



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

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TITLE PAGE

TITLE : IRON METABOLISM AND RELATED GENETIC DISEASES : A CLEARED LAND, KEEPING MYSTERIES

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ABSTRACT

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 receptor2 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.

Iron is the best and the worst thing for the human body. Iron being deeply involved in a number of critical metabolic processes, 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 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, related either to iron deficiency or to 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.

1. 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).

1.1. 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 hemoglobin 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 billions of new red blood cells(6). Therefore, iron plays a major role in the respiratory process, and without iron, the human body could not breath. This is as more true as iron is also involved, at the cellular and molecular levels, in the respiratory chain which serves to the production 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.

1.2. IRON IS NOT PRODUCED BY THE BODY WHICH IS THEREFORE EXPOSED TO IRON DEFICIENCY

The only iron source is alimentary. Normal diet provides 10-20 mg per day, of which one tenth (1-2mg) only 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 to decrease 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)).

1.3. 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 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 to many metabolic processes (respiration, xenobiotic biotransformation, DNA synthesis).

1.4. 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 linked to the ability of iron to generate Reactive Oxygen Species (ROS).

1.4.1. 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 so far as, 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).

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

1.5. 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).

1.5.1. 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.

1.5.2. 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 necessary 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.

1.5.3. 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).

1.5.4. 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.

1.6. 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 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 anemia(30)) or repeated transfusions(31) (for hemoglobinopathies or myelodysplastic syndromes).

1.7. IRON RECYCLING IS A CRUCIAL PERMANENT PROCESS

Since the daily quantity of iron entering and leaving the body is minimal (1-2mg), as compared to the daily body iron needs (of the order of 20mg), 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). Therefore, the « ecological attitude », and therefore energy preservation, are hallmarks of iron metabolism.

1.8. 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 consist 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 ERK (*extracellular signal regulating kinase*) MAPK (*mitogen activated protein kinase*) pathway, and the BMP (*bone morphogenetic proteins*)/ SMAD (*son of mothers against decapentaplegic homologues*) 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 transferrin saturation levels would act within a few hours versus 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, 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 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 an 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 down-regulating the expression of DMT1, that 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 erythroferrone (*ERFE*)(51, 52). A further mechanism is hepatocellular failure since hepcidin is synthesized by the hepatocytes(53, 54).

1.9. 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 IRE (*Iron Responsive Element*)-IRP 1 and 2 (*Iron Regulatory Protein*) system(55, 56). In case of decreased cellular iron, an IRP1 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 receptor1 m-RNA, IRP hyperfixation on IREs inhibits transferrin degradation. These two combined mechanisms result in decreased iron storage capacity (decreased ferritin synthesis) and in increased iron entry capacity (increased transferrin receptor 1 expression), a « logical » process for counteracting the initial cellular iron decrease in cell. The reverse phenomenon occurs in case of increased cellular iron concentration.

1.10. MAJOR ADVANCES IN IRON METABOLISM UNDERSTANDING DO 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 HCP1 (heme carrier protein 1) 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 PCB1 (Polyr©-Binding Protein1(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 erythroferrone is acting, and ix) the mechanisms accounting for «brain protection» in most situations of systemic iron overload.

2. IRON-RELATED GENETIC DISEASES

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

2.1. GENETIC IRON OVERLOAD DISORDERS

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

2.1.1. DISEASES WITH TOTAL BODY IRON OVERLOAD

2.1.1.1. HAEMOCHROMATOSES

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

2.1.1.1.1. 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 receptor2 (*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 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 macrophages (Kupffer cells, splenic macrophages). This means, on liver biopsy performed at early stages, exclusive hepatocytic iron deposition with Perls staining, and, on MRI (magnetic resonance imaging), 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 >200µg/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 case 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 blood-letting therapy(79) due 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 transferrin saturation and ferritin) in the 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) Type1 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 patho-physiology, 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).

2.1.1.1.2. 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 transferrin saturation 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) bloodletting 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.

2.1.1.2. 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 transferrin saturation 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 GPI (glycosylphosphatidylinositol) 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 : i) to 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) to 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.

2.1.1.3. OTHER DISEASES WITH SYSTEMIC IRON EXCESS

2.1.1.3.1. HEREDITARY ATRANSFERRINEMIA (HAT)

Rare recessive disease due to transferrin (*TF*) mutations on chromosome 3, HAT affects young individuals(104). In the absence of transferrin, anemia develops due to lack of transferrin-iron delivery to the bone marrow, and iron overload occurs due to circulating NTBI.

