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Update on iron metabolism and molecular perspective of common genetic and acquired disorder, hemochromatosis

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Contents

Abstract

Iron is an essential component of erythropoiesis and its metabolism is tightly regulated by a variety of internal and external cues including iron storage, tissue hypoxia, inflammation and degree of erythropoiesis. There has been remarkable improvement in our understanding of the molecular mechanisms of iron metabolism past decades. The classical model of iron metabolism with iron response element/iron response protein (IRE/IRP) is now extended to include hepcidin model. Endogenous and exogenous signals funnel down to hepcidin via wide range of signaling pathways including Janus Kinase/Signal Transducer and Activator of Transcription 3 (JAK/STAT3), Bone

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Morphogenetic Protein/Hemojuvelin/Mothers Against Decapentaplegic Homolog (BMP/HJV/SMAD), and Von Hippel Lindau/Hypoxia-inducible factor/Erythropoietin (VHL/HIF/EPO), then relay to ferroportin, which directly regulates intra- and extracellular iron levels. The successful molecular delineation of iron metabolism further enhanced our understanding of common genetic and acquired disorder, hemochromatosis. The majority of the hereditary hemochromatosis (HH) patients are now shown to have mutations in the genes coding either upstream or downstream proteins of hepcidin, resulting in iron overload. The update on hepcidin centered mechanisms of iron metabolism and their clinical perspective in hemochromatosis will be discussed in this review.

Keywords: Iron metabolism; Hemochromatosis; Hepcidin; Ferroportin; Bone morphogenetic protein; Erythroferrone

1. Introduction

Iron plays a pivotal role in cell survival and proliferation by regulating enzymatic activity and oxidation-reduction reactions. Accordingly, iron deficiency may lead to cell growth arrest or death [1]. Excessive iron also induces catastrophic results by generating cytotoxic hydroxyl and lipid free radicals that activate reticuloendothelial system, aggravating inflammatory process [1]. Therefore, iron metabolism needs to be tightly regulated in a physiologic range to prevent adverse effects from either iron deficiency or overload. Iron metabolism is composed of two major parts: sensors and effectors (Fig. 1). A wide range of endogenous and exogenous signals including iron demand from hemorrhage, erythropoiesis, inflammation, and tissue ischemia can turn on and off signaling pathways that are involved in iron metabolism. These signals converge into protein called hepcidin that orchestrates inputs from sensors and relays them into three main cellular processes: iron uptake, storage and export (Fig. 1) [2]. The upstream and downstream signaling pathways of hepcidin are composed of a variety of proteins such as HIF, BMP, transferrin, ferritin, ferroportin, and hemochromatosis protein (HFE), and their activities and expressions are tightly coordinated in interdependent manner [3]. Accordingly, any genetic or acquired disorder in one of these proteins can potentially disrupt intra- and extracellular iron balance and result in pathologic conditions from either iron deficiency or iron overload [1].

2. Effector pathways in iron metabolism

The human body requires 25 mg of iron a day and 80% of iron is used in erythropoiesis in the bone marrow (Fig. 2) [1]. 18–19 mg of iron are recycled from damaged or old red blood cells and 1–2 mg are newly absorbed through gastrointestinal system, mainly duodenal entrerocytes [1]. The homeostasis depends on three main cellular processes: iron absorption, iron storage and iron uptake.

2.1. Iron absorption

Iron can be absorbed into cells in two major forms: protein bound iron (i.e. transferrin, hemoglobin) and free iron. Transferrin is one of the major iron binding proteins, which is an 80 kD glycoprotein composed of 679 amino acids circulating in the plasma [4,5]. Two iron ions reversibly bind to transferrin with high affinity and intracellular uptake of transferrin and iron complex is regulated by transferrin receptors 1 (TfR1) (Fig. 3) [6,7]. Once transferrin and iron complex binds to TfR1 on the cell surface, the plasma membrane is isolated into the cytoplasm and forms early endosome (Fig. 3) [7]. Proton pumps maintain an acidic environment inside the endosome to induce conformational changes of transferrin and TfR1, and facilitate the dissociation of iron from transferrin and transferrin receptor [7]. Free Fe^{3+} iron in the endosome is reduced to Fe^{2+}, then transferred back into cytosol via the divalent metal transporter 1 (DMT-1) [8,9]. TfR1

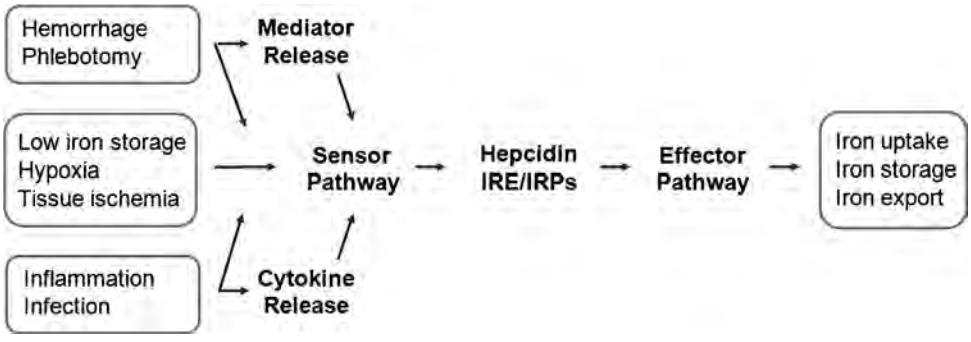

Fig. 1. **Iron Metabolism.** Iron metabolism is composed of sensor and effector pathways. Four major inputs from iron storage level, hypoxia, inflammation and erythropoiesis converge into hepcidin that relays a coordinated signal to effector proteins, ferroportion that maintain iron balance through iron uptake, storage and export. Any genetic or acquired disorder in these processes can potentially cause iron metabolic disorder.

