobin; HAMP, human antimicrobial peptide; HC, hemochromatosis; Hep25, hepcidin25; HFE, hemochromatosis protein; HJV, hemojuvelin; M, male; nd, not determined; Ph, phlebotomy; Ref, references; SLC40A1, gene name of ferroportin1; TFR2, transferrin receptor2; TSAT, transferrin saturation.

significant differences were observed in the clinical features of HFE-HC between Japanese and Caucasian patients (Table 4). Furthermore, there were no new patients with TFR2-HC in the revised version, but *TFR2* was one of the major HC gene as well as *HJV*. HJV-HC was also a major genotype of juvenile HC. Three patients were reported in the first version[18] and three were enrolled in the revised version.[34,35] As previously reported,[18] two siblings of a family received two medical examinations because of the long-term interruption of phlebotomy treatment. The ages of onset of their iron diseases were 13 and 17 years. The ages of onset of HJV-HC in the three new patients were 37, 39, and 52 years (Table 3). Two female patients in a family had DM and hypogonadism[34] and a male patient had severe hyperferritinemia of 6,421 ng/mL but iron-induced organ damage was limited to biochemical liver dysfunction.[35] A 45-year-old male patient appeared with the HC triad.[18] The second examination after phlebotomy treatment at 58 years of age had 16.5 ng/mL for ferritin and BDL for Hep25, and revealed disease-causing mutations in the *HAMP* gene. As summarized in Table 3, HJV-HC had a wide range of onset ages of between 13 and 52 years. In addition, Japanese patients with HJV- and HAMP-HC had a wide clinical spectrum from juvenile HC to adult-onset traditional HC with cirrhosis, DM, and cardiac failure.

Three patients with SLC40A1-PIOSs were reported in the first version[18] and five were newly enrolled in the revised version (Table 2).[36–39] Phenotypically, they were divided into five patients with SLC40A1-HC and three with FP-D. Four new patients with SLC40A1-HC were already complicated with multiorgan damage at presentation. All five patients with SLC40A1-HC had high serum ferritin and Hep25 levels and also had advanced chronic liver diseases associated with combined parenchymal and reticuloendothelial iron loading. Four of the five patients were also affected by DM and one had hypopituitarism. In contrast, the three patients with FP-D had selective hyperferritinemia without iron-induced organ damage. An 81-year-old male patient and his 49-year-old son were described in the first study[18] and were followed up for more than 10 years.[22] The last physical examinations of the father and son were performed at the ages of 90 and 59 years, respectively and found hyperferritinemia, hyperhepcidinemia, and no iron-induced organ damage. A few years later, the father died from malnutrition associated with dysphagia. A 66-year-old woman from another family (Table 3, FP-D-2) who was first enrolled in the revised version remained asymptomatic for 10 years. However, her liver histology showed heavy iron deposits in hepatocytes and Kupffer cells but neither inflammation nor fibrosis. Serum levels of ferritin and Hep25 were high in SLC40A1-PIOSs, and the difference between SLC40A1-HC and FP-D was iron-induced organ damage owing to gain-of-function FP molecules in the former and no organ damage resulting from the loss-of-function FP molecules in the latter (Table 4).

Discussion

The original classification based on the Hep25/FP system included ACP as a prehepatic PIOS.[18] In the revised version, the new disease entity of ILA[19–21] was included as the second prehepatic PIOS. It is important to note that the number of our patients with ILA was small and their genetic backgrounds were limited. However, iron overload has been found in a number of anemias ranging from traditional thalassemia and CDA to new DHS associated with *PIEZO1* mutations.[31] ACP[11,16,26] and ILA[19–21] are now recognized as major prehepatic PIOSs with an impaired Hep/FP system. The terminology of SLC40A1-PIOSs was adjusted in the recent proposal