2.1.1.3.2. DIVALENT METAL TRANSPORTER ONE (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.

2.1.2. DISEASE WITH RELATIVE IRON EXCESS

2.1.2.1. 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.

2.1.2.2. 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), *ABCB7* (109), glutaredoxine 5 (110)), and ii) hereditary porphyrias(111).

2.2. GENETIC IRON DEFICIENCY DISORDER : IRIDA

IRIDA (iron refractory iron-deficiency anemia) is caused by mutations of *TMPRSS6* (chromosome 22) which encodes matriptase-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 coreceptor of BMPs(112). *TMPRSS6* mutations are responsible for chronic hyperhepcidinemia which leads to decrease plasma iron level, 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).

2.3. GENETIC IRON METABOLISM-RELATED DISORDERS WITHOUT IRON EXCESS : GENETIC HYPERFERRITINEMIAS

L-ferritin mutations (chromosome 19) are responsible for a dominant inherited disorder expressed by serum ferritin elevation (often >1000 µg/L), with normal transferrin saturation and without cellular iron excess. Depending on the mutation location on the L-ferritin m-RNA, 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, 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 extra-hepatic 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, transferrin saturation 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.

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 haemochromatoses).
- . The liver is less impacted by iron overload in the usual form of the ferroportin disease (type 4 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.

FIGURE LEGENDS

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

FIGURE 2. Main cell types involved in iron metabolism.

2A (*upper left panel*) Enterocyte. Fe : iron ; DCytb : duodenal cytochrome ; DMT1 : divalent metal transporter1 ; HCP1 : Heme carrier protein 1 ; TFR1 : transferrin receptor 1.

2B. (*upper right panel*) Hepatocyte. NTBI : non-transferrin bound iron ; ZIP14 (SLC39A14 –solute carrier 39A14).

2C (*lower left panel*) Macrophage. Senescent erythrocyte (in red) ; Fe : iron ; HRG1 : heme-responsive gene protein 1 .

2D (*lower right panel*) Erythroblast. Fe-S : iron-sulfur clusters ; DMT1 : divalent metal transporter 1 ; STEAP3 : six-transmembrane epithelial antigen of the prostate.

FIGURE 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) (the green dotted arrow indicates that hepcidin also impacts duodenal iron uptake via DMT1). Increased (plasma or hepatocytic) iron load stimulates hepcidin production (TRF2 : transferrin receptor 2 ; ERK/MAK : extracellular signal regulating kinase/mitogen activated protein kinase ; HJV (or HFE2) : hemojuvelin ; BMP6: bone morphogenetic protein 6 (induced by intracellular iron) ; BMP-R : BMPreceptor ; SMAD : son of mothers against decapentaplegic homologs). Inflammation is also a positive hepcidin regulator (IL-6 : interleukin6 ; 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. TMPRSS6 (*transmembrane serine protease S6*) is also a negative hepcidin regulator (HIF : hypoxia inducible factor ; JAK2 : Janus kinase 2).

FIGURE 4. Local (cellular) iron regulation. Decreased cellular iron content activates IRE (iron responsive element) fixation on IRP (iron regulatory protein), leading to decreased ferritin synthesis and to increased TFR1 (transferrin receptor1) expression. Inverse situation in case of increased cellular iron content.

FIGURE 5. Mechanisms of iron overload in haemochromatosis. A) (Left panel) 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) (Right panel) 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 (no iron  load ; moderat iron overload ; heav iron overload).