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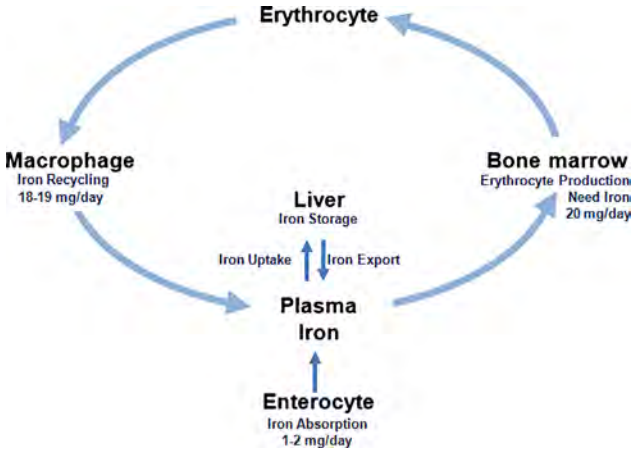

Fig. 2. **Iron distribution in the body.** Human body requires 25 mg of iron a day and 80% of iron is used in erythropoiesis. 18–19 mg of iron is recycled from old red blood cells and 1–2 mg is absorbed through gastrointestinal system. The iron homeostasis depends on iron absorption, storage and uptake in different organs and cells.

is expressed in a wide range of cells including enterocytes and erythroid precursors that consume the majority of iron for hemoglobin synthesis.

Absorption of iron bound to proteins other than transferrin is also important in iron recycling, especially in congenital or acquired disorders such as sickle cell anemia, thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulopathy (DIC) and hemolysis (Fig. 3). Hemoglobin released into plasma forms complex with haptoglobin and undergoes endocytosis after they are captured by CD163 surface molecule on macrophage or monocyte [10].

Most of the irons from diet are non-soluble free Fe^{3+} and non-protein bound free ion can be directly absorbed into duodenal enterocytes. Fe^{3+} is reduced into Fe^{2+} by ctochrome *b*-like ferrireductase (Dcytb) residing on the luminal surface of duodenal enterocyte, then transferred into cytosol via DMT1 (Fig. 3) [11]. Collective, iron from plasma or diet can be absorbed into cells in a various forms and abnormality in any step of absorption may cause pathologic sequel of iron deficiency or overload.

2.2. Iron storage

Hepatocyte and reticuloendothelial cells including macrophage and monocyte are the main iron storage sites in human body. Iron is stored as ferritin bound form in these cells. Ferritin is a ubiquitous protein composed of 12 light chain and additional 12 heavy chain subunits [12]. Ferritin multimeric apoprotein shell can hold total 4500 iron in the core, serving as intracellular iron storage machinery. It is downregulated in a setting of iron deficiency, rendering more iron to be available to meet the demand. Conversely, iron overload induces ferritin upregulation to protect cells from radical induced cytotoxicity [3].

2.3. Iron export

Extracellular iron export is an important axis of iron homeostasis that is mediated through iron specific transporter, ferroportin. Ferroportin is mainly expressed in the basolateral membrane of duodenal enterocyte and plasma membrane of macrophage (Fig. 3). Iron deficiency induces ferroportin expression, thereby enhancing iron export into plasma, and this is facilitated by downregulation of intracellular ferritin to release more iron through ferroportin [3]. The importance of ferroportin regulation by hepcidin has been extensively studied over the past decade, and the detailed mechanisms will be discussed below [2,13].

3. Molecular mechanisms of iron metabolism

A variety of endogenous and exogenous signals activates or deactivates molecular pathways to maintain iron balance within physiologic level. Proteins participating in iron metabolism can be indirectly regulated through hepcidin which integrates signals from multiple sources as well as directly by iron level via IRE and IRPs (Fig. 3).

3.1. Hepcidin regulates ferroportin expression

Hepcidin is a major coordinator of iron metabolism and its role has been extensively studied over the past decade. Pathologic conditions including inflammation, hemorrhage, tissue ischemia and erythropoiesis had been shown to regulate the expression level of hepcidin [14–19], and BMP was identified as its upstream regulator in response to inflammation and iron storage level [20–23]. In animal models, hepatic hepcidin mRNA level was found to be upregulated under iron overload [24], and inverse correlation between hepcidin and duodenal ferroportin expression was observed [25]. Subsequent studies with upstream stimulatory factor-2 (USF2) knockout mice proved hepcidin as an inhibitor of iron release from hepatocytes and macrophages [26,27], and these findings were confirmed in human studies [13]. Collectively, the *in vitro* and *in vivo* findings suggest hepcidin as a key protein in iron metabolism. Two recent studies elucidated bone BMP6/SMAD1/5/8 pathway as an upstream pathway that transcriptionally regulates hepcidin expression [28,29]. Upon binding of BMP6 to BMP receptor and its co-receptor HJV [30], SMAD1/5/8 is activated to phosphorylate SMAD4. Then, phosphorylated SMAD 4 and SMAD1/5/8 complex localizes in the nucleus to transcriptionally activate hepcidin (HAMP) [28,29].

In the following study, Nemeth et al demonstrated that hepcidin negatively regulates iron export by direct interaction with ferroportin [31]. The authors in this study used HEK293 cell lines stably expressing mouse ferroportin-green fluorescent protein (GFP) protein. They expressed hepcidin 25, hepcidin 20 (hepcidin lacking five N-terminal residue) and protegrin (non-specific cationic antimicrobial peptide)

