

# Iron metabolism and related genetic diseases: A cleared land, keeping mysteries

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## Summary

Body iron has a very close relationship with the liver. Physiologically, the liver synthesizes transferrin, in charge of blood iron transport; ceruloplasmin, acting through its ferroxidase activity; and hepcidin, the master regulator of systemic iron. It also stores iron inside ferritin and serves as an iron reservoir, both protecting the cell from free iron toxicity and ensuring iron delivery to the body whenever needed. The liver is first in line for receiving iron from the gut and the spleen, and is, therefore, highly exposed to iron overload when plasma iron is in excess, especially through its high affinity for plasma non-transferrin bound iron. The liver is strongly involved when iron excess is related either to hepcidin deficiency, as in HFE, hemojuvelin, hepcidin, and transferrin receptor 2 related haemochromatosis, or to hepcidin resistance, as in type B ferroportin disease. It is less involved in the usual (type A) form of ferroportin disease which targets primarily the macrophagic system. Hereditary aceruloplasminemia raises important pathophysiological issues in light of its peculiar organ iron distribution.

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## Introduction

Iron is the best and the worst thing for the human body. Iron is deeply involved in a number of critical metabolic processes, a lack of this metal impairs body functioning, especially in the haematological domain. Conversely, excessive body iron is the source of multiple cellular and visceral damage. These two

“mirror” hazards explain why iron homeostasis is a crucial need for the body. For this physiological purpose, a myriad of metabolic actors, particularly proteins, are involved in iron metabolism. Structural and/or functional disturbances of these actors, of acquired or genetic origin, may cause severe diseases relating to either an iron deficiency or an iron overload. The liver plays a key role in iron homeostasis, not only as the source of major protein actors, among which transferrin, ceruloplasmin, and mostly hepcidin, but also as the main iron storage organ and a preferential target of iron overload toxicity [1]. Although the iron domain has benefited from major advances, a number of issues remain to be solved.

### Key points

- The liver produces most proteins of systemic iron metabolism: transferrin (plasma iron transport), ceruloplasmin (plasma iron delivery), haptoglobin (linkage with haemoglobin), hemopexin (linkage with free heme), and hepcidin, the master regulator of iron homeostasis.
- The liver is a major iron storage organ, concerned mostly by parenchymal (hepatocytic) but also by macrophagic (Kupffer cell) iron deposition.
- Non-transferrin bound iron (NTBI) is avidly taken up by hepatocytes and is toxic through its reactive form (labile plasma iron-LPI).
- The liver accumulates iron and undergoes its toxicity mainly in hepcidin deficiency-related haemochromatosis (types 1, 2, and 3 haemochromatosis).
- The liver is less impacted by iron overload in the usual form of the ferroportin disease (type 4-A haemochromatosis).
- The mechanisms whereby hepatocytic iron deposition occurs in hereditary aceruloplasminemia are not fully elucidated.
- Hyperferritinemia is the usual diagnostic call sign for iron overload, and its interpretation requires a rigorous approach.

**Keywords:** Iron; Transferrin; Ferritin; Hepcidin; Ceruloplasmin; Ferroportin; Erythroferrone; Non-transferrin bound iron; Hemojuvelin; Transferrin receptor; Haemochromatosis; Liver.

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## Review

### Iron metabolism: “The ten iron laws”

Iron homeostasis is governed by inescapable laws. A failure to follow these rules, especially due to inborn errors, favors the development of iron metabolism diseases [2–4].

#### *Iron is not dispensable*

Total body iron load normally approximates three to four grams. Two-thirds of this iron quantity are contained in red blood cells, within the haemoglobin molecules. Iron (Fe) is part of the porphyrin ring of the heme molecule, and has a major ability for linking oxygen [5]. Erythrocytic iron circulates in the plasma and delivers oxygen to all cells, while being itself delivered to the bone marrow in order to contribute to the daily production of approximately 200 billion of new red blood cells [6]. Therefore, iron plays a major role in the respiratory process, and without iron, the human body could not breathe. This is as truer as iron is also involved at the cellular and molecular levels, in the respiratory chain which serves to the generation of energy through ATP production. The muscle, through iron incorporation inside myoglobin, has a special place in this energy process. Iron is also involved in multiple enzyme activities catalysing metabolic processes such as xenobiotics biotransformation, lipid metabolism, collagen production, or DNA synthesis.

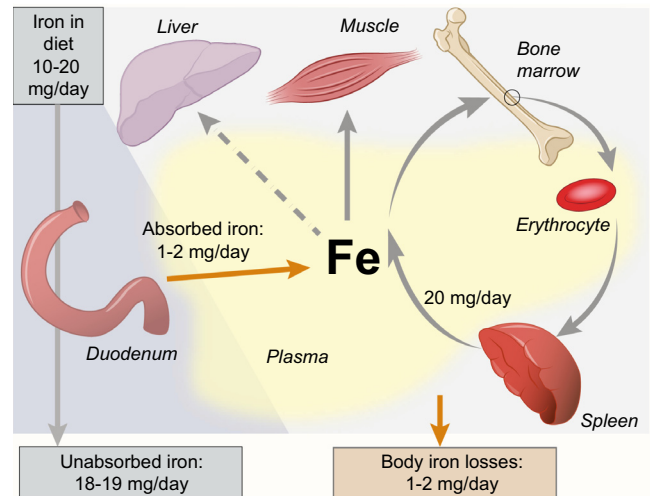
#### *Iron is not produced by the body which is therefore exposed to iron deficiency*

The only iron source is alimentary. A normal diet provides 10–20 mg per day, of which only one tenth (1–2 mg) is absorbed [4]. Within the digestive tract, iron exists under two forms: heme iron (meat, fish) and non-heme iron (cocoa, cereals with the highest content in lentils). As to spinach, its iron content is far from initially (erroneously) reported (the “Popeyes’ syndrome”...), but remains significant since it is close to that of meat. Iron is absorbed at the duodenal level and this absorption process is approximately five times more efficient for heme iron than for non-heme iron.

Chronic lack of dietary iron unavoidably leads to iron deficiency. Two main situations are concerned. If digestive absorption is normal, deficient alimentary input is either “absolute” (malnutrition) or “relative” (increased physiological iron needs, especially during infancy, adolescence, pregnancy, and lactation). The second mechanism is defective iron absorption. It may be due to alimentary co-factors which are capable of decreasing iron absorption (for example, tannins contained especially in tea and at a lesser degree in coffee, or phytates contained in seeds, legumes, and nuts) or to increase it (vitamin C [7]). These co-factors interfere preferentially with non-heme iron absorption. Beside the role of co-factors, defective iron absorption may be related to damage of the absorption process itself (corresponding to malabsorption, such as occurring in coeliac disease [8]).

#### *The fate of iron after intestinal absorption is mainly the erythrocyte (Fig. 1)*

Once iron has crossed the digestive barrier, at the duodenal level, it reaches the blood, is linked to its carrier protein transferrin, and



**Fig. 1. Iron homeostasis.** Plasma iron comes from duodenal absorption and from the spleen (iron recycling following erythrophagocytosis).