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

ACCEPTED MANUSCRIPT

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FIGURE 1

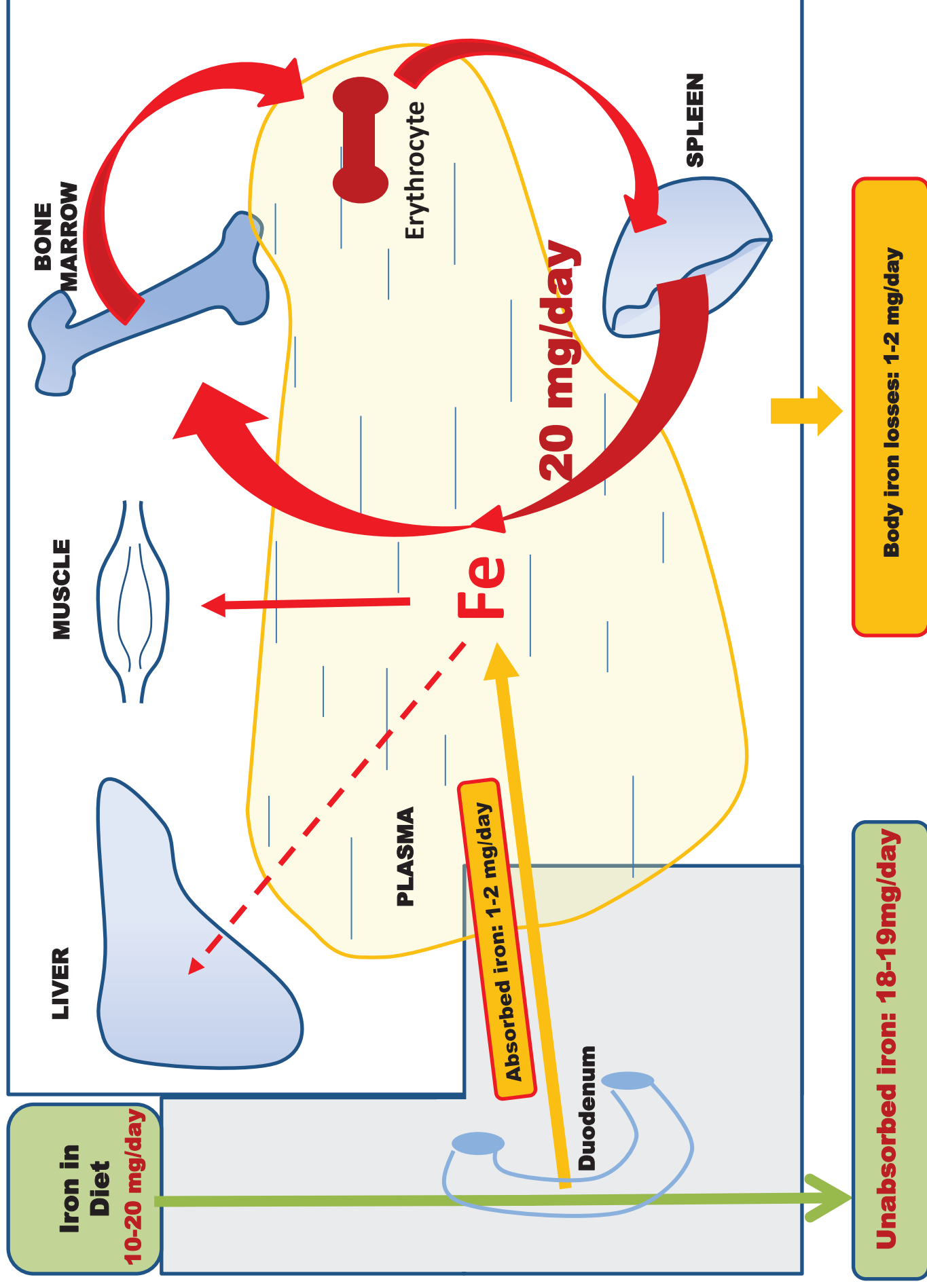


FIGURE 2A

2A ENTEROCYTE

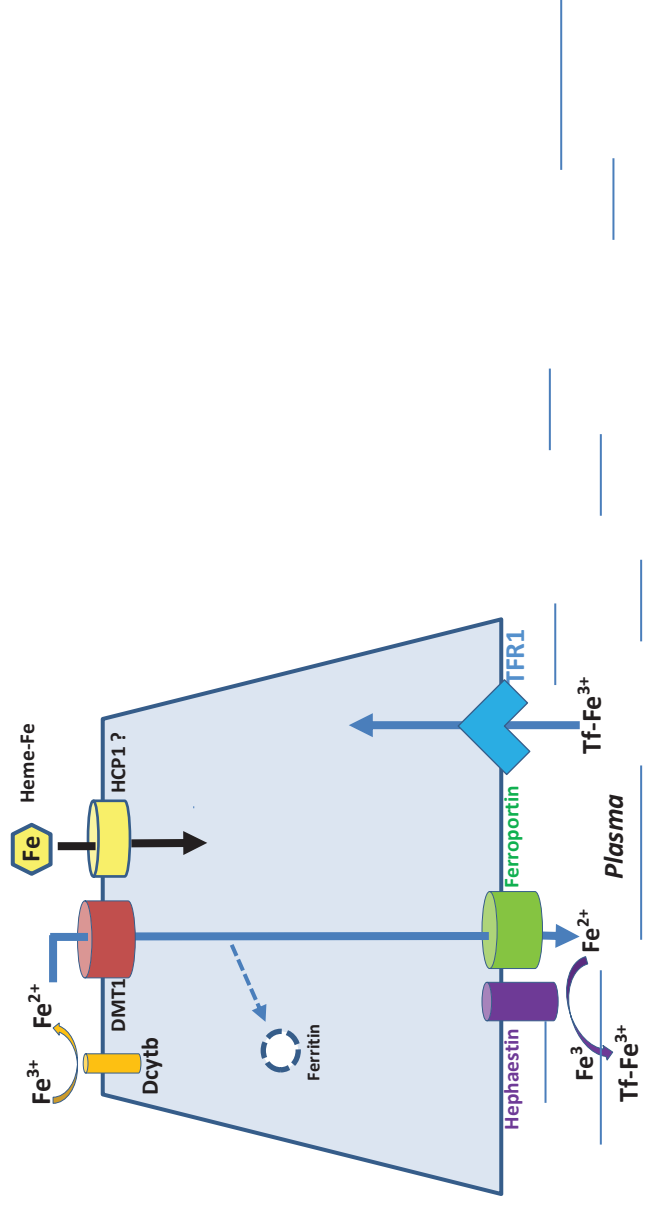


FIGURE 2B

2B HEPATOCYTE