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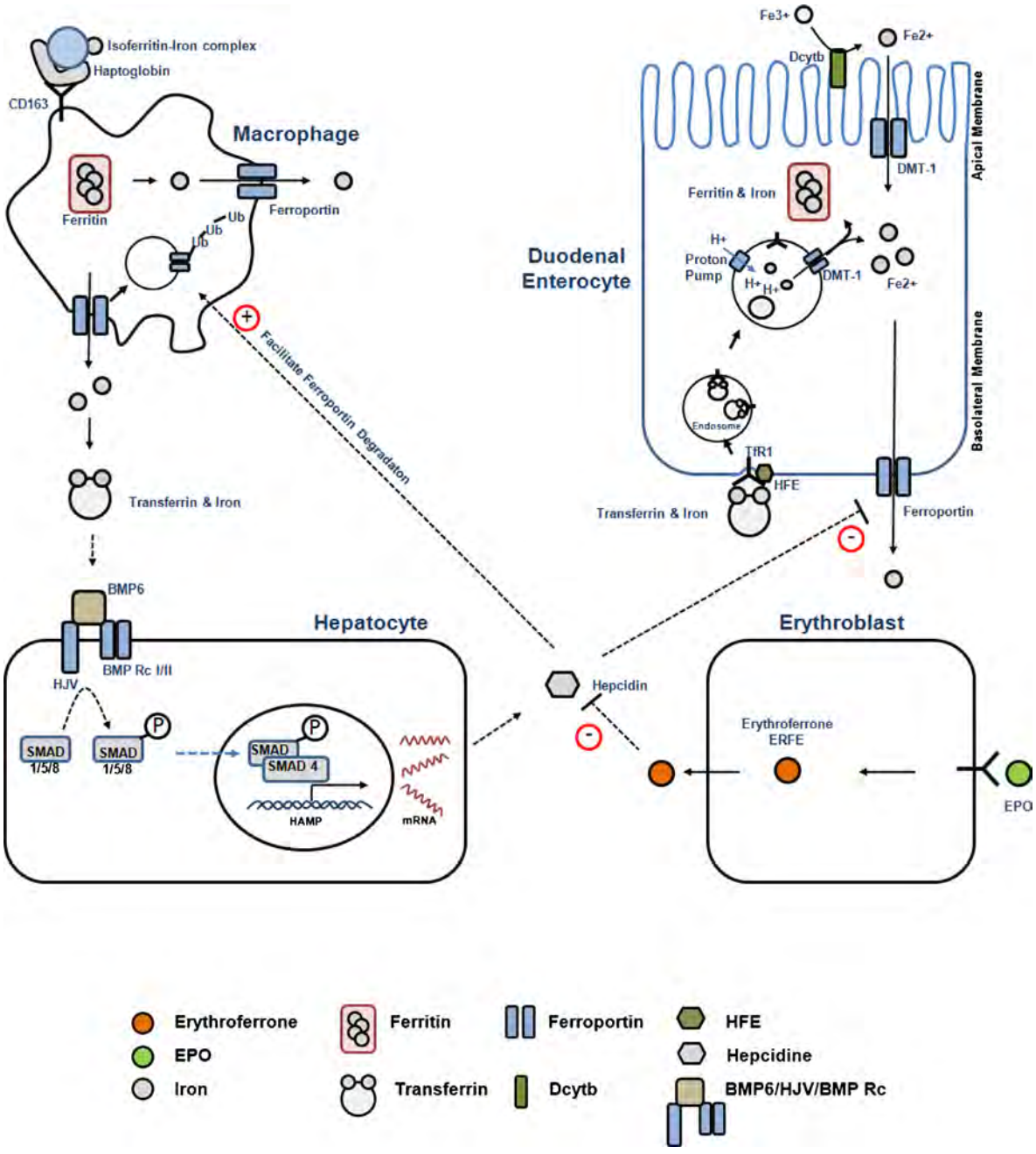

Fig. 3. **Molecular mechanisms of iron homeostasis.** Hepcidin is mainly synthesized in hepatocytes. Upregulation of hepcidin upon a wide range of intra- and extracellular signals facilitates ubiquitination and degradation of ferroportin, inhibiting iron export. Conversely, decreased hepcidin level induces iron transport via ferroportin.

in the cells and demonstrated that only bioactive hepcidin 25 induces ferroportin internalization and subsequent degradation via direct interaction with ferroportin [31]. In a following study by Qiao et al., ubiquitination mainly on lysines located in the intracellular loop domain of ferroportin was shown to play a key role in its internalization and degradation [32]. Using stable cell lines expressing mutant ferroportin-GFP proteins on lysine 229, 240, 247 and 258, the authors demonstrated delayed internalization of mutant ferroportins upon hepcidin treatment and resultant higher intracellular ferritin levels in the cell lines with mutant ferroportins. Accordingly, any mutation or dysfunction in Smad4, BMP6, HJV and ferroportin can lead to phenotypes of hemochromatosis that is consistent with findings in animal and human genetic studies [29,30,33–36].

3.2. Cellular response to iron storage

The question as to how iron storage levels regulate hepcidin remained unanswered until a recent study suggested HFE and TfR2 complex as a candidate upstream regulator of hepcidin [37]. In previous studies, liver biopsy samples

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from HFE knockout mice and HFE mutant hemochromatosis patients were shown to have lower hepcidin and higher ferroportin levels compared to control group. Also, many other studies suggested HFE as a possible upstream regulator of hepcidin [38–43]. Moreover, high levels of ferritin were shown to be correlated with increased hepcidin mRNA, indicating that HFE may regulate hepcidin by directly sensing ferritin level [22,44]. Gao et al. proved this hypothesis by demonstrating that HFE/TfR2/ferritin complex relays signal to induce hepcidin mRNA [37]. Although the molecular connection between HFE and BMP signaling pathway was shown in several other studies, it is still unresolved whether HFE/TfR2/ferritin complex regulates hepcidin via BMP/SMAD pathway [43].