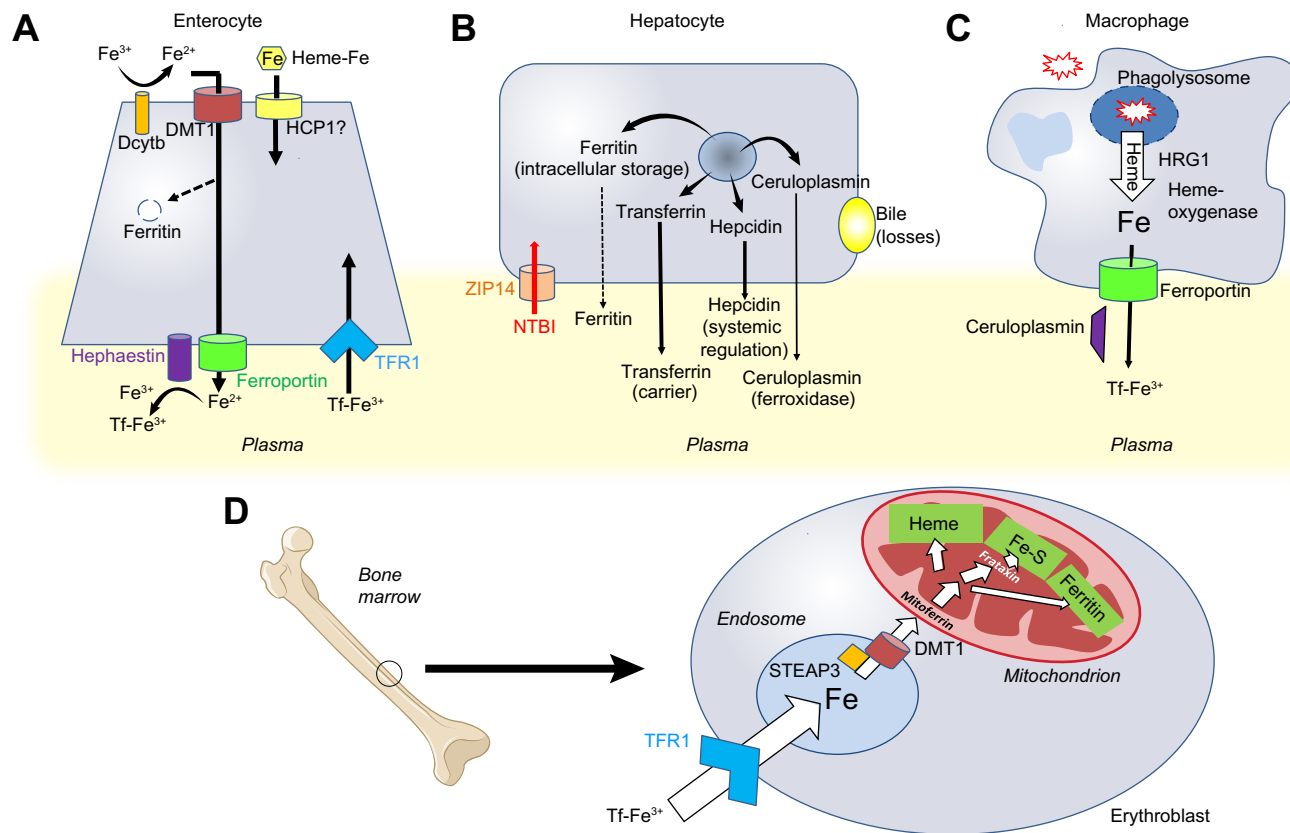
is predominantly (up to 80%) directed toward the bone marrow. It enters the erythroblasts via transferrin receptor 1 and undergoes the classical transferrin iron cycle. The remnant part (20%) goes into the various extramedullary cells in order to participate in many metabolic processes (respiration, xenobiotics biotransformation, DNA synthesis).

#### *Iron cannot circulate within the body or be stored in a free form*

Being a metal, iron is neither soluble in the plasma nor in the cytosol. Therefore, it must be linked to other molecules in order to avoid toxicity due to the ability of iron to generate reactive oxygen species (ROS).

In the blood, plasma iron is physiologically taken up by transferrin, with a normal linkage ratio between the theoretical capacity of iron binding to transferrin (2 iron atoms per transferrin molecule) and plasma iron concentration of less than 45% (transferrin saturation [TS]). Whenever TS increases over 45%, new circulating iron species can appear, named non-transferrin bound iron (NTBI) [9]. NTBI has a very special kinetics. In contrast with transferrin iron, it targets preferentially – and with very high affinity – the parenchymal cells, especially the hepatocytes [10,11]. NTBI uptake by the hepatocytes involves mostly solute carrier SLC39A14 (ZIP14) [12,13]. This NTBI is not a “free” iron but is likely linked to low molecular weight ligands (citrate, acetate) or to carboxylic groups of albumin [14]. When TS exceeds 75%, a peculiar NTBI form, called labile plasma iron (LPI) or reactive plasma iron, defined by its capacity for producing ROS, may appear. It corresponds to a potentially toxic form of circulating iron [15–19]. Iron can also be transported by indirect systems, such as haptoglobin, binding haemoglobin, and hemopexin, binding free heme (coming from intravascular hemolysis).

In the cytosol, iron is essentially stored inside the ferritin molecules. Each ferritin molecule may store up to 4500 iron atoms. Ferritin acts as an iron “sponge”, storing the metal in case



**Fig. 2. Main cell types involved in iron metabolism.** (A) Enterocyte. Fe, iron; Dcytb, duodenal cytochrome; DMT1, divalent metal transporter1; HCP1, Heme carrier protein 1; TFR1, transferrin receptor 1. (B) Hepatocyte. NTBI, non-transferrin bound iron; ZIP14 (SLC39A14 – solute carrier 39A14). (C) Macrophage. Senescent erythrocyte (in red); Fe, iron; HRG1, heme-responsive gene protein 1. (D) Erythroblast. Fe-S, iron-sulfur clusters; DMT1, divalent metal transporter 1; STEAP3, six-transmembrane epithelial antigen of the prostate.

of excessive influx for avoiding cellular iron toxicity, and releasing iron in case of body iron deficiency to avoid anemia.

*The iron redox state maintenance is functionally critical*

The iron property to exist under two redox forms, the oxidized one; ferric iron ( $Fe^{3+}$ ) and the reduced one; ferrous iron ( $Fe^{2+}$ ) is of major functional importance at four main levels (Fig. 2).

*Transmembrane iron transport*

Whatever the cell (enterocyte, hepatocyte, macrophage), iron crosses its plasma and intracellular membranes under the ferrous form ( $Fe^{2+}$ ). This explains the importance of iron reducing proteins. The main ferro-reductases are DCYTB (duodenal cytochrome B) [20], which reduces alimentary non-heme iron to permit its luminal entry into the enterocyte through DMT1 (divalent metal transporter 1) [20], and STEAP3 (six-transmembrane epithelial antigen of the prostate 3) [21]. STEAP3 reduces intra-endosomal iron thus permitting, through DMT1 expressed on the endosomal membrane, its cytosolic delivery for cellular metabolism or storage.

*Plasma iron delivery and transport*

Iron is carried by transferrin under the ferric form. Since it is released from the cells in the ferrous form, ferroxidases are nec-

essary for enabling ferrous iron to be taken up by transferrin. This role is ensured by multicopper oxidase proteins: ceruloplasmin [22] for macrophages, and hephaestin [23] for enterocytes.

*Cellular iron storage*

Iron is stored within ferritin [24] under its ferric form ( $Fe^{3+}$ ) and needs reduction to be released. Ferritin is formed by 24 subunits of two forms (L and H, encoded by two different genes). The H form possesses a ferroxidase activity permitting iron internalization. Vitamin C, when acting as a reducing agent, can favor iron delivery from ferritin [25].

*Iron toxicity [26]*

The transition from ferric to ferrous iron, through the Fenton reaction, generates the production of ROS which can damage cellular membranes and nuclei. This mechanism is recognized as the main cause of cellular and organ damage in iron overload.

*The iron body has limited excretory capacities and is therefore exposed to iron overload*

The main exit pathways of iron are represented by intestinal exfoliation, skin desquamation, sweat, urine, bile and, in women, menstruations. Although biliary iron excretion may undergo some adaptation to body iron load [27], it is globally admitted

Review

## Review

that the ability of the human body to regulate its iron stores through iron excretion is very limited.

As a consequence of its poor excretory capacity, the human body is prone to iron overload, whatever the iron source. These sources can be either enteral as in hepcidin-deficient related haemochromatosis [28] or dyserythropoiesis [29], or parenteral as caused by uncontrolled iron injections (for iron deficiency anaemia [30]) or repeated transfusions [31] (for haemoglobinopathies or myelodysplastic syndromes).