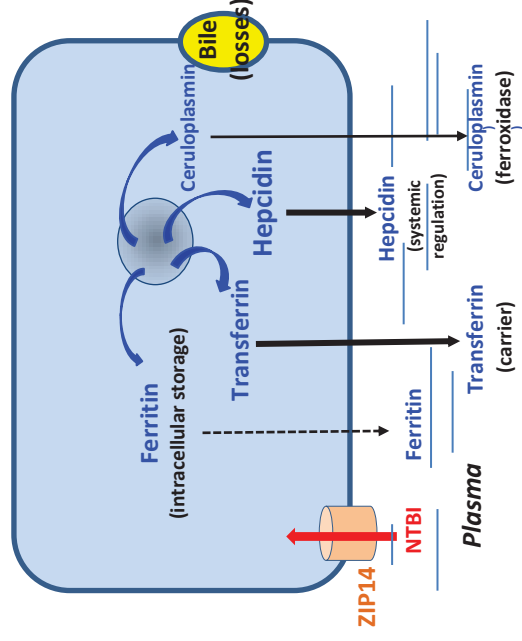


FIGURE 2C

2C MACROPHAGE

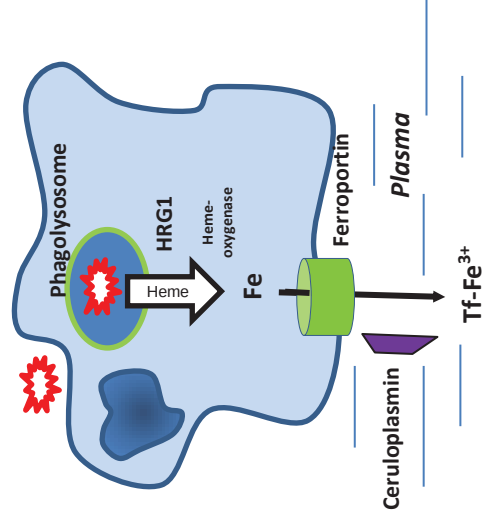
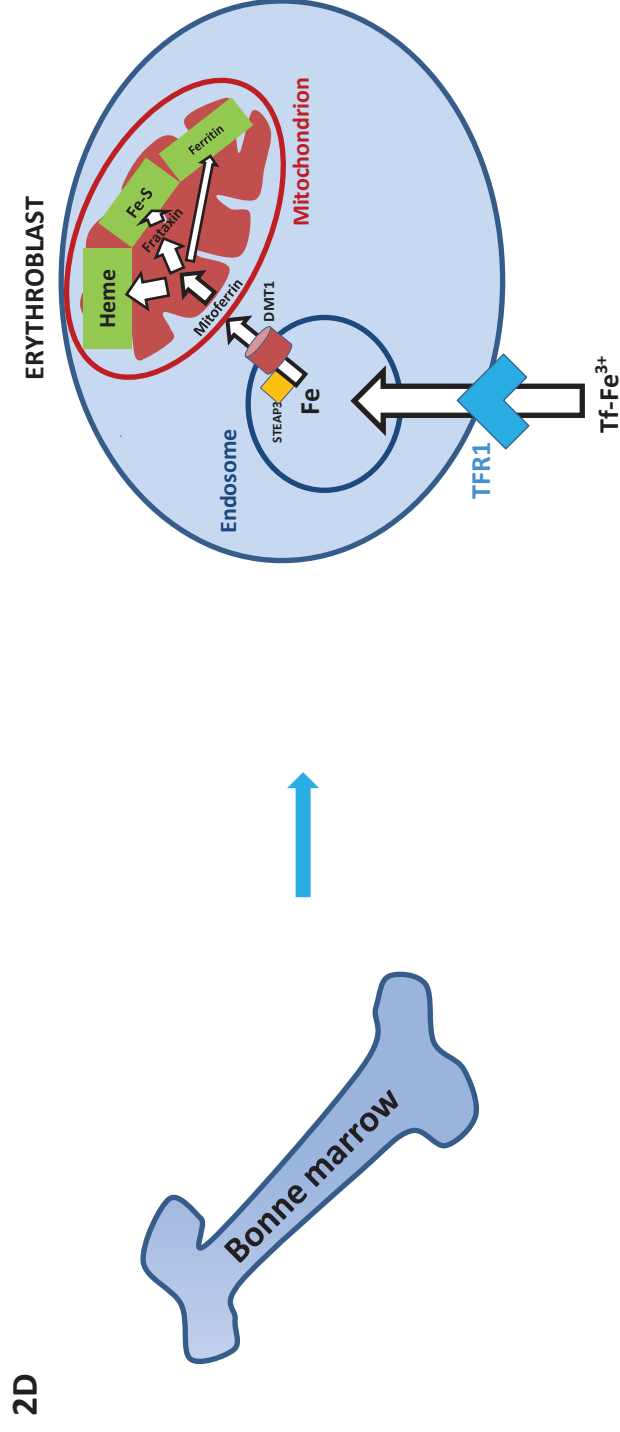