Table 4. Summary of the characteristics of primary iron overload syndromes

Class	Disease	Serum ferritin	Serum Hep25	Responsible gene and detection of disease-causing mutations/patients	Other diagnostic index and main organ damage	Phlebotomy and other options
		reference: 13–277 ng/mL for M; 5–15 ng/mL for F	reference: 1.6–12.7 ng/mL			
Prehepatic	ACP	High	Low	*CP*, 5/5	CP deficiency, low TSAT, central DM, CNS disorders	Intolerance, Oral chelation
	ILA	High	Low	Various genetic anemias: *PIEZO1*: 3/4, *CDAN1*:1/1, nd: 0/1	Ineffective erythropoiesis and hemolysis, Overproduction of ERFE, Cirrhosis, DM, pigmentation, cardiac failure	No indication; Oral chelation; Transfusion/ oral chelation
Hepatic	HC	High	Low or BDL[*2]	*HFE*, 3/3; *TFR2*, 5/5; *HIV*, 6/6; *HAMP, 1/1*	HC-liver, DM, pigmentation, cardiac failure	Good tolerance
Post-hepatic	SLC40A1-HC	High	High	*SLC40A1* (Gain of function) 4/5	FP-liver, Mixed-cell iron cirrhosis, DM, pigmentation, cardiac failure	Good tolerance; Oral chelation tolerance
	SLC40A1-FP-D	High	High	*SLC40A1* (Loss of function) 3/3	mixed-cell iron	None needed

The classification of PIOS revised from the original classification[18] included the new disease entity, ILA[19–21] and the terminology of SLC40A1-PIOSs was adjusted to the new proposal of HC by the BIOIRON Society.[23] Serum levels of ferritin were increased in PIOSs. Serum levels of Hep25 were low in prehepatic and hepatic PIOSs and high in posthepatic PIOSs. Hep25 was not detected in HAMP-HC.[18] Severe anemias due to ineffective erythropoiesis and hemolysis resulted in ILA via the overproduction of ERFE, while low levels of TSAT were characteristic of ACP. DM was the most common complication of PIOSs. It may be pancreatic, associated with iron deposits in Langerhans islands of ILA, the four genotypes of HC, and SLC40A1-HC, and central involving the brain, pituitary gland, and pancreas in ACP. Full marks of the triad of HC consisting of cirrhosis, DM, and pigmentation, and the two major features of juvenile HC of cardiac failure and hypogonadism, appeared in the late stages of ILA, HC, and SLC40A1-HC. Hepatic iron appeared to be nontoxic in ACP and FP-D. HC had good tolerance to phlebotomy. It was not indicated for ILA. Underlying bone marrow dysfunction appeared after phlebotomy in ACP and FP-D. ACP, aceruloplasminemia; BDL, below the detection limit; CNS, central nervous system; CP, ceruloplasmin; DM, diabetes mellitus; ERFE, erythroferrone; FP, ferroportin; FP-D, ferroportin disease; HAMP, human antimicrobial peptide; HC, hemochromatosis; Hep25, hepcidin25; HFE, hemochromatosis protein; HJV, hemojuvelin; ILA, iron-loading anemia; nd, not determined; SLC40A1, gene name of ferroportin1; TFR, transferrin receptor; TSAT, transferrin saturation.

for HC by the BIOIRON Society.[23] However, we renamed FP-D in the posthepatic PIOSs. The new FP-D has similar iron loading, but different organ toxicity than SLC40A1-HC. Comparative study of the two phenotypes of SLC40A1-PIOSs is still needed. It is particularly important to clarify why an age-dependent iron accumulation in the liver occurs without change of structure and function in FP-D.[18,22]

HFE-HC also appeared in the revised version because a Japanese family associated with non-C282Y-HFE-HC was identified in Japan.[25] Therefore, the current list of HC was changed from three to four genotypes, which was similar in Caucasians and non-Caucasians. The only difference between the two ethnicities is the markedly higher prevalence of C282Y-HFE-HC in Caucasians.[3,4] Regarding the relationship between HC and its genetic background, C282Y *per se* is a mutation in the *HFE* gene of HC of PIOSs.[1,24] However, accumulated evidence on HC, particularly C282Y-HFE-HC in Caucasians[23,28] is important for a more detailed understanding of PIOSs in non-Caucasians. The revised classification of PIOS is now more useful for both Caucasians and non-Caucasians.