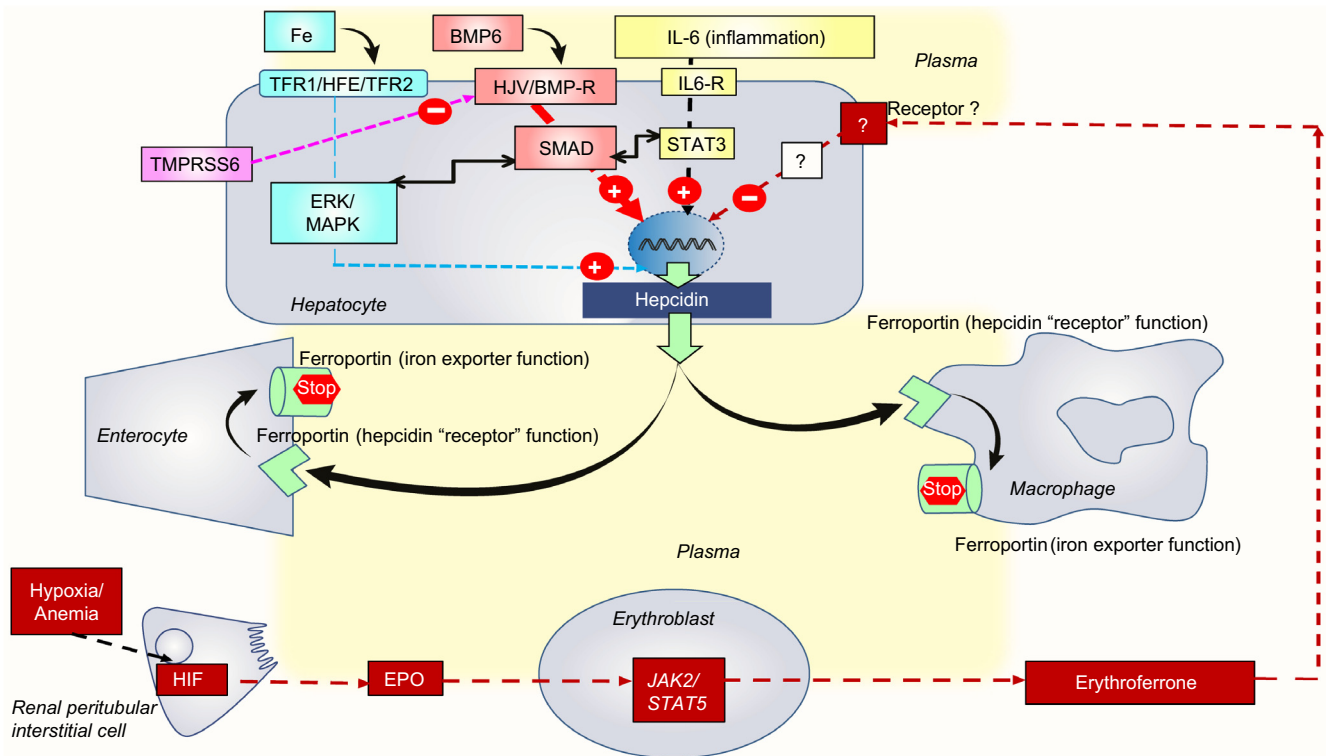
### Iron recycling is a crucial permanent process

Since the daily quantity of iron entering and leaving the body is minimal (1–2 mg), as compared to the daily body iron needs (of the order of 20 mg), an intense and constant recycling process, involving a bone marrow-spleen-bone marrow “virtuous” circle, occurs to ensure plasma iron sufficiency. It is estimated that one billion iron atoms are required daily for producing the hemoglobin of new red blood cells [32]. The “ecological attitude”, and therefore energy preservation, are hallmarks of iron metabolism.

### Systemic iron homeostasis necessitates a finely tuned regulation: the hepcidin-ferroportin duo (Fig. 3)

The master regulator of iron metabolism is the protein hepcidin [33–36] which acts in close connection with ferroportin.

Hepcidin is mainly produced by the liver (hepatocytes). This hormone is a small peptide whose mature and active part consists of 25 aminoacids. Body iron load is a main regulator of hepcidin synthesis [37]. Physiologically, iron homeostasis functions as follows: when plasma or hepatocyte iron concentration increases, there is an activation of signalling pathways, including the extracellular signal regulating kinase (ERK)/mitogen activated protein kinase (MAPK) pathway, and the bone morphogenetic proteins (BMP)/son of mothers against decapentaplegic homologues (SMAD) pathway, respectively. There are likely crosstalks between these two pathways [38–40]. As to HFE, which may be primarily concerned by the ERK–MAPK pathway, it has been reported to interact with the BMP type 1 receptor ALK3 to regulate hepcidin expression [41]. It is likely that these two types of signals (plasma iron and hepatocytic iron) correspond to differential chronological reactivity. Thus, the regulation initiated by plasma TS levels would act within a few hours vs. several days for hepatocyte iron excess [42–44]. Whatever its cause, increased signalling pathway activation induces hepcidin mRNA expression, leading in turn to increased plasma hepcidin concentration which has a double consequence: on the one hand, a decreased duodenal iron absorption, and on the other hand a decreased release from the spleen of the iron coming from the normal red blood cell degradation (erythrophagocytosis). The overall result is a decrease of plasma iron concentration aiming to counteract the initial plasma and/or cellular iron



**Fig. 3. Systemic iron regulation.** Hepcidin decreases the iron entry into the plasma at the duodenal and splenic levels through its impact on ferroportin (the only known cellular iron exporter). Increased (plasma or hepatocytic) iron load stimulates hepcidin production (TRF2, transferrin receptor 2; ERK/MAPK, extracellular signal regulating kinase/mitogen activated protein kinase; HJV (or HFE2), hemojuvelin; BMP6, bone morphogenetic protein 6 (induced by intracellular iron); BMP-R, BMP receptor; SMAD, son of mothers against decapentaplegic homologues). Inflammation is also a positive hepcidin regulator (IL-6, interleukin-6; STAT3, signal transducer and activator of transcription 3). Hypoxia and/or anemia decrease hepcidin production through erythroferrone, produced by the erythroblasts in response to EPO (erythropoietin) synthesis by the kidney. TMRSS6 (transmembrane serine protease S6) is also a negative hepcidin regulator (HIF, hypoxia inducible factor; JAK2, Janus kinase 2).

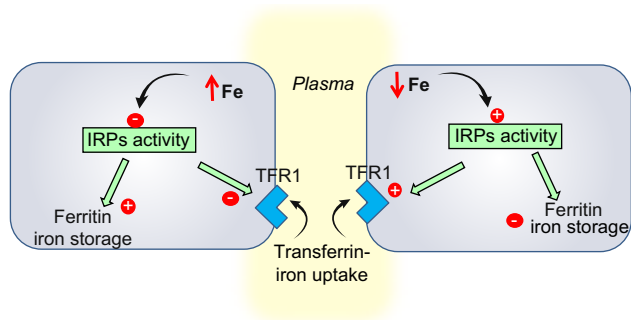
increase. A mirror situation occurs in case of decreased plasma and/or cellular iron.

For exerting its biological effect, hepcidin interacts with ferroportin which is mainly localised in the cell membrane of enterocytes and macrophages. This results in hepcidin internalization followed by lysosomal degradation of ferroportin [45,46]. Ferroportin, besides acting as a hepcidin receptor, is the only known cellular iron exporter, so that the final biological consequence is a decreased iron delivery into the plasma. Hepcidin also interferes with intestinal iron absorption by downregulating the expression of DMT1, which is involved in non-heme iron uptake at the apex of the enterocyte [47].

It should be noted that, beside iron load, several factors are able to regulate hepcidin synthesis. One major mechanism is represented by inflammation, which stimulates hepcidin production through the interleukin-6 (IL-6) [48] (and IL-22 [49])-STAT3 (signal transducer and activator of transcription) signalling pathway, and through activin B which likely implicates the BMP-SMAD pathway [50]. The other key mechanism is dyserythropoiesis which decreases hepcidin synthesis via the action of the bone marrow hormone erythropoietin (ERFE) [51,52]. A further mechanism is hepatocellular failure since hepcidin is synthesized by the hepatocytes [53,54].

*Local intracellular iron regulation completes systemic iron regulation to ensure body iron homeostasis (Fig. 4)*

A local regulation exists as a complement of this hepcidin-driven systemic regulation. It involves the iron responsive element (IRE)-iron regulatory protein (IRP) 1 and 2 system [55,56]. In case of decreased cellular iron, an IRP-1 conformational change and an IRP-2 level modulation occur which enhance physical interaction of IRPs with the IRE nucleotidic sequence located at the 5' non-coding region of L-ferritin mRNA. This, in turn, inhibits ferritin translation. Simultaneously, at the 3' extremity of transferrin receptor 1 mRNA, IRP hyperfixation on IREs inhibits transferrin degradation. These two combined mechanisms result in decreased iron storage capacity (decreased ferritin synthesis) and increased iron entry capacity (increased transferrin receptor 1 expression), a "logical" process for counteracting the initial cellular iron decrease in cells. The reverse phenomenon occurs in case of increased cellular iron concentration.