FIGURE 2D



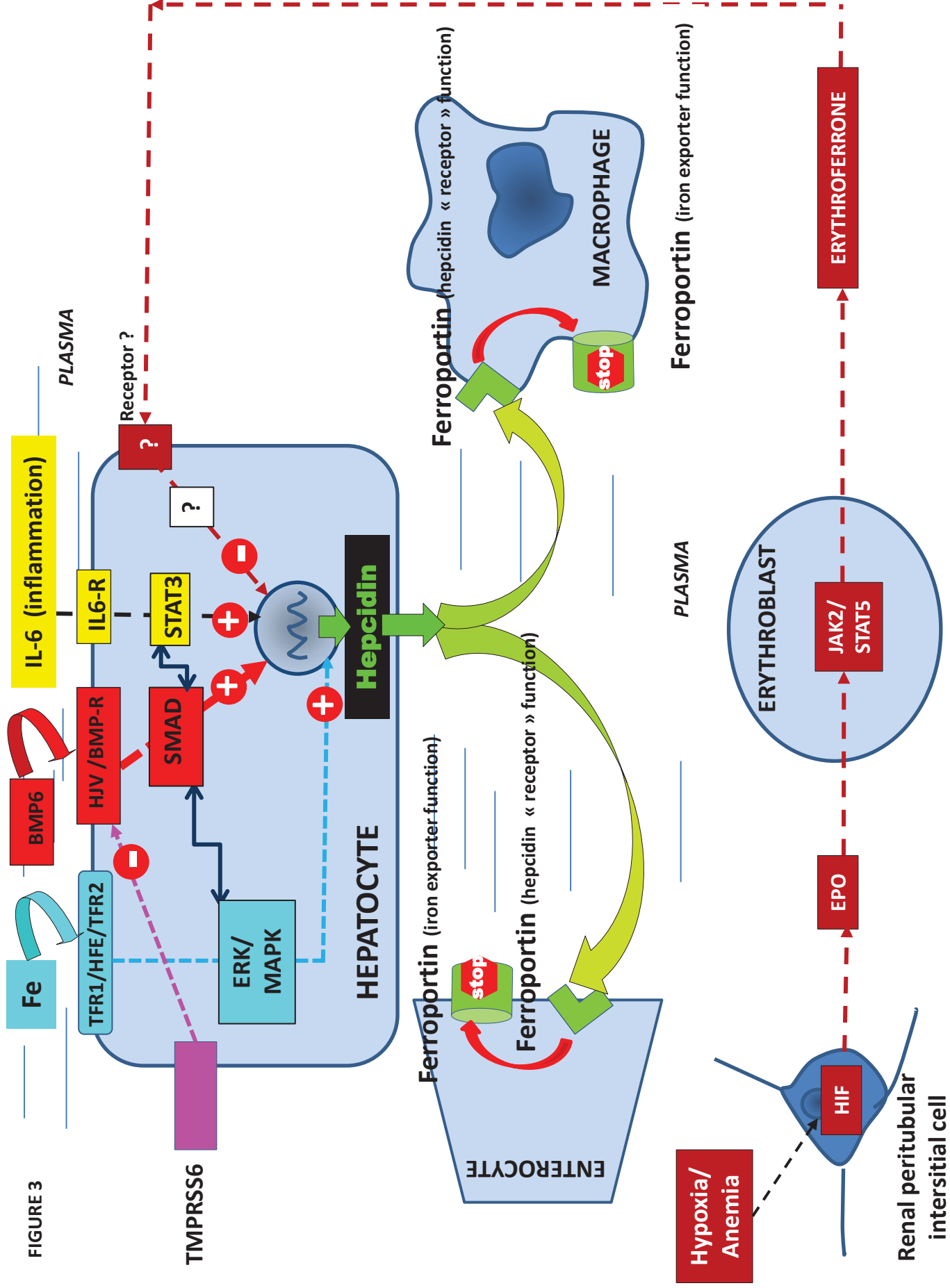
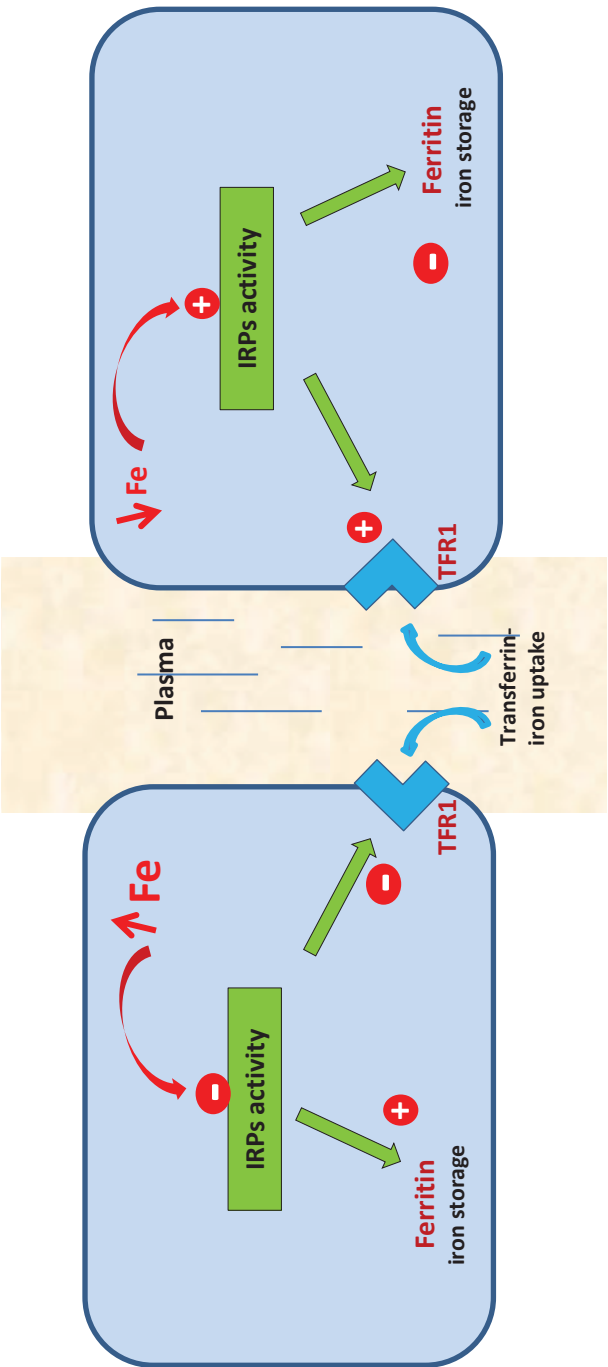