In an adult man with an iron overload condition, TFR1 and TFR2 receive iron-saturated TF and release signals that induce the synthesis of a large amount of Hep25 coded by HAMP (Fig. 1). The Hep25/FP system suppresses the iron export function of FP after Hep25-FP complex formation, its internalization, and lysosomal degradation. Enterocytes may then transport a signal to divalent metal transporter1 (DMT1) in order to replace iron stores. As absorbed iron is less than its daily loss, a negative balance is maintained during an iron overload state.[13,14]

In the prehepatic PIOSs of ACP and ILA, false signals from outside the liver suppress the synthesis of Hep25 (Fig. 2).[16,19–21] These false signals are circulating TF with a low TSAT in ACP[10] and the increased production of ERFE by erythroblasts in ILA.[19–21] Serum levels of Hep25 are low in contrast to hyperferritinemia in patients with ACP[11,16] and ILA.[19–21] Therefore, the hepatic production of Hep25 and its release into the circulation are suppressed in a prehepatic manner. The four genotypes of HFE, TFR2, HJV, and HAMP-HC are known as hepatic PIOSs.[16,18] All HC genotypes have highly saturated TF, but their serum Hep25 levels are reduced to a low level or nearly zero in a hepatic manner[13,16,18] as shown in Figure 3A–D. In prehepatic and hepatic PIOSs (Fig. 4), inactivation of the iron exporter FP by Hep25 is negligible; therefore, active FP exports a large amount of iron into the circulation from enterocytes. Iron-vacant enterocytes require DMT1 to absorb a large amount of iron from the diet.[13,14] Therefore, the body iron store continues to increase with time.[18] In posthepatic PIOSs with SLC40A1 mutants (Fig. 5), the liver releases adequate amounts of Hep25 into the circulation. However, FP, the receptor of Hep25 in enterocytes and macrophages, resists circulating Hep25 without Hep25/FP complex formation, being free from Hep25 control. Gain

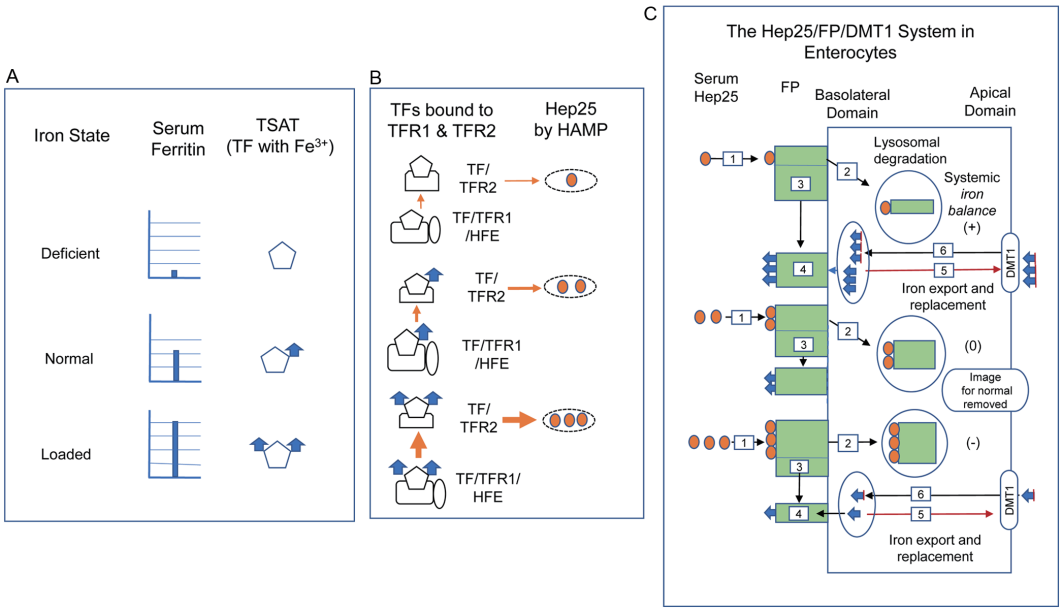

Fig. 1. Iron homeostasis by the Hep25/FP system. Iron homeostasis is regulated by iron export and replacement in the gut under the control of the Hep25/FP/DMT1 system. (A) Prehepatic stage: In the case of an iron deficiency (the upper portion), serum ferritin is low and TF is mostly free from Fe^{3+}, while in iron overloading (the lowest portion), serum ferritin is high and TF is mostly saturated with two molecules of Fe^{3+}. (B) Hepatic stage: When TFRs in the liver receive iron-deficient signals (the upper portion), Hep25 synthesis and release are reduced to minimum levels, while the response of the liver to the iron overload signal (the lowest portion) is the release of a large amount of Hep25. (C) Post-hepatic stage: In enterocytes, FP at the basolateral domain receives Hep25 signals from the circulation (1), followed by lysosomal degradation of Hep25/FP complexes (2). Remained active FP (3) exports the cytosolic Fe^{3+} into the circulation (4). Iron depleted signals in enterocytes (5) are transferred to DMT1 at the apical domain to replace the cytosolic Fe^{3+} from the circulation (5, 6). Therefore, the systemic iron balance maintains plus (+) in iron-deficient state, zero (0) in normal, and minus (–) in iron overloaded. DMT1, divalent metal transporter1; FP, ferroportin; HAMP, human antimicrobial peptide; Hep25, hepcidin25; TF, transferrin; TFR, transferrin receptor; TSAT, transferrin saturation.