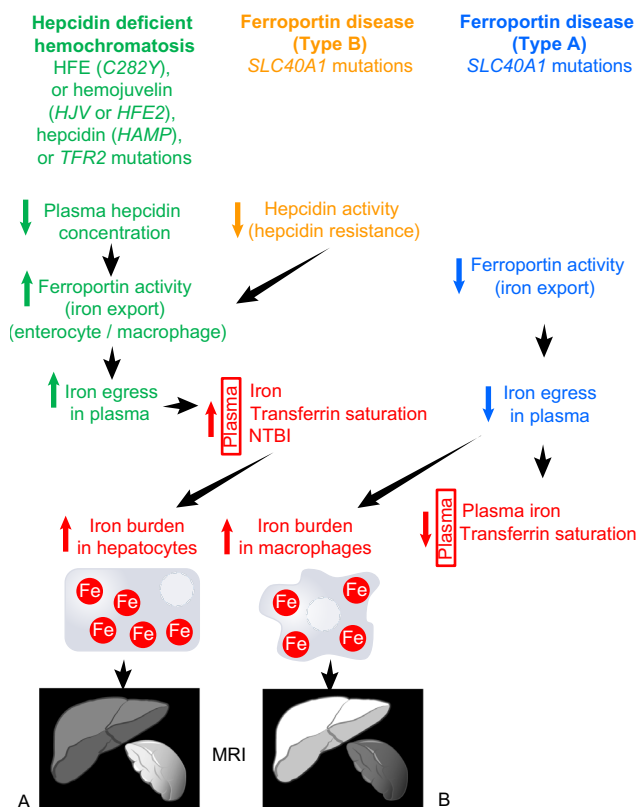
**Fig. 4. Local (cellular) iron regulation.** Decreased cellular iron content activates iron responsive element (IRE) fixation on iron regulatory protein (IRP), leading to decreased ferritin synthesis and to increased transferrin receptor 1 (TFR1) expression. Inverse situation in case of increased cellular iron content.

*Major advances in iron metabolism understanding does not mean complete knowledge*

A number of issues remain to be solved. Among them: (i) the mechanism whereby heme iron is taken up by the enterocyte, the precise role of the candidate protein heme carrier protein 1 (HCP1) remaining to be identified [57,58]; (ii) the biochemical nature of NTBI [59]; (iii) the way iron circulates within the cytosol with the possible role of chaperone molecules such as Polyr<sup>®</sup>-Binding Protein1 (PCBP) [60–62]; (iv) the factors determining transferrin gene expression [63]; (v) the interactions between immunity and iron metabolism [64]; (vi) the mechanisms underlying the metabolic connections between iron and non-iron metals [35]; (vii) the mechanisms which drive cellular ferritin delivery into the plasma [65]; (viii) the precise mechanisms by which erythropoietin is acting; and (ix) the mechanisms accounting for "brain protection" in most situations of systemic iron overload.

**Iron-related genetic diseases**

The maintenance of iron homeostasis requiring multiple actors and regulators, iron metabolism can be impacted by mutations



**Fig. 5. Mechanisms of iron overload in haemochromatosis.** (A) Hepcidin deficiency- and hepcidin resistance-related haemochromatosis: increased plasma iron generates NTBI (non-transferrin bound iron) which is quickly taken up by the parenchymal cells (here: one hepatocyte); (B) Ferroportin disease: impairment of the iron exporter ferroportin at the macrophagic level causes cellular iron retention (here: one macrophage) together with low plasma iron levels. MRI: magnetic resonance imaging (white: no iron overload; light grey: moderate iron overload; dark grey: heavy iron overload).

Review

## Review

occurring in a large number of genes. Many of them have been identified and contribute to most iron-related genetic diseases resulting in iron overload or iron deficiency. However, the phenotypic expression variability despite similar mutations in the same gene, the discrepancies sometimes observed between phenotypic expression of a disease and the theoretical impact of the involved mutation in the considered gene, together with the existence of unexplained iron overload phenotypes, do suggest that yet unrecognized elements remain to be identified.

### Genetic iron overload disorders

Iron excess can be found at the systemic level or involve only specific cellular structures.

#### Diseases with total body iron overload:

##### Haemochromatoses

Two main types of haemochromatoses (HC) should be considered [66,67] (Fig. 5).

##### Hepcidin deficiency related HC

- Hepcidin deficiency is the common denominator and is responsible for organ iron excess through increased cellular iron entry. The involved iron species is NTBI which, as previously mentioned, occurs in the plasma following increased transferrin saturation, which is itself due to elevated serum iron concentration. This may correspond to quantitative hepcidin deficiency or to hepcidin resistance. Quantitative hepcidin deficiency is by far the most frequent situation. In this setting, decreased hepatic synthesis is responsible for chronic hypo-hepcidinemia. The related diseases are: (i) primarily, HFE-related HC. It is due, most often, to homozygote mutation in the *HFE* gene (located on chromosome 6) *p.Cys282Tyr/pCys282Tyr (C282Y/C282Y)* and corresponds to type 1 HC; some rare HFE mutations in association with *C282Y* (compound heterozygosity) may give a similar phenotypic profile; (ii) much more rarely, non-HFE-related HC are involved. They are related to mutations in genes also coding proteins involved in hepcidin expression induction such as hemojuvelin (*HJV*) or transferrin receptor 2 (*TFR2*)-related HC (type 3 HC) (chromosome 7), or in hepcidin gene (*HAMP*) leading to decreased hepcidin production and/or activity. Mutations in hemojuvelin or hepcidin genes, which concern chromosomes 1 and 19 respectively, induce juvenile HC (types 2A and 2B HC). Hepcidin resistance corresponds to a refractory state of the cells to circulating hepcidin. This resistance state is related to ferroportin (*SLC40A1*: solute carrier family 40, member 1) mutations altering the "hepcidin receptor" function of ferroportin [68–71]. The corresponding disorder, which involves chromosome 2, is sometimes referred to as ferroportin disease type B (type 4B HC), but should rather be named "hepcidin resistance-related HC".
- All HC forms related to quantitative hepcidin deficiency correspond to endocrine disorders [72] involving the liver as source and/or target [73].
- The phenotype of these various HC forms shares numerous features which can be grouped under the concept of "hepcidin deficiency syndrome": (i) increased serum iron concentration and TS; (ii) iron deposition within the parenchymal cells (mostly hepatocytes, but also pancreatic, pituitary, and cardiac cells), contrasting with the lack of iron in the macro-

phages (Kupffer cells, splenic macrophages). This means on liver biopsy performed at early stages, exclusive hepatocytic iron deposition with Perls staining, and on magnetic resonance imaging (MRI) diffuse hepatic iron excess without splenic iron (aspect of "black liver and white spleen") [74–77]; (iii) serum ferritin is well correlated to liver and body iron overload, and is therefore a valuable parameter for the indication of venesection therapy (>300 µg/L in men and >200 µg/L in women), for following its efficacy (on a monthly basis) and for reaching and maintaining the desaturation target (50 µg/L) [78]; (iv) chronic iron overload progressively damages the hepatocytes and is responsible for moderate cytolysis (serum transaminases less than 3 times the upper normal limit), hepatomegaly, and progressive fibrosis (especially in cases of co-factors such as alcoholism or fatty liver), leading to cirrhosis with the risk of hepatocellular carcinoma. This risk persists despite total iron removal if the treatment was initiated while cirrhosis was already present; (v) a further feature of this hepcidin-deficiency syndrome is the strong efficiency of bloodletting therapy [79] due to the effectiveness of phlebotomies for enhancing iron recycling which is needed to ensure post-venesection induced erythropoiesis.

- Family studies follow the rules of a recessive disease and is mainly based on *C282Y* testing (together with plasma TS and ferritin) in major siblings. However, the high mutation prevalence (of the order of 1/10 in the Caucasian population) justifies to check also the major offspring [80].
- Some important differential aspects exist between hepcidin-related HC forms: (i) type 1 HC is only present in Caucasian populations; (ii) type 1 HC has a low penetrance [81] and a major issue is to identify the factors which modulate phenotypic expression, both in terms of iron excess and organ damage. Alcoholism is an acquired factor which may both accentuate liver fibrosis [82] and favor iron overload possibly through an hepcidin-decreasing effect. Overweight which attenuates disease expression in women possibly through increased hepcidin production [83]. Genetic factors are increasingly identified, including the roles of digenism, specific mutations [84] or various polymorphisms [85]. Nevertheless, much remains to be discovered to fully explain the basis of disease expression [86–88]; (iii) type 2 HC (or juvenile HC) correspond to severe disorders with predominant heart, pituitary and liver damage and their treatment may require, besides venesections, the use of chelation therapy.
- In the future, apart from the hepcidin resistance syndrome, these hepcidin-related HC will benefit from innovative therapeutic approaches, based on the underlying pathophysiology, and aiming to increase hepcidin by using mini or complete hepcidins, hepcidin agonists or by modulating actors of the BMP-SMAD pathway which could stimulate hepcidin synthesis. Another way could be to favor ferroportin internalization and/or degradation [72,89].