3.3. Hepcidin as a sensor of erythropoeisis

Erythropoiesis is another main signal in iron homeostasis [45]. Hypoxia, anemia, hemorrhage, and phlebotomy were shown to be associated with low hepcidin and high EPO level [46–48]. In additional studies with thalassemia major patients, ineffective erythropoiesis and chronic anemia were shown to be associated with low hepcidin, elevated EPO and high ferroportin levels compared to normal population [49–52]. Moreover, transfusion in these patients was shown to downregulate and upregulate EPO and hepcidin, respectively [18]. Collectively, these observations suggested that EPO change upon various conditions may regulate iron metabolism via hepcidin. Subsequent studies demonstrated that EPO can suppress hepcidin transcription which indicates the presence of a direct mediator of hepcidin regulation in response to eythropoiesis (Fig. 3) [15,17,19]. Growth differentiation factor 15 (GDF-15) and twisted gastrulation 1 (TWSG1) that are BMP family proteins had been proposed as erythroid regulators [53,54], however, their role as a hepcidin regulator upon hemorrhage has been controversial [55,56]. Kautz et al. recently identified erythroferrone (ERFE) as a BMP6/SMAD independent erythroid regulator and demonstrated that EPO upregulates ERFE mRNA in erythroblast populations of bone marrow and spleen via JAK/STAT pathway (Fig. 3) [57]. ERFE knockout mice failed to downregulate hepcidin mRNA upon phlebotomy or recombinant EPO administration, and ERFE overexpression resulted in hepcidin mRNA downregulation in mouse hepatocytes, confirming the direct role of ERFE on iron metabolism. Moreover, hypoxia and inflammation failed to induce ERFE mRNA indicating that ERFE is a unique hepcidin regulator exclusively responding to the demand of erythropoiesis. Further investigation to identify ERFE receptor and its downstream signaling pathways will be the next step.

3.4. Role of hepcidin in anemia of chronic disease

Anemia of chronic disease is the second most common form of anemia. It is associated with acute or chronic infections, autoimmune diseases and malignancies [58]. The release of cytokines upon infection or inflammation mobilizes iron into storage cells, leading to serum iron decrease and impairment of erythropoiesis. Tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin 1/6 (IL-1/6) induce H-ferritin, but inhibit TfR1 transcription [12,59,60]. Additionally, IFN-γ and lipopolysaccharide (LPS) can induce the expression of DMT-1 and reduce ferroportin in active monocytes, preventing iron from being used by foreign pathogens [12,59,60]. As such, anemia of chronic disease shows characteristic laboratory findings of low serum iron, low serum iron binding capacity, and increased serum ferrtin. In recent studies, hepcidin was identified as an alternative mechanism of iron regulation under inflammation [61]. IL-6 was shown to induce hepcidin transcription through JAK/STAT [62,63,64,65] and BMP/SMAD [21,23] pathways and attenuating these pathways reversed anemia of chronic disease, confirming the connection between hepcidin and iron metabolism under inflammatory conditions [23,66].

3.5. Iron response element (IRE) and iron response proteins (IRPs)

The 7Me-GTP cap dependent translation of transferrin, ferritin, and ferroportin is one of the well known mechanisms of iron metabolism. Eukaryotic translation initiation factor 4 gamma (eIF4G) is a translational activator which binds to 5′UTR of mRNA and it forms complex with eIF4E to activate 7Me-GTP dependent translation. eIF4E/eIF4G complex formation is antagonized by eukaryotic translation initiation factor 4E binding protein 1 (4EBP1). Alternatively, translation of mRNA also depends on 3 prime untranslated region (3′UTR). MicroRNAs and RNA binding proteins have been shown to interact with 3′UTR to regulate mRNA stability and subsequent protein expression [67,68]. The 5′UTR and 3′UTR regions in the mRNAs of transferrin, ferritin, and ferroportin contain IREs and regulates their translation by interacting with IRP1/2 [3]. Under iron deficiency, IRP binds to the hairpin structure of IRE on the 5′UTR of ferritin and ferroportin mRNAs, thereby prevents 7Me-GTP cap dependent translation of these proteins [69]. Conversely, high iron level inactivates IRP by inducing the assembly of cubane [4Fe–4S] cluster in IRP, preventing its binding to IRE hairpin structure [69]. Moreover, high iron level upregulates TfR1 expression by stabilizing mRNA through the interaction of IRP and IRE on the 3′UTR [69]. Collectively, iron regulates the translation of proteins involved in iron metabolism by direct interaction of IRPs and IRE on their mRNAs.

4. Molecular pathophysiology hemochromatosis

4.1. Primary (hereditary) hemochromatosis

Hemochromatosis is one of the most common benign hematologic disorders that results from iron overload. Both genetic and acquired disorders can cause hemochromatosis.

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Table 1
Classification of HH.

Type	Genes	Molecular mechanism	Chromosome	Inheritance	Onset	Severity	Response to phlebotomy
I	HFE [82]	MHCI like molecule Bind to β2 microglobulin and TfR1 to facilitate TfR1 uptake into cells from cell membrane	6p21.3	AR	40–50 yr	Mild	Good
IIA	HJV (HFE2) [36,84]	Co-receptor of BMP6 receptor Binds to BMP6 and activates SMAD/Hepcidin pathway	1q21	AR	20–30 yr	Severe	Good
IIB	HAMP (Hepcidin) [2]	Negatively regulate ferrofortin expression in the membrane	19q13.1	AR	20–30 yr	Severe	Good
III	TfR2 [37]	Form complex with HFE and ferritin	7q22	AR	40–50 yr	Intermediate	Good
IV	Ferrofortin (SLC40A1) [31]	Iron exporter in basolateral membrane of enterocyte and plasma membrane of reticuloendothelial cells	2q32	AD	40–50 yr	Mild	Susceptible to anemia

The typical presentation of HH includes diabetes, liver cirrhosis and bronze pigmentation of the skin which are the consequence of excessive iron accumulation in the peripheral organs [70,71], although these classical manifestations are rare nowadays due to early genetic screening. As explained earlier, new molecular findings of hepcidin pathway extended our understanding of pathogenesis in hereditary hemochomatosis, classifying the disease into 4 different categories (Table 1) [70,71].