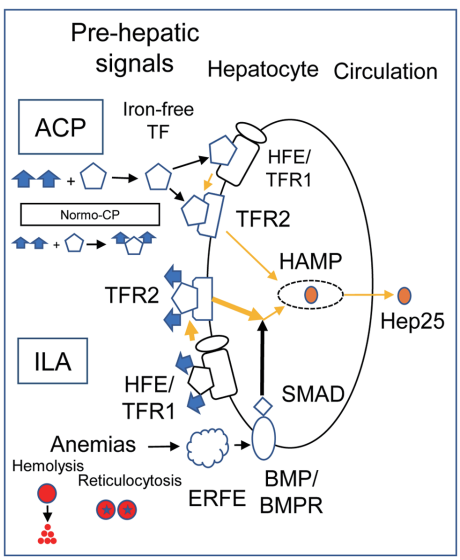

Fig. 2. Prehepatic PIOS. In the prehepatic PIOSs of ACP and ILA, false signals from outside the liver suppress the synthesis of Hep25. One of these signals (upper part) is low TSAT because of ceruloplasmin (ferroxidase) deficiency in ACP[16] and the other (lower part) is the increased production of ERFE by erythroblasts in genetic anemias associated with ineffective erythropoiesis and hemolysis.[19–21] Therefore, the hepatic production of Hep25 and its release into the circulation are suppressed in a prehepatic manner. ACP, aceruloplasminemia; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CP, ceruloplasmin; ERFE, erythroferrone; HAMP; human antimicrobial peptide; Hep25, hepcidin25; ILA, iron-loading anemia; PIOS, primary iron overload syndrome; TF, transferrin; TFR, transferrin receptor; TSAT, transferrin saturation.

of function-FP may independently export a large amount of toxic iron and is responsible for SLC40A1-HC, while loss of functionFP may also export a wide range of non-toxic iron in FP-D.[17] Therefore, iron absorption does not discontinue in a posthepatic manner.[18] Owing to low Hep25 levels in prehepatic and hepatic PIOSs and resistant FP against adequate levels of Hep25 in posthepatic PIOSs, the impaired control of iron export by the Hep25/FP system results in the accumulation of excess iron.[18]

As summarized in Table 4, the classification may be an excellent base for the diagnosis and treatment of PIOSs in clinical practice.[16,18] The first test is an assessment of serum ferritin, which is high in all patients with PIOSs prior to the initiation of treatment. Serum Hep25, a key molecule in PIOSs, needs be measured in all patients. We assessed serum levels of Hep25 in Japanese patients with PIOSs and related iron disorders using LC/ESI-MS/MS.[29] It was low in prehepatic and hepatic PIOSs, but became elevated in parallel with ferritin in posthepatic PIOSs.[16,18] TSAT is also high in all PIOSs, except for ACP with an inappropriate low TSAT due to a CP (ferroxidase) deficiency.[10,16,18] The two prehepatic PIOSs, ACP and ILA, are characterized by deficient CP[10,11] and increased ERFE production,[19–21] respectively. Hb and CP are all normal in the four genotypes of HC but Hep25 is low or zero in contrast to a high ferritin level.[16,18] Posthepatic PIOSs and iatrogenic iron overload, such as repeated transfusions and long-term iron supplementation, are both associated with high serum levels of Hep25 in parallel with serum ferritin levels. A detailed medical history is essential for a differential diagnosis of these two conditions.[16,18] We confirmed that FP-D in the three patients was not a premature form of SLC40A1-HC.[22] The former may be a selective type of hyperferritinemia,[22] while the latter may be compli-