##### The ferroportin disease [90–92]

This term should be reserved for the usual form of genetic iron overload related to ferroportin mutations (*SLC40A1*) and preferred to the designation "type 4A HC". Those mutations, by altering the iron export property of the protein, cause iron overload by an intracellular retention mechanism. The ferroportin-related HC phenotypic profile is almost point by point opposed to that of hepcidin-related HC: (i) serum iron and TS are not elevated

(and sometimes decreased); (ii) iron deposition occurs essentially in the macrophages, due to the decrease of iron export activity related to ferroportin dysfunction in those cells, so that, on liver biopsy, iron predominates in the Kupffer cells, and, on MRI, iron overload prevails in the spleen as compared to the liver (“black spleen and grey liver”); (iii) serum ferritin, probably due its prevailing macrophagic origin in this setting, is usually much higher than in hepcidin-related HC and has not the same predictive value of total body iron load. This should lead to special attention in the use of this parameter both for diagnostic and therapeutic purposes; (iv) there is limited damaging effect of this macrophagic iron, making this disease a relatively benign one; (iv) blood-letting may be moderately tolerated with the risk of anemia due to poor recycling capacity.

As to family studies, they should follow the rules applied to a disease with a dominant mode of transmission.

*The hereditary aceruloplasminemia (HAC) case*

- This rare recessive iron overload disease is due to mutations within the ceruloplasmin (CP) gene (chromosome 3) [93,94]. The disease phenotype of the disease is a mixed one. On the one hand, it shares a major feature of hepcidin-related HC that is hepatocytic iron deposition without macrophagic iron overload (MRI shows a picture of “black liver and white spleen” on the T2 sequence) [95–99]; on the other hand, serum iron and TS are extremely low with a frequent profile of iron-deficient anemia, suggesting intracellular iron retention, similar to anemia of chronic diseases. Moreover, iron deposition in the central nervous system, namely beyond the blood brain barrier, is very peculiar to the disease.
- The classical mechanistic explanation for the development of iron overload is not fully satisfactory. Indeed, it is frequently advocated that the impairment of the ceruloplasmin-related ferroxidase activity prevents ferrous iron from being oxidized in order to be taken up by plasma transferrin in plasma. This could favor a disturbance in the export activity of ferroportin causing intracellular iron retention and decreasing serum iron and TS [100], similarly to the ferroportin disease. However, this mechanism does not explain the dramatically low level of plasma iron and, more importantly, the parenchymal type of iron deposition with macrophage sparing. The development of brain iron overload is likely related to the expression, in the brain, of a glycosylphosphatidylinositol/inositol (GPI) ceruloplasmin isoform, anchored in cell membrane and resulting from an alternative splicing of the CP gene (in contrast with the secretory form expressed in the hepatocytes) [93]. It is noteworthy that the mutations within the CP gene may lead to: (i) decreased secretion of the mutated ceruloplasmin form through retention of the protein within the endoplasmic reticulum, thus leading to the classical form of HAC, with very low or undetectable serum ceruloplasmin; or (ii) altered association of apoceruloplasmin to the copper atoms that are essential for the ferroxidase activity of holoceruloplasmin, thus leading to a biological picture where ferroxidase activity of the ceruloplasmin is strongly decreased whereas serum ceruloplasmin levels are less or not affected compared to the classical HAC form (Review in [94]).
- Considering that hepcidin deficiency has been reported both clinically [101,102] and experimentally [103], it cannot be excluded that some degree of duodenal iron hyperabsorption

occurs, especially if, like in the brain, hephaestin could partially compensate the lack of ceruloplasmin-related ferroxidase activity.

*Other diseases with systemic iron excess*

*Hereditary atransferrinemia (HAT)*

HAT is a rare recessive disease due to transferrin (TF) mutations on chromosome 3, affecting young individuals [104]. In the absence of transferrin, anemia develops due to a lack of transferrin iron delivery to the bone marrow, and iron overload occurs due to circulating NTBI.

*DMT1-related iron disorder [105,106]*

Given the dual role of this protein in dietary iron uptake at the apical membrane of the duodenal enterocyte and in iron egress from cytosolic endosomes, DMT1 (SLC11A2: solute carrier family 11, member 2) mutations (located on chromosome 12) lead to a peculiar picture. Indeed, this rare recessive disease associates microcytic anemia, present from birth and resistant to oral supplementation, associated with visceral iron overload.

*Diseases with relative iron excess*

*Friedreich ataxia*

This recessive disease is due to mutations of the frataxin (FXN) gene (chromosome 9) [94]. These mutations lead to mitochondrial iron accumulation without total body/organ iron overload. The clinical consequences are spinocerebellar degeneration and frequent cardiomyopathy.

*Other diseases*

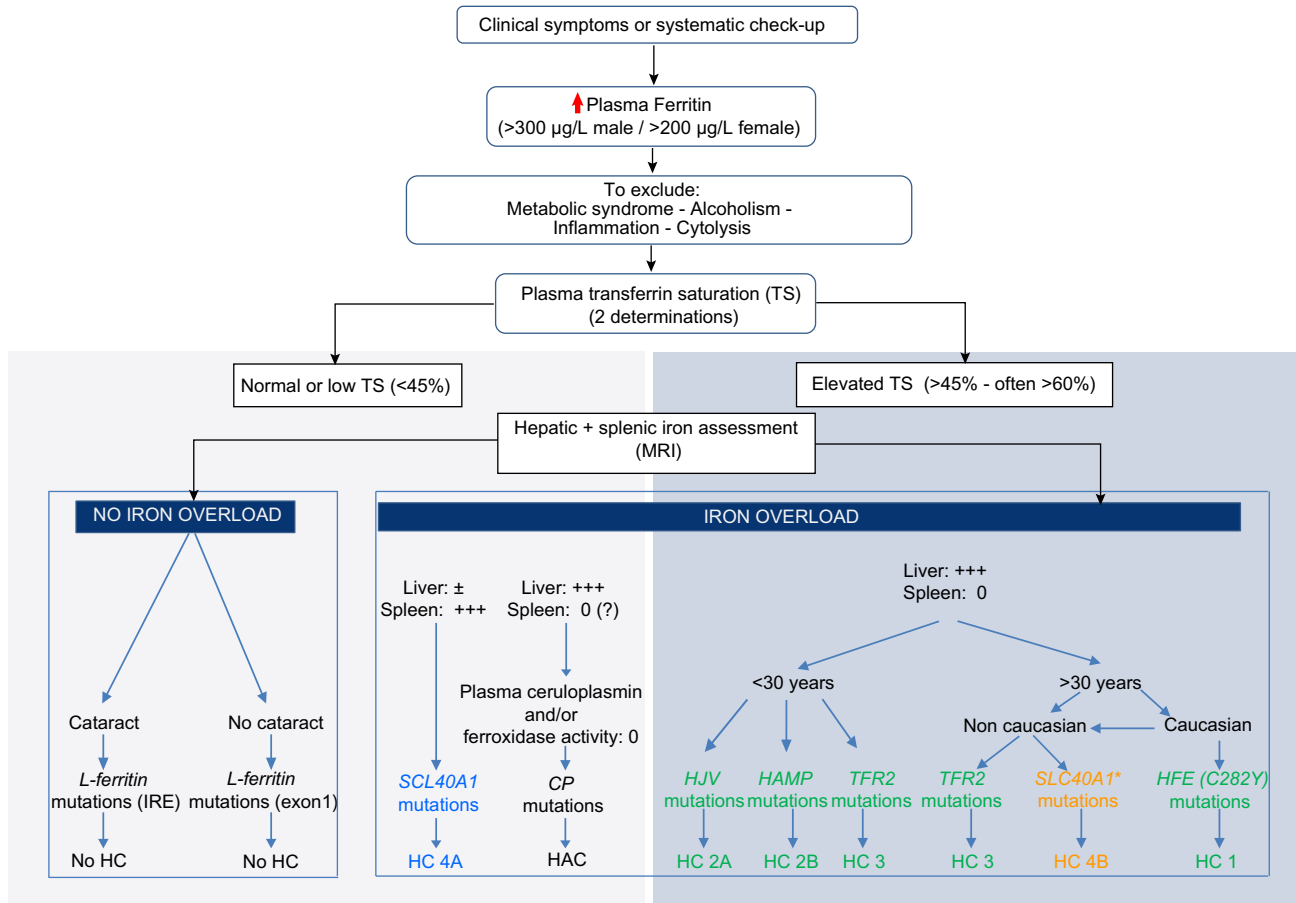
They correspond to disturbances in heme synthesis, encompassing some forms of: (i) congenital sideroblastic anemias (by mutations of the following genes *ALAS2* [107], *SLC25A38* [108], *ABC7* [109], glutaredoxine 5 [110]); and (ii) hereditary porphyrias [111].