Type 1 is the most common genetic disorder with a prevalence of 0.5% in northern European descendants [72,73]. It is inherited as an autosomal recessive pattern and associated with HFE C282Y and/or H63D gene mutations on chromosome 6 [72,73]. The prevalence of C282Y homozygote and C282Y/WT heterozygote are 1 in 150–300 and 1 in 10 in general population, respectively, and they are the most common mutations in HH [74–77]. Although 70% of patients with C282Y homozygote have elevated ferritin level, less than 10% of these patients present with phenotypes of hemochromatosis [74–77]. HFE is a major histocompatibility complex class I (MHC I) like protein lacking antigen presenting capability [72,78]. Previous studies suggested that HFE may interact with TfR1 to regulate iron import and export [79–81], and Waheed et al. demonstrated that binding of wild type HFE to TfR1 facilitates TfR1-mediated transferrin uptake into enterocytes (Fig. 3) [82]. C282Y mutation disrupts disulfide bond in HFE and prevents it from interacting with β-2-microglobulin that is critical for binding of HFE to TfR1 on the cell surface [83]. The detailed mechanism as to how the disruption of HFE and TfR1 interaction causes iron overload remains elusive although “crypt programming model” was suggested to explain constitutively activated iron absorption in enterocytes [70,71]. Recently, HFE was shown to directly sense the level of iron by forming HFE/TfR2/ferritin complex as previously described, and HFE mutation or deficiency resulted in hepcidin downregulation and subsequent iron overload [37].

Type 2 HH includes two different subtypes: type 2A with HJV mutation [36,84] and type 2B with hepcidin mutation [85]. The importance of these proteins in iron metabolism has been well demonstrated [86]. Since hepcidin diminishes iron release from enterocytes by degrading ferroportin [31], and HJV functions as a co-receptor for BMP6, mutations or deficiency of hepcidin and HJV result in excessive iron absorption through enterocytes to increase plasma iron concentration and iron overload in the peripheral organs [2,25].

Type 3 is TfR2 related HH. TfR2 is a homologous protein of TfR1 and its expression is limited in hepatocyte and duodenal crypt cells [87]. Although the binding affinity of TfR2 to transferrin is 30 fold lower than that of TfR1, TfR2 knockout mice were shown to develop hemochromatosis as well [88]. Additional familial case studies of HH proved their association with TfR2 mutation [88–90] and these findings suggested that TfR2 plays an independent role in iron metabolism. As discussed earlier, the role of TfR2 in hemochromatosis was recently resolved by one study that showed HFE/TfR2/ferritin complex can regulate hepcidin transcription [37], however, it is still unclear whether the signaling pathway works through BMP/SMAD dependent manner.

Type 4 indicates HH secondary to ferroportin mutation [71,91,92]. Since ferroportin is an iron exporter expressed on basolateral membrane of enterocyte and plasma membrane of macrophage (Fig. 3) [93], dysregulation of ferroportin leads to iron overload in these cells. As hepcidin directly interacts with ferroportin and facilitates its internalization and degradation via ubiquitination [31,32], mutations on lysine residues prevent ubiquitination and result in ferroportin retention on the plasma membrane, causing higher intracellular ferritin levels. In accord to these molecular findings, human genetic studies with HH patients proved compatible mutations in lysine residues of ferroportin [94–98]. Because ferroportin is mainly expressed on the plasma membrane of reticuloendothelial cells of liver and spleen rather than hepatocytes,

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Table 2
Diseases in secondary hemochromatosis.

Class	Disease	Mechanisms of iron overload	Treatment of iron overload
Hereditary	Thalassemia	Enteric iron absorption	Iron chelating agents
	Sickle cell anemia	Chronic transfusion	Phlebotomy no effect
	Diamond-Blackfan anemia		
	Pyruvate kinase deficiency		
	X-linked sideroblastic anemia		
Acquired	Myelodysplastic syndrome	Chronic transfusion	Iron chelating agents
	Myelofibrosis		Phlebotomy no effect
	Aplastic anemia		
	Acquired sideroblastic anemia		

this explains the milder phenotype of type 4 HH compared to other types [70]. Moreover, type 4 HH patients tend to have lower plasma iron and transferrin saturation due to iron accumulation in reticuloendothelial system. As such, anemic presentation in postmenarchal female or patients with aggressive phlebotomy are relatively a common finding in type 4 HH.

In summary, various mutations in genes involved in iron metabolism can cause HH. However, the severity of phenotype depends of types of genes involved and the role of proteins in iron metabolism. In general, patients with homozygous mutations of HAMP and HJV tend to present in early age with severe clinical phenotypes including organ damage, however, HFE and TfR2 related HH is associated with relatively later disease onset and milder symptoms (Table 1) [70].

4.2. Secondary hemochromatosis

Secondary hemochromatosis indicates iron metabolic disorder that is not from HH (Table 2) [99]. Dysfunctional erythropoiesis in many hereditary and acquired diseases including thalassemia major, myelodysplastic syndrome (MDS) and sideroblastic anemia enhances enteric iron absorption, causing positive iron balance. Moreover, these patients require periodic red blood cell transfusions that often result in a further disruption of iron balance. Ultimately, patients develop secondary hemochromatosis from iron overload upon chronic transfusion. The clinical manifestation of secondary hemochromatosis is similar to that of HH. Hepatomegaly, splenomegaly, cardiac disease, diabetes and liver cirrhosis are the most common clinical presentation although cirrhosis is rare in patients with MDS because their life expectancy is relatively shorter to develop long term complication from iron overload [99].

5. Clinical manifestation of hemochromatosis

Iron can accumulate in multiple organs including pituitary gland, parathyroid gland, heart, pancreas and liver. As such, hemochromatosis patients can present with various clinical manifestations such as arthralgia, ataxia, diabetes, impotence, heart failure, and liver cirrhosis although the onset of symptoms and clinical severity can vary depending on underlying genetic mutation, environmental and host factors (Table 1 and 3) [70]. Hepatocyte (except type 4 HH) is the major iron storage site. Therefore, liver is one of the most frequently affected organs in hemochromatosis. 60% and 10–90% of patients with HH present with abnormal liver function and liver cirrhosis at the time of diagnosis, respectively [100–104]. Iron deposits induce iron mediated oxidative damage via lipid peroxidation and subsequent mitochondrial dysfunction in the hepatocytes [105–107]. Moreover, intracellular byproducts released from damaged hepatocytes activate Kupffer cells to release cytokines and exacerbate the inflammatory process including hepatic fibrosis [105–107]. Since liver cirrhosis and subsequent hepatocellular carcinoma is one of the most serious complications of hemochromatosis with high mortality [108], hemochromatosis should always be considered as one of differential diagnosis in patients with symptoms described above for an early diagnosis and appropriate intervention to delay disease progression and increase survival [109].