FIGURE 3

FIGURE 4



A

B

FIGURE 5

Hepcidin deficient hemochromatosis HFE (C282Y), or hemojuvelin (*HJV* or *HFE2*), hepcidin (*HAMP*), or *TFR2* mutations

Ferroportin disease (Type B) *SLC40A1* mutations

Ferroportin disease (Type A) *SLC40A1* mutations

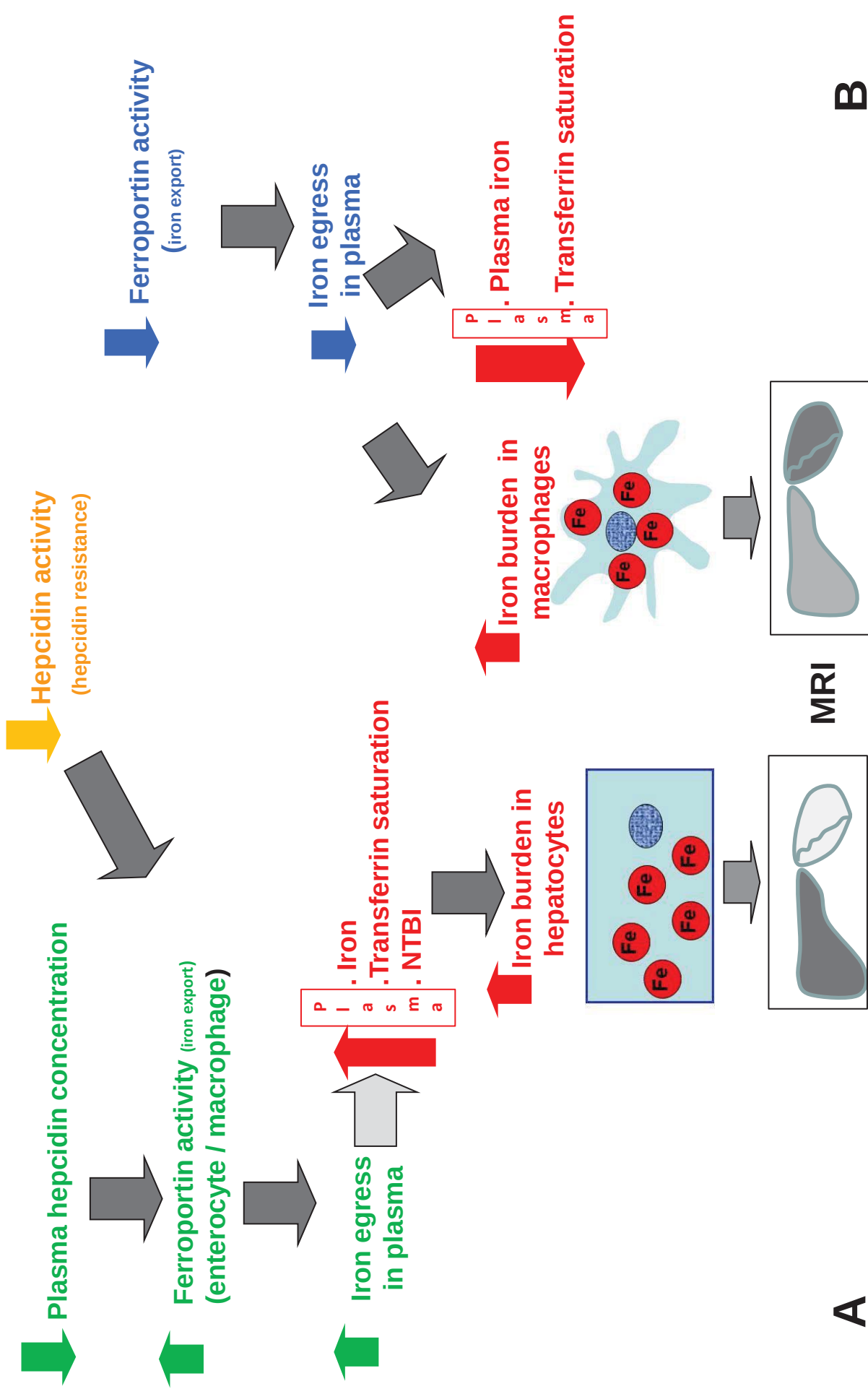


FIGURE 6