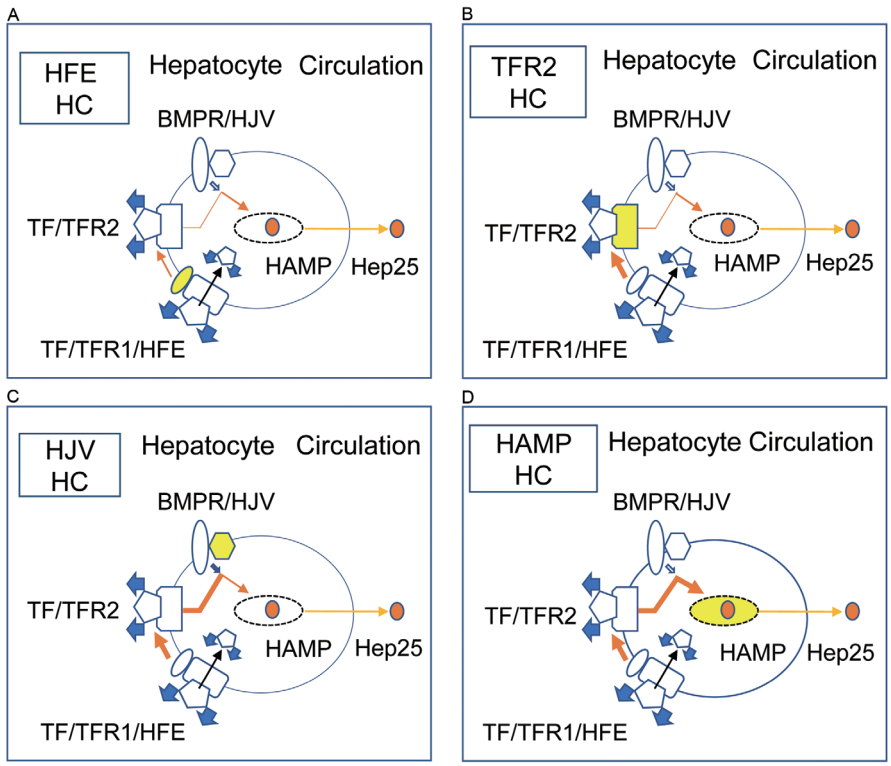

Fig. 3. Hepatic PIOS (A–D). TF/TFR1/HFE in HFE-HC and TF/TFR2 in TFR2-HC release a false signal of iron deficiency to HAMP (A, B, respectively). In HJV-HC, the BMPR/HJV complex may suppress the iron-saturated TF signal to an iron deficiency level (C), while in HAMP-HC, mutant HAMP may lose the ability to synthesize Hep25 (D). In all genotypes of HC, a small or absent amount of Hep25 is released into the circulation. These figures are not metabolic maps, but images of iron transporters that suppress Hep25 synthesis. Body iron signals from TF/TFR1/HFE, TF/TFR2, and BMPR/HJV may independently regulate Hep25 synthesis. BMPR, bone morphogenetic protein receptor; HAMP, human antimicrobial peptide; HC, hemochromatosis; Hep25, hepcidin25; HFE, hemochromatosis protein; HJV, hemojuvelin; PIOS, primary iron overload syndrome; TF, transferrin; TFR, transferrin receptor.

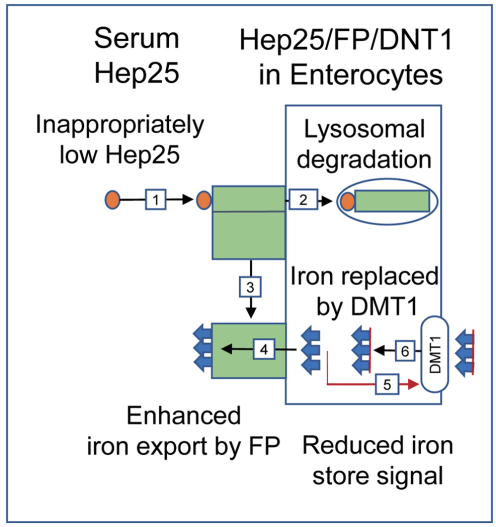

Fig. 4. Hep25/FP/DMT1 in prehepatic and hepatic PIOS. The key factors in these PIOSs are the suppressed synthesis of Hep25 (1), followed by the incomplete internalization and lysosomal degradation of the Hep25/FP complex (2, 3). Hypohepcidinemia because an impaired Hep25/FP system results in the export of a large amount of Fe^{+3} into the circulation (4), followed by the replacement of the enterocyte iron store by the active absorption of dietary iron through DMT1 (5, 6). Therefore, body iron accumulates daily in these PIOSs. DMT1, divalent metal transporter1; FP, ferroportin; Hep25, hepcidin25; PIOS, primary iron overload syndrome.

cated by multiorgan damage in the late stage[40] similar to hepatic HC.