Table 3
Affected systems and clinical manifestation in hemochromatosis.

Affected System	Clinical Manifestation
Neural system	Weakness
	Lethargy
	Ataxia
Cardiovascular system	Heart failure
	Adrenal insufficiency
Endocrine system	Diabetes
	Gynacomastia
	Testicular atrophy
	Impotence
Gastrointestinal system	Abdominal pain
	Liver cirrhosis
	Hepatocellular carcinoma
Musculoskeletal system	Arthralgia
Skin system	Skin pigmentation
	Loss of skin hair

6. Diagnosis of hemochromatosis

Early diagnosis of hemochromatosis is very important since non-treated hemochromatosis eventually results in

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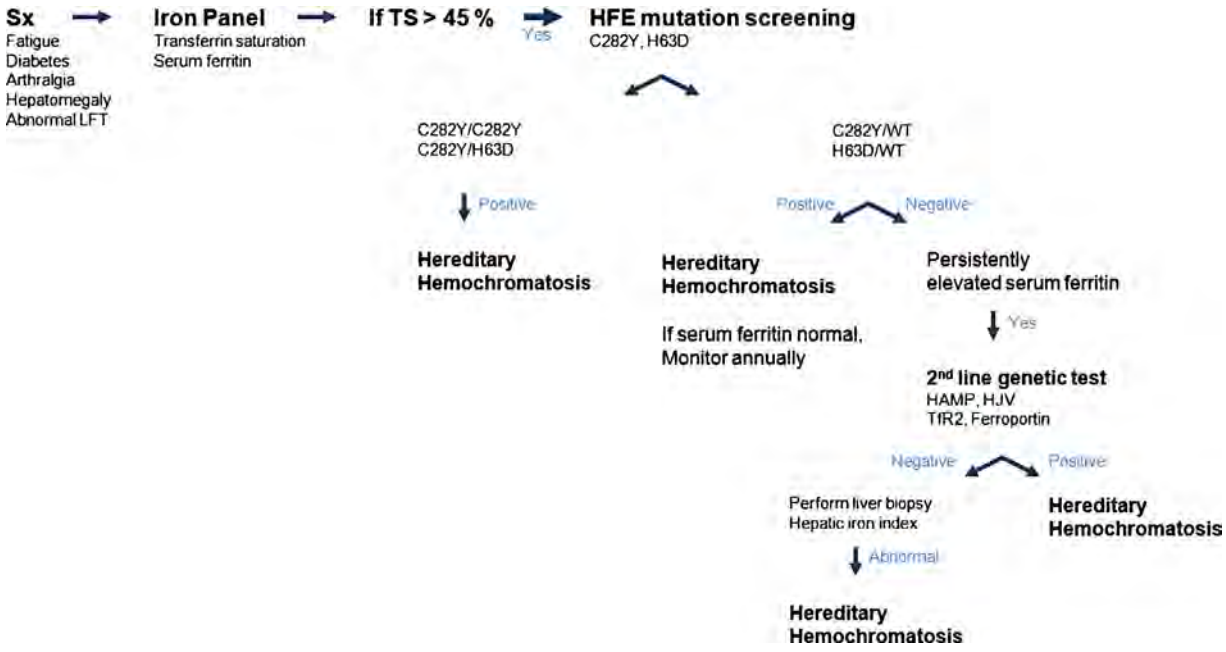

Fig. 4. **Diagnostic flow.** Genetic testing of HFE C282Y or H63D should be considered in patients with transferrin saturation higher than 45%. Second line genetic testing is appropriate for negative result for HFE mutation. When ferritin level is higher than 1000 μg/dL or in the presence of abnormal liver enzymes, liver biopsy should be performed to identify any liver disease and to evaluate the degree of fibrosis.

organ failure that contributes to the mortality [108]. The American association for the study of liver disease (AASLD) recommends iron study, especially ferritin and transferrin saturation (TS), as an initial screening test in patients with evidence of liver disease, suggestive symptoms, family history of hemochromatosis [109]. Also, HFE genetic screening is recommended in asymptomatic patients with transferrin saturation (TS) >45% (Fig. 4) [109]. The cutoff value of 45% in TS has been used as an initial screening because of its high sensitivity. Higher cutoff values were shown to have better specificity and positive predictive value, however, they were associated with lower sensitivity missing the diagnosis of HH of heterozygotic C282Y/WT or other genotypes with milder phenotypes [110].

Serum ferritin level provides additional power to rule in or out hemochromatosis since it reflects the degree of iron storage. Hemochromatosis and iron overload screening (HEIRS) study with 99,711 North American population demonstrated 88% and 57% of male and female C282Y homozygotic patients to have elevated serum ferritin (>300 μg/L in men and >200 μg/L in women) [74]. Moreover, 77% and 56% of male and female with elevated ferritin (>250 μg/L in men and >200 μg/L in women) were shown to have positive C282Y homozygotes in one study within a population in California [75]. Accordingly, combination of TS <45% and normal range of serum ferritin showed 97% of negative predictive values to exclude iron overload [111]. In non-HFE related or C282Y/H63D heterozygous hemochromatosis, ferritin provides valuable information since elevated ferritin levels can be the only abnormal laboratory finding without elevation of TS [112]. However, the level of ferritin has a significant limitation as a diagnostic criteria of hemochromatosis since it has high false positive rate and can be non-specifically elevated in inflammatory conditions including acute infection, chronic hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, and lymphoma [3]. As such, careful interpretation is mandatory when ferritin level is used as a surrogate value to rule in or out hemochromatosis.