*Genetic iron deficiency disorder: IRIDA*

Iron refractory iron deficiency anemia (IRIDA) is caused by mutations of *TMPRSS6* (chromosome 22) which encodes matrilysin-2, a transmembrane serine protease expressed on cell membranes of hepatocytes which is involved in the BMP/SMAD hepcidin regulatory pathway by processing hemojuvelin protein, a co-receptor of BMPs [112]. *TMPRSS6* mutations are responsible for chronic hyperhepcidinemia which leads to decreased plasma iron levels, thus inducing severe iron deficiency anemia that is refractory to oral iron supplementation and only partially responsive to parenteral iron [113,114]. It should be noticed that *TMPRSS6* polymorphisms have been associated to iron deficiency anemia partially responsive to oral treatment [115].

*Genetic iron metabolism-related disorders without iron excess or iron deficiency*

L-ferritin mutations (chromosome 19) are responsible for a dominant inherited disorder expressed by serum ferritin elevation (often >1000 µg/L), with normal TS and without cellular iron excess. Depending on the mutation location on the L-ferritin mRNA, clinical consequences are either expressed by early cataract [116–118] or totally absent [119]. These syndromes are different from other situations involving L-ferritin mutations,



**Fig. 6. Overall diagnostic strategy for hyperferritinemia.** TS, transferrin saturation; MRI, magnetic resonance imaging; HC, haemochromatosis; HAC, hereditary aceruloplasminemia.

called neuroferritinopathies [120]. This exceptional dominant disease is an adult-onset neurodegenerative disorder related to iron overload in the basal ganglia which, clinically, is expressed by extrapyramidal neurological features with low serum ferritin values.

In conclusion, from the hepatologist viewpoint, the liver is a key organ in iron metabolism. It is the source of multiple proteins playing major roles in plasma iron transport (transferrin), in transmembrane iron passage (ceruloplasmin), and in systemic iron regulation (hepcidin). This means that the synthesis of all these proteins can be affected by hepatocellular failure. The liver is also a major iron storage organ and, when overwhelmed by chronic and massive iron burden, can be severely damaged, opening the way to extrahepatic iron-related complications. Increased serum ferritin is the usual initial biochemical finding leading to evoke iron overload. A careful diagnostic strategy should drive the interpretation of hyperferritinemia (Fig. 6), based on four types of key data: clinical data, TS levels, MRI assessment of liver and spleen iron load, and targeted genetic searches with the help of reference centers.

However, despite tremendous advances in the iron pathophysiological domain, a number of molecular mechanisms remain to be elucidated, with the stimulating perspective of

finding novel potential targets which could be valuable for the diagnostic and therapeutic management of patients affected by iron-related disorders.

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**Conflict of interest**

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

**Authors' contributions**

PB and OL contributed equally to the design and the writing of the manuscript.



## References

- [1] Meynard D, Babitt JL, Lin HY. The liver: conductor of systemic iron balance. *Blood* 2014;123:168–176.
- [2] Andrews NC, Schmidt PJ. Iron homeostasis. *Annu Rev Physiol* 2007;69:69–85.
- [3] Lane DJ, Merlot AM, Huang ML, Bae DH, Jansson PJ, Sahni S, et al. Cellular iron uptake, trafficking and metabolism: key molecules and mechanisms and their roles in disease. *Biochim Biophys Acta* 2015;1853:1130–1144.
- [4] Brissot P, Cavey T, Troadec MB, Ropert M, Loréal O. *Métabolisme du fer*. EMC Endocrinol Métabol 2015:1–11.
- [5] Chiabrando D, Vinchi F, Fiorito V, Mercurio S, Tolosano E. Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. *Front Pharmacol* 2014;5:61.
- [6] Lawen A, Lane DJ. Mammalian iron homeostasis in health and disease: uptake, storage, transport, and molecular mechanisms of action. *Antioxid Redox Signal* 2013;18:2473–2507.
- [7] Davidsson L. Approaches to improve iron bioavailability from complementary foods. *J Nutr* 2003;133:1560S–1562S.
- [8] Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. *Blood* 2007;109:412–421.
- [9] Hershko C, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. *Br J Haematol* 1978;40:255–263.
- [10] Brissot P, Wright TL, Ma WL, Weisiger RA. Efficient clearance of non-transferrin-bound iron by rat liver. Implications for hepatic iron loading in iron overload states. *J Clin Invest* 1985;76:1463–1470.
- [11] Craven CM, Alexander J, Eldridge M, Kushner JP, Bernstein S, Kaplan J. Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proc Natl Acad Sci U S A* 1987;84:3457–3461.
- [12] Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. *Proc Natl Acad Sci U S A* 2006;103:13612–13617.
- [13] Jenkitkasemwong S, Wang CY, Coffey R, Zhang W, Chan A, Biel T, et al. SLC39A14 is required for the development of hepatocellular iron overload in murine models of hereditary hemochromatosis. *Cell Metab* 2015;22:138–150.
- [14] Hider RC. Nature of nontransferrin-bound iron. *Eur J Clin Invest* 2002;32:50–54.
- [15] Brissot P, Ropert M, Le Lan C, Loreal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta* 2012;1820:403–410.
- [16] Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. *Blood* 2003;102:2670–2677.
- [17] Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. *Best Pract Res Clin Haematol* 2005;18:277–287.
- [18] Le Lan C, Loreal O, Cohen T, Ropert M, Glickstein H, Laine F, et al. Redox active plasma iron in C282Y/C282Y hemochromatosis. *Blood* 2005;105:4527–4531.
- [19] Cabantchik ZI. Labile iron in cells and body fluids: physiology, pathology, and pharmacology. *Front Pharmacol* 2014;5:45.
- [20] McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 2001;291:1755–1759.
- [21] Ohgami RS, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. *Blood* 2006;108:1388–1394.
- [22] Mukhopadhyay CK, Attieh ZK, Fox PL. Role of ceruloplasmin in cellular iron uptake. *Science* 1998;279:714–717.
- [23] Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999;21:195–199.
- [24] Arosio P, Ingrassia R, Cavadini P. Ferritins: a family of molecules for iron storage, antioxidation and more. *Biochim Biophys Acta* 2009;1790:589–599.
- [25] Lane DJ, Richardson DR. The active role of vitamin C in mammalian iron metabolism: much more than just enhanced iron absorption! *Free Radic Biol Med* 2014;75:69–83.
- [26] Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011;283:65–87.
- [27] Brissot P, Deugnier Y, Guyader D, Zanninelli G, Loreal O, Moirand R, et al. Iron overload and the biliary route. *Adv Exp Med Biol* 1994;356:277–283.
- [28] Brissot P, Bardou-Jacquet E, Jouanolle AM, Loreal O. Iron disorders of genetic origin: a changing world. *Trends Mol Med* 2011;17:707–713.
- [29] Tolosano E. Increasing serum transferrin to reduce tissue iron overload due to ineffective erythropoiesis. *Haematologica* 2015;100:565–566.
- [30] Fishbane S, Mathew A, Vaziri ND. Iron toxicity: relevance for dialysis patients. *Nephrol Dial Transplant* 2014;29:255–259.
- [31] Porter JB, Garbowski M. The pathophysiology of transfusional iron overload. *Hematol Oncol Clin North Am* 2014;28:683–701, vi.
- [32] Crichton RR. Iron metabolism: from molecular mechanisms to clinical consequences. Wiley edits; 2009.
- [33] Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol Chem* 2001;276:7811–7819.
- [34] Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, et al. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci U S A* 2001;98:8780–8785.
- [35] Loreal O, Cavey T, Bardou-Jacquet E, Guggenbuhl P, Ropert M, Brissot P. Iron, hepcidin, and the metal connection. *Front Pharmacol* 2014;5:128.
- [36] Ganz T. Systemic iron homeostasis. *Physiol Rev* 2013;93:1721–1741.
- [37] Zhao N, Zhang AS, Enns CA. Iron regulation by hepcidin. *J Clin Invest* 2013;123:2337–2343.
- [38] Verga Falzacappa MV, Casanovas G, Hentze MW, Muckenthaler MU. A bone morphogenetic protein (BMP)-responsive element in the hepcidin promoter controls HFE2-mediated hepatic hepcidin expression and its response to IL-6 in cultured cells. *J Mol Med (Berl)* 2008;86:531–540.
- [39] Ramey G, Deschemin JC, Vaulont S. Cross-talk between the mitogen activated protein kinase and bone morphogenetic protein/hemojuvelin pathways is required for the induction of hepcidin by holotransferrin in primary mouse hepatocytes. *Haematologica* 2009;94:765–772.
- [40] Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. *Cell* 2010;142:24–38.
- [41] Wu XG, Wang Y, Wu Q, Cheng WH, Liu W, Zhao Y, et al. HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. *Blood* 2014;124:1335–1343.
- [42] Corradini E, Meynard D, Wu Q, Chen S, Ventura P, Pietrangelo A, et al. Serum and liver iron differently regulate the bone morphogenetic protein 6 (BMP6)-SMAD signaling pathway in mice. *Hepatology* 2011;54:273–284.
- [43] Ramos E, Kautz L, Rodriguez R, Hansen M, Gabayan V, Ginzburg Y, et al. Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology* 2011;53:1333–1341.
- [44] Zhang AS, Anderson SA, Wang J, Yang F, DeMaster K, Ahmed R, et al. Suppression of hepatic hepcidin expression in response to acute iron deprivation is associated with an increase of matriptase-2 protein. *Blood* 2011;117:1687–1699.
- [45] McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 2000;5:299–309.
- [46] De Domenico I, Ward DM, Nemeth E, Vaughn MB, Musci G, Ganz T, et al. The molecular basis of ferroportin-linked hemochromatosis. *Proc Natl Acad Sci U S A* 2005;102:8955–8960.
- [47] Brasse-Lagnel C, Karim Z, Letteron P, Bekri S, Bado A, Beaumont C. Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. *Gastroenterology* 2011;140:e1261.
- [48] Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;113:1271–1276.
- [49] Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. *Blood* 2011;118:4129–4139.
- [50] Besson-Fournier C, Latour C, Kautz L, Bertrand J, Ganz T, Roth MP, et al. Induction of activin B by inflammatory stimuli up-regulates expression of the iron-regulatory peptide hepcidin through Smad1/5/8 signaling. *Blood* 2012;120:431–439.
- [51] Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet* 2014;46:678–684.
- [52] Kautz L, Nemeth E. Molecular liaisons between erythropoiesis and iron metabolism. *Blood* 2014;124:479–482.
- [53] Detivaud L, Nemeth E, Boudjema K, Turlin B, Troadec MB, Leroyer P, et al. Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. *Blood* 2005;106:746–748.
- [54] Maras JS, Maiwall R, Harsha HC, Das S, Hussain MS, Kumar C, et al. Dysregulated iron homeostasis is strongly associated with multiorgan failure and early mortality in acute-on-chronic liver failure. *Hepatology* 2015;61:1306–1320.