Various human genetic analyses have been introduced in clinical practice. Serum levels of CP are reduced under many pathological conditions, including Wilson's disease.[16,18] Therefore, direct sequencing of the *CP* gene may be indicated for a patient with hyperferritinemia associated with hypoceruloplasminemia, for example, a male patient with Wilson's disease treated by long-term copper chelation.[41] ILA is an ERFE-induced PIOS, but not anemia-induced iron overloading.[19–21] A wide range of genetic and acquired diseases result in anemias associated with ineffective erythropoiesis and hemolysis. However, the identification of disease-causing mutations in the genes associated with anemias may not be necessary in clinical practice. The direct sequencing of the four HC genes is not an absolute test of genotyping hepatic PIOSs. The C282Y mutation in HFE, which is common in Caucasian HC,[3] may be detected by a simple, inexpensive kit.[42] When this test is negative, it is necessary to examine all candidate HC genes for mutations,[3] as in the case of non-Caucasians.[4] It is also important to note that the *HJV* gene is not only associated with juvenile HC, but also with adult-onset HC.[4,18] Mutant-dependent phenotypes have been reported in SLC40A1-PIOSs; therefore, genetic testing for a mutation in the *SLC40A1* gene may be clinically useful.[16,18] Genetic information on PIOSs may be important in clinical practice, but may not be needed for the clinical diagnosis or management of patients. Even though a patient may have the typical clinical features of a phenotype, a genetic analysis