In patients with TS higher than 45%, genetic testing of HFE C282Y or H63D should be considered. When HFE mutation is negative, second line genetic testing such as HJV, HAMP, TfR2 are warranted to rule out other types of HH. Once a patient is diagnosed with C282Y homozygote hemochromatosis and if serum ferritin level is lower than 1000 μg/dL, they need therapeutic phlebotomy with periodic ferritin or hemoglobin monitor only [109]. However, when ferritin level is higher than 1000 μg/dL at the time of diagnosis or in the presence of elevated liver enzymes, liver biopsy should be performed to identify any presence of liver disease and evaluate the degree of fibrosis (Fig. 4). Liver biopsy also can be performed in patients with heterozygote C282Y/H63D or other HFE non-related hemochromatosis to determine the severity of liver disease as well (Fig. 4).

7. Treatment of hemochromatosis

The logic behind the treatment of iron overload is to reduce iron mediated free radical generation and prevent tissue damage. However, most of the animal studies were performed with supraphysiologic iron concentration, making the mechanisms of iron induced cytotoxicity to be

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Table 4
Treatment options in hemochromatosis.

Treatment	Mechanism	Advantage	Disadvantage	Preference
Phlebotomy [114,116]	Hemoglobin removal	Effective Safe Low cost Long experience	Only effective in normal erythropoiesis Require clinic visit Temporary hypotension	Standard
Erythrocytapheresis [121]	Hemoglobin removal	Rapid Safe	Limited experience Needs specialized facility Temporary hypotension	Severe iron overload
Deferoxamine [123–126]	Chelation Urinary iron excretion	Safe Long experience in other iron overload diseases May reduce liver damage and have anti-cancer effect in hepatocellular carcinoma	High cost Infusion reaction Limited experience in hemochromatosis	Phlebotomy intolerance
Deferasirox [129]	Chelation Iron excretion via GI	Orally available Effective in hepatic iron chelation	Limited experience High cost Toxicity	Phlebotomy intolerance
Proton pump inhibitor [132]	Inhibits enteric absorption of non-heme iron	Safe Long experience Orally available	Insufficient evidence for the efficacy	Not recommended

inconclusive in physiologic condition. Also, no randomized controlled trials comparing treatment vs. non-treatment has been performed to prove the survival benefit of iron depletion therapies. However, initiation of treatment before developing liver cirrhosis or diabetes was shown to be associated with improved survival in HH in many retrospective studies [113,114]. Therefore, it is widely accepted that hemochromatosis patients with elevated serum ferritin need iron depleting treatment [115].

Phlebotomy is the most common and effective treatments to reduce iron overload (Table 4). The benefit from phlebotomy mainly results from stimulation of erythropoiesis and mobilization of iron from peripheral iron storing tissues to the bone marrow. The clinical benefits such as reversal of hepatic fibrosis, improvement in blood glucose control and reduction in hemochromatosis related symptoms have been shown in many studies [114,116]. Additionally, several studies showed that maintaining normal range of ferritin with periodic phlebotomy could increase survival by lowering cancer risk [113,117,118]. In patients with elevated serum ferritin, 500 mL of phlebotomy or smaller volume in patients with low body weight should be performed weekly until hemoglobin becomes less than 12 g/dL or serum ferritin lower than 200 μg/dL. Then the treatment schedule can be modified to every two weeks phlebotomy until serum ferritin level becomes less than 50 μg/dL (Fig. 5). One study demonstrated the negative correlation between serum ferritin level and enteric absorption of non-heme iron [119]. In this study, non-heme iron absorption was 42%, 12% and 9% in patients treated with full, partial and no phlebotomy, respectively. In other words, excessive phlebotomy enhances enteric non-heme iron absorption, rendering the benefit of phlebotomy to be compromised by unnecessary phlebotomy. As such, phlebotomy should be discontinued in patients who achieved serum ferritin lower than 50 μg/dL (Fig. 5). The change of TS is much slower than that of ferritin level and TS may remain elevated even with ferritin of normal range [120]. Consequently, using TS as an indicator of phlebotomy increases the risk of developing iron deficiency anemia. Some clinicians prefer hemoglobin as a treatment indicator. In this case, weekly phlebotomy can be performed until hemoglobin becomes lower than 12 g/dL and it can be stopped when hemoglobin level is lower than 11 g/dL for three weeks in a row [115]. Collectively, phlebotomy is one of the most efficient, safe and inexpensive therapeutic modalities in hemochromatosis although it can cause temporary hypotension and requires frequent visits to clinic.

Erythrocytapheresis is an alternative iron depletion treatment. It removes iron as hemoglobin bound form like phlebotomy [121]. Erythrocytapheresis has benefit by saving other components of blood including coagulation factors and plasma proteins. Moreover, larger amounts of red blood cell can be removed in shorter period of time [122]. As such, it is preferably used in patients with phlebotomy intolerance or severe iron overload requiring rapid iron depletion. However, our experience with erythrocytapheresis in hemochromtosis patients is relatively limited and it requires a special facility for the procedure, further limiting wide application (Table 4).

Iron chelating agents are alternative pharmacological options that can be used in phlebotomy intolerant patients. The efficacy and safety of deferoxamine, an intravenous agent, had been extensively studied in patients with transfusion induced iron overload (Table 5) [123–126], however the evidence in hemochromatosis is relatively limited. One study demonstrated deferoxamine is as effective as weekly phelebotomy of 500 mL in hemochromatosis patients, however, its practicability is limited due to long infusion time and adverse effects [127]. Deferasirox is an alternative orally available iron chelating agent, and its efficacy has been shown to be comparable to that of deferoxamine in