## Review

- [55] Zhang DL, Ghosh MC, Rouault TA. The physiological functions of iron regulatory proteins in iron homeostasis – an update. *Front Pharmacol* 2014;5:124.
- [56] Wilkinson N, Pantopoulos K. The IRP/IRE system in vivo: insights from mouse models. *Front Pharmacol* 2014;5:176.
- [57] Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, et al. Identification of an intestinal heme transporter. *Cell* 2005;122:789–801.
- [58] Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* 2006;127:917–928.
- [59] Evans RW, Rafique R, Zarea A, Rapisarda C, Cammack R, Evans PJ, et al. Nature of non-transferrin-bound iron: studies on iron citrate complexes and thalassemic sera. *J Biol Inorg Chem* 2008;13:57–74.
- [60] Richardson DR, Lane DJ, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, et al. Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. *Proc Natl Acad Sci U S A* 2010;107:10775–10782.
- [61] Shi H, Bencze KZ, Stemmler TL, Philpott CC. A cytosolic iron chaperone that delivers iron to ferritin. *Science* 2008;320:1207–1210.
- [62] Leidgens S, Bullough KZ, Shi H, Li F, Shakoury-Elizeh M, Yabe T, et al. Each member of the poly-r(C)-binding protein 1 (PCBP) family exhibits iron chaperone activity toward ferritin. *J Biol Chem* 2013;288:17791–17802.
- [63] Gkouvatso K, Papanikolaou G, Pantopoulos K. Regulation of iron transport and the role of transferrin. *Biochim Biophys Acta* 2012;1820:188–202.
- [64] Porto G, De Sousa M. Iron overload and immunity. *World J Gastroenterol* 2007;13:4707–4715.
- [65] Cohen LA, Gutierrez L, Weiss A, Leichtmann-Bardoogo Y, Zhang DL, Crooks DR, et al. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. *Blood* 2010;116:1574–1584.
- [66] Brissot P, Bardou-Jacquet E, Jouanolle AM, Loreal O. Iron disorders of genetic origin: a changing world. *Trends Mol Med* 2011;17:707–713.
- [67] Bardou-Jacquet E, Ben Ali Z, Beaumont-Epinette MP, Loreal O, Jouanolle AM, Brissot P. Non-HFE hemochromatosis: pathophysiological and diagnostic aspects. *Clin Res Hepatol Gastroenterol* 2013.
- [68] Drakesmith H, Schimanski LM, Ormerod E, Merryweather-Clarke AT, Viprakasit V, Edwards JP, et al. Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. *Blood* 2005;106:1092–1097.
- [69] Sham RL, Phatak PD, West C, Lee P, Andrews C, Beutler E. Autosomal dominant hereditary hemochromatosis associated with a novel ferroportin mutation and unique clinical features. *Blood Cells Mol Dis* 2005;34:157–161.
- [70] Sham RL, Phatak PD, Nemeth E, Ganz T. Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation. *Blood* 2009;114:493–494.
- [71] Fernandes A, Preza GC, Phung Y, De Domenico I, Kaplan J, Ganz T, et al. The molecular basis of hepcidin-resistant hereditary hemochromatosis. *Blood* 2009;114:437–443.
- [72] Pietrangelo A. Genetics, Genetic Testing and Management of Hemochromatosis: 15 years since hepcidin. *Gastroenterology* 2015.
- [73] Bardou-Jacquet E, Philip J, Lorho R, Ropert M, Latournerie M, Housset-Debry P, Guyader D, et al. Liver transplantation normalizes serum hepcidin level and cures iron metabolism alterations in HFE hemochromatosis. *Hepatology* 2013.
- [74] Gandon Y, Olivie D, Guyader D, Aube C, Oberti F, Sebille V, et al. Non-invasive assessment of hepatic iron stores by MRI. *Lancet* 2004;363:357–362.
- [75] St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855–861.
- [76] Wood JC. Impact of iron assessment by MRI. *Hematology Am Soc Hematol Educ Program* 2011;2011:443–450.
- [77] Deugnier Y, Turlin B. Pathology of hepatic iron overload. *Semin Liver Dis* 2011;31:260–271.
- [78] HAS. French recommendations for management of HFE hemochromatosis. Haute Autorité de Santé; 2005. [www.has-sante.fr](http://www.has-sante.fr).
- [79] Brissot P, Ball S, Rofail D, Cannon H, Jin VW. Hereditary hemochromatosis: patient experiences of the disease and phlebotomy treatment. *Transfusion* 2011;51:1331–1338.
- [80] Porto G, Brissot P, Swinkels DW, Zoller H, Kamarainen O, Patton S, Alonso I, et al. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). *Eur J Hum Genet* 2015.
- [81] Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008;358:221–230.
- [82] Fletcher LM, Powell LW. Hemochromatosis and alcoholic liver disease. *Alcohol* 2003;30:131–136.
- [83] Desgrippes R, Laine F, Morcet J, Perrin M, Manet G, Jezequel C, et al. Decreased iron burden in overweight C282Y homozygous women: Putative role of increased hepcidin production. *Hepatology* 2013;57:1784–1792.
- [84] Island ML, Jouanolle AM, Mosser A, Deugnier Y, David V, Brissot P, et al. A new mutation in the hepcidin promoter impairs its BMP response and contributes to a severe phenotype in HFE related hemochromatosis. *Haematologica* 2009;94:720–724.
- [85] Valenti L, Fracanzani AL, Rametta R, Fraquelli M, Soverini G, Pelusi S, et al. Effect of the A736V TMPRSS6 polymorphism on the penetrance and clinical expression of hereditary hemochromatosis. *J Hepatol* 2012;57:1319–1325.
- [86] McLaren CE, Emond MJ, Subramaniam VN, Phatak PD, Barton JC, Adams PC, et al. Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload. *Hepatology* 2015;62:429–439.
- [87] de Tayrac M, Roth MP, Jouanolle AM, Coppin H, le Gac G, Piperno A, et al. Genome-wide association study identifies TF as a significant modifier gene of iron metabolism in HFE hemochromatosis. *J Hepatol* 2015;62:664–672.
- [88] Milet J, Dehais V, Bourgain C, Jouanolle AM, Mosser A, Perrin M, et al. Common variants in the BMP2, BMP4, and HJV genes of the hepcidin regulation pathway modulate HFE hemochromatosis penetrance. *Am J Hum Genet* 2007;81:799–807.
- [89] Fung E, Nemeth E. Manipulation of the hepcidin pathway for therapeutic purposes. *Haematologica* 2013;98:1667–1676.
