

Showcase on Research

Inherited Disorders of Iron Metabolism

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Almost all eukaryotic cells have an absolute requirement for iron as the metal is a vital component of many enzymes in critical metabolic pathways. Despite the essential nature of iron, it is toxic when present in excess due to its propensity to catalyse the formation of reactive oxygen species. To cater for this dual nature, cells and organisms have developed elaborate mechanisms for regulating iron intake and efflux.

While some aspects of iron homeostasis have been well understood for many years, it is only in the last few years, with the application of genetic and molecular approaches to inherited disorders of iron metabolism, that we have begun to understand the vectorial transport of iron in any detail. The contribution of some inherited disorders of iron metabolism to these exciting recent developments will be described below, following a brief review of normal iron trafficking pathways in the body.

Normal iron homeostasis

The basic pathways of body iron metabolism are shown in **Fig. 1**. Iron is

absorbed from the diet across the epithelial cells of the proximal small intestine as either haem or in a non-haem form.

Little is known about the absorption of haem iron but the major protein facilitating the uptake of non-haem iron is the proton-coupled brush border transporter DMT1 (1). DMT1 transports only ferrous iron, and there is evidence that the more abundant ferric form must be reduced by a plasma membrane reductase before it can be utilized.

The subsequent passage of iron across the basolateral membrane is likely to be mediated by the recently identified integral membrane protein IREG1 (2). The membrane-bound ceruloplasmin homologue hephaestin is also required for efficient basolateral transport (3), but whether it interacts with IREG1 or operates independently is unknown.

After iron traverses the intestinal epithelium it is bound by plasma transferrin (Tf) and is distributed to tissues throughout the body. Transferrin binds to cell surface transferrin receptors (TfRs) and the resulting complex is internalised by receptor-mediated endocytosis (4). Iron

is released from transferrin following endosomal acidification and is transported to the cytoplasm via DMT1 where it can be utilised for metabolic processes, or stored in ferritin if it is in excess of cellular requirements.

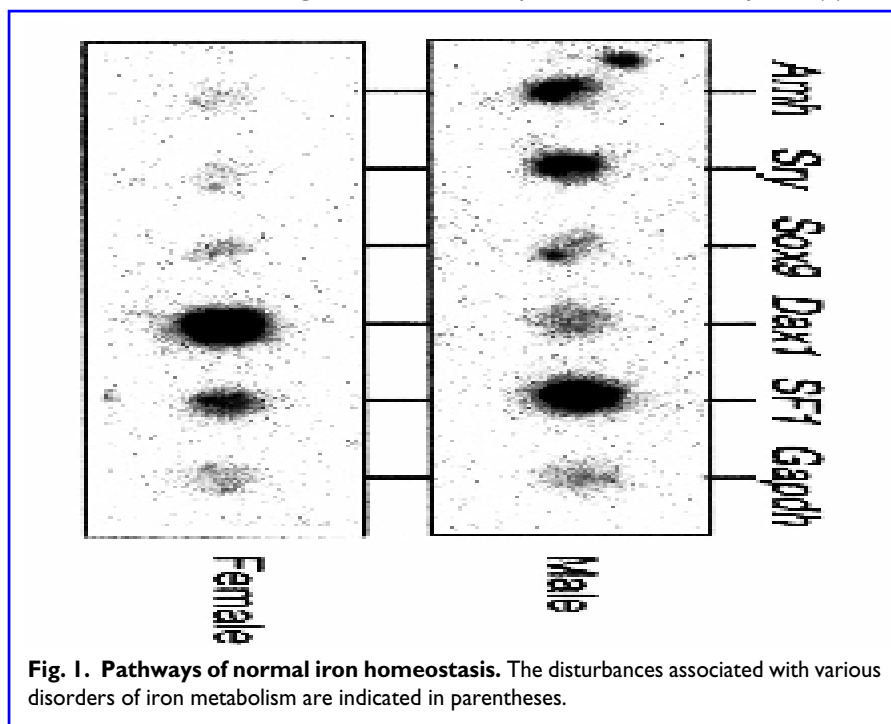
Quantitatively, the most important site of iron utilization is the TfR-rich erythroid marrow where immature erythrocytes or reticulocytes incorporate the iron into haemoglobin. At the end of their life, red cells are phagocytosed by macrophages and the iron is liberated from haemoglobin and returned to the circulation. The transporter IREG1 may provide the pathway for iron return to the plasma. To facilitate this return, extracellular ceruloplasmin appears to be required.

Iron uptake and release by cells is tightly regulated. The best characterised regulatory system is the post-transcriptional control of the mRNAs for ferritin