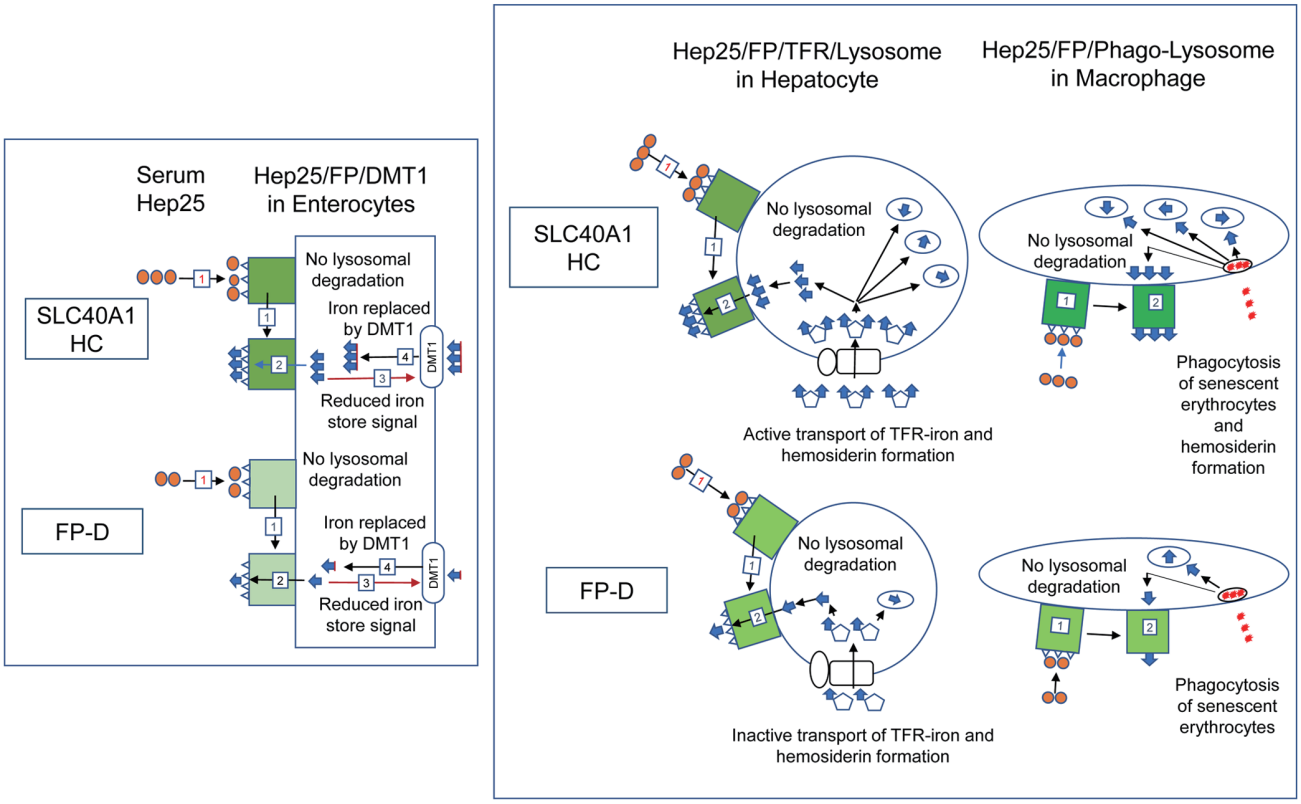

Fig. 5. Posthepatic PIOS. The left square is a model of resistant FP to Hep25, while the right one is two models for the iron homeostasis in SLC40A1 HC, and FP-D. In the posthepatic PIOS SLC40A1-HC and FP-D, appropriate amounts of Hep25 are released in the circulation, while FP molecules resist circulating Hep25 (1, 2). Binding of Hep25 to FP is disrupted to regulate iron homeostasis by two approaches via the Hep25/FP/DMT1 system in enterocytes (3, 4): gain-of-function FP in SLC40A1-HC (dark green), and loss-of-function FP in FP-D (light green). In addition to the different directions of FP function, iron-induced cytotoxicity differs: SLC40A1-HC exhibits iron-induced systemic organ damage including the triad of HC, heart diseases, and endocrine disorders, and FP-D exhibits asymptomatic iron accumulation in the liver and spleen without biochemical damage. DMT1, divalent metal transporter1; FP, ferroportin; FP-D, ferroportin disease; Hep25, hepcidin25; PIOS, primary iron overload syndrome; SLC40A1, gene name of ferroportin1.

may fail to detect the disease-causing mutation in the postulated gene.[40]

Patients with HC have excellent tolerance for standard phlebotomy[3,4,25] Milder phlebotomy may be recommended for SL40A1-HC[3,38] and other phenotypes.[3] New oral iron chelators are now available for patients intolerant of phlebotomy. However, the effects of iron chelators on brain iron levels in ACP are controversial.[30,43] In ILA, oral iron chelation is the first-line treatment, and transfusion, where needed, needs to be combined with oral chelation. Treatment may not be necessary for FP-D.[22]

Serum Hep25 is undoubtedly a key molecule in PIOSs, and there are a few laboratories that measure Hep25 in Japan. However, 28% of new patients (5/18) in the revised version did not receive Hep25 testing. A large number of patients with complete clinical data on the Hep25/FP system need to be enrolled for the classification of PIOSs in the future.

Conclusions

The genetic background of PIOS differs in Caucasians and non-Caucasians.[2] Therefore, physicians have to overcome differences in the global prevalence of PIOS in clinical practice. The Hep25/FP system classifies PIOSs as prehepatic ACP and ILA, hepatic HC, and posthepatic SLC40A1-HC and FP-D. The revised classification for PIOSs may be useful worldwide.

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None to declare.

Conflict of interest

KH has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (YT, MY, HM, SI, KK, HH, KH), methodology (YT, SW, TN, WN, AK), investigation (YT, WN, AK), writing original draft (MY, TI, SI, AT, WN, AK), writing review, and editing (YT, SW, KK, HH, KY, KH), and supervision (YT, MY, SW, HM, KK, HH, KY, KH).

Ethical statement

The protocol of the present study was prepared in accord with

the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects issued by the Ministry of Economy, Trade, and Industry of Japan (revised in 2023.3.27). Informed consent was obtained from each patient before the assessment of serum Hep25 levels and a genetic analysis of iron overload syndromes. Instead of informed consent, OPTOUT was permitted for patients. The draft of the present study was prepared in accordance with the STROBE statement. Finally, the statement of the classification of PIOSs in an international journal was approved by the Ethics Committees of the Aichi-Gakuin University School of Pharmacy and other institutes. The new classification of PIOSs is not duplicated, but revised from the original classification published in 2012. The new disease entity ILA is registered in the revised version and the terminology is renewed according to the proposal for HC by the BIOIRON Society. This manuscript is not under consideration for publication by any other journal.

Data sharing statement

No additional data are available.

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