- [90] Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. *Nat Genet* 2001;28:213–214.
- [91] Montosi G, Donovan A, Totaro A, Garuti C, Pignatti E, Cassanelli S, et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. *J Clin Invest* 2001;108:619–623.
- [92] Pietrangelo A. The ferroportin disease. *Blood Cells Mol Dis* 2004;32:131–138.
- [93] Miyajima H. Aceruloplasminemia. *Neuropathology* 2015;35:83–90.
- [94] Kono S. Aceruloplasminemia: an update. *Int Rev Neurobiol* 2013;110:125–151.
- [95] Finkenstedt A, Wolf E, Hofner E, Gasser BI, Bosch S, Bakry R, et al. Hepatic but not brain iron is rapidly chelated by deferasirox in aceruloplasminemia due to a novel gene mutation. *J Hepatol* 2010;53:1101–1107.
- [96] Hattori A, Tomosugi N, Tatsumi Y, Suzuki A, Hayashi K, Katano Y, et al. Identification of a novel mutation in the HAMP gene that causes non-detectable hepcidin molecules in a Japanese male patient with juvenile hemochromatosis. *Blood Cells Mol Dis* 2012;48:179–182.
- [97] Tai M, Matsuhashi N, Ichii O, Suzuki T, Ejiri Y, Kono S, et al. Case of presymptomatic aceruloplasminemia treated with deferasirox. *Hepatology Res* 2014;44:1253–1258.
- [98] Rusticeanu M, Zimmer V, Schleithoff L, Wonney K, Viera J, Zimmer A, et al. Novel ceruloplasmin mutation causing aceruloplasminemia with hepatic iron overload and diabetes without neurological symptoms. *Clin Genet* 2014;85:300–301.
- [99] Loreal O, Turlin B, Pigeon C, Moisan A, Ropert M, Morice P, et al. Aceruloplasminemia: new clinical, pathophysiological and therapeutic insights. *J Hepatol* 2002;36:851–856.
- [100] De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, et al. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J* 2007;26:2823–2831.
- [101] Kaneko Y, Miyajima H, Piperno A, Tomosugi N, Hayashi H, Morotomi N, et al. Measurement of serum hepcidin-25 levels as a potential test for diagnosing hemochromatosis and related disorders. *J Gastroenterol* 2010;45:1163–1171.
- [102] Kono S, Yoshida K, Tomosugi N, Terada T, Hamaya Y, Kanaoka S, et al. Biological effects of mutant ceruloplasmin on hepcidin-mediated internalization of ferroportin. *Biochim Biophys Acta* 2010;1802:968–975.
- [103] Guo P, Cui R, Chang YZ, Wu WS, Qian ZM, Yoshida K, et al. Hepcidin, an antimicrobial peptide is downregulated in ceruloplasmin-deficient mice. *Peptides* 2009;30:262–266.
- [104] Aslan D, Crain K, Beutler E. A new case of human atransferrinemia with a previously undescribed mutation in the transferrin gene. *Acta Haematol* 2007;118:244–247.
- [105] Iolascon A, Camaschella C, Pospisilova D, Piscopo C, Tchernia G, Beaumont C. Natural history of recessive inheritance of DMT1 mutations. *J Pediatr* 2008;152:136–139.

- [106] Bardou-Jacquet E, Island ML, Jouanolle AM, Detivaud L, Fatih N, Ropert M, et al. A novel N491S mutation in the human SLC11A2 gene impairs protein trafficking and in association with the G212V mutation leads to microcytic anemia and liver iron overload. *Blood Cells Mol Dis* 2011;47:243–248.
- [107] Ducamp S, Kannengiesser C, Touati M, Garcon L, Guerci-Bresler A, Guichard JF, et al. Sideroblastic anemia: molecular analysis of the ALAS2 gene in a series of 29 probands and functional studies of 10 missense mutations. *Hum Mutat* 2011;32:590–597.
- [108] Guernsey DL, Jiang H, Campagna DR, Evans SC, Ferguson M, Kellogg MD, et al. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. *Nat Genet* 2009;41:651–653.
- [109] Bekri S, Kispal G, Lange H, Fitzsimons E, Tolmie J, Lill R, et al. Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron-sulfur protein maturation. *Blood* 2000;96:3256–3264.
- [110] Camaschella C, Campanella A, De Falco L, Boschetto L, Merlini R, Silvestri L, et al. The human counterpart of zebrafish shiraz shows sideroblastic-like microcytic anemia and iron overload. *Blood* 2007;110:1353–1358.
- [111] Besur S, Hou W, Schmeltzer P, Bonkovsky HL. Clinically important features of porphyrin and heme metabolism and the porphyrias. *Metabolites* 2014;4:977–1006.
- [112] Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nat Genet* 2008;40:569–571.
- [113] Heeney MM, Finberg KE. Iron-refractory iron deficiency anemia (IRIDA). *Hematol Oncol Clin North Am* 2014;28:637–652. v.
- [114] De Falco L, Silvestri L, Kannengiesser C, Moran E, Oudin C, Rausa M, et al. Functional and clinical impact of novel TMPRSS6 variants in iron-refractory iron-deficiency anemia patients and genotype-phenotype studies. *Hum Mutat* 2014;35:1321–1329.
- [115] Poggiali E, Andreozzi F, Nava I, Consonni D, Graziadei G, Cappellini MD. The role of TMPRSS6 polymorphisms in iron deficiency anemia partially responsive to oral iron treatment. *Am J Hematol* 2015;90:306–309.
- [116] Girelli D, Corrocher R, Bisceglia L, Olivieri O, De Franceschi L, Zelante L, et al. Molecular basis for the recently described hereditary hyperferritinemia-cataract syndrome: a mutation in the iron-responsive element of ferritin L-subunit gene (the “Verona mutation”). *Blood* 1995;86:4050–4053.
- [117] Yin D, Kulhali V, Walker AP. Raised serum ferritin concentration in hereditary hyperferritinemia cataract syndrome is not a marker for iron overload. *Hepatology* 2014;59:1204–1206.
- [118] Bowes O, Baxter K, Elsey T, Snead M, Cox T. Hereditary hyperferritinaemia cataract syndrome. *Lancet* 2014;383:1520.
- [119] Kannengiesser C, Jouanolle AM, Hetet G, Mosser A, Muzeau F, Henry D, et al. A new missense mutation in the L ferritin coding sequence associated with elevated levels of glycosylated ferritin in serum and absence of iron overload. *Haematologica* 2009;94:335–339.
- [120] Martelli A, Puccio H. Dysregulation of cellular iron metabolism in Friedreich ataxia: from primary iron-sulfur cluster deficit to mitochondrial iron accumulation. *Front Pharmacol* 2014;5:130.