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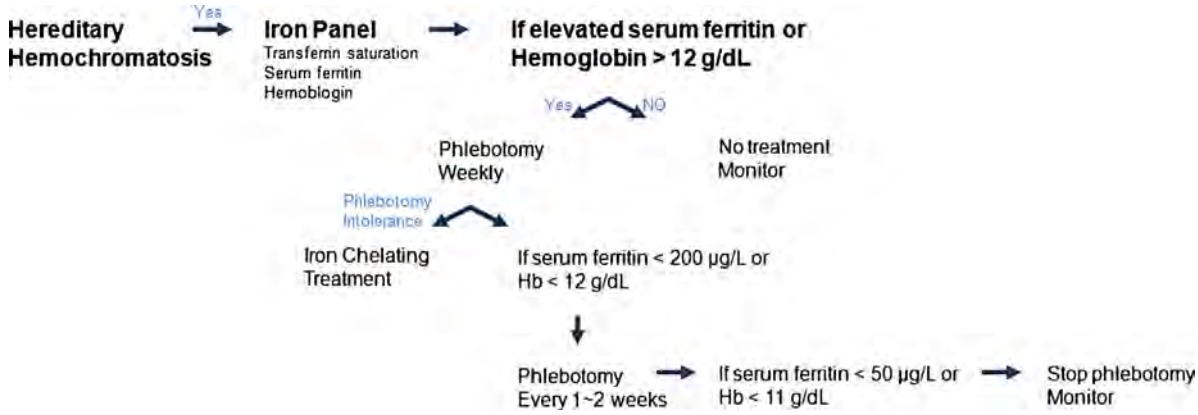
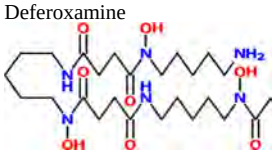
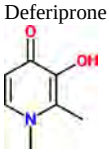
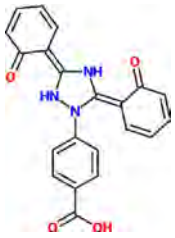


Fig. 5. **Treatment flow.** Weekly phlebotomy should be performed until hemoglobin becomes less than 12 g/dL or serum ferritin lower than 200 μg/dL. Then, treatment schedule can be modified to every two weeks phlebotomy and it should be stopped when hemoglobin becomes lower than 11 g/dL or serum ferritin level less than 50 μg/dL. For patients with phlebotomy intolerance, iron chelating agents can be an alternative option.

Table 5
Iron chelating agents.

Agent	Route	Chelating	Metabolism	Adverse effects	Indications
Deferoxamine 	Subcutaneous Intravenous	1:1	RenalBiliary	Infusion reaction Allergic reaction Visual disturbance Auditory disturbance Zinc deficiency Bone growth abnormality	Thalassemia Sickle cell anemia Diamond-Blackfan anemia Pyruvate kinase deficiency MDS
Deferiprone 	Oral	3:1	Renal	GI symptoms Liver enzyme elevation Neutropenia Arthralgia	Thalassemia major with deferoxamine intolerance
Deferasirox 	Oral	2:1	Biliary	GI symptoms Skin rash Serum creatinine elevation Visual disturbance Auditory disturbance Liver toxicity Marrow toxicity	Thalassemia major Other types of anemia with deferoxamine intolerance

randomized phase III clinical trial with thalassemia patients (Table 5) [128]. In accord to this result, phase I/II clinical trial of deferasirox in patients with HFE C282Y homozygotes demonstrated promising results [129]. Additional *in vivo* studies showed that iron chelating agent can prevent liver damage and development of pre-neoplastic hepatic lesions [130,131], implicating that randomized clinical trials to compare the long term survival of iron chelating agents vs. conventional phlebotomy may be interesting.

Proton pump inhibitors were shown to inhibit gastric acid secretion, thereby diminish gastric absorption of non-heme iron [132]. Also, iron transport via DMT-1 requires proton co-transport [133]. As such, proton pump inhibitors were assumed to be effective in iron overload, however, it is not on the list of current recommendations due to insufficient clinical data to prove its efficacy in hemochromatosis patients.

Phlebotomy is not indicated in patients with chronic transfusion induced secondary hemochromatosis. Since the main effect of phlebotomy depends on redistribution of iron from peripheral storage sites to bone marrow where erythropoiesis occurs, phlebotomy has no effect in diseases with impaired erythropoiesis. Moreover, these patients have underlying anemia which may be exacerbated upon phlebotomy. Accordingly, iron chelating agents are treatment of choice in secondary hemochromatosis [99].

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8. Conclusion

Iron is one of the pivotal components of erythropoiesis and the molecular mechanisms of its metabolism have been extensively investigated over the past decades. Hepcidin was identified as a key protein to orchestrate intra- and extracellular signals and directly regulate ferroportin expression. Additional pathways including JAK/STAT3, BMP/HJV/SMAD, and VHL/HIF/EPO were also shown to play an important role as upstream or downstream pathways of hepcidin. These findings resolve the mysterious puzzles of mutations that had been observed in many types of HH. Despite the successful establishment of the hepcidin centered model, many questions still remained to be answered. In a recent study, ERFE was identified as a mediator that connect EPO signal to hepcidin. Increased EPO upon hemorrhage or phlebotomy stimulates erythroblast to induce ERFE mRNA expression. However, the mechanism that ERFE signal is transferred to JAK/STAT pathway as well as the identity of ERFE receptor remain elusive. In view of the inflammatory response, hepcidin was also shown to facilitate iron storage in response to cytokines. These findings suggest an important role of hepcidin in anemia of chronic disease. Accordingly, BMP6, HJV, SMAD or hepcidin can be a reasonable target for the treatment of anemia chronic disease and future studies with small inhibitors may revolutionize our approach to anemia of chronic disease. In an effort to establish the detailed mechanism, HFE/TfR2/ferritin complex was shown to regulate hepcidin transcription, suggesting an independent role of HFE. However, it is not yet tested if the signal from this complex is relayed to ferroportin in BMP6 dependent manner. Lastly, phlebotomy has been the standard treatment for iron overload in hemochromatosis. Though, several studies proved the efficacy of iron chelating agents as an alternative iron depletion modality in secondary hemochromatosis. Randomized clinical trials comparing the long-term outcome of iron chelating agents vs. phlebotomy are needed in order to establish better care standards and thus ultimately contribute to the quality of life in hemochromatosis patients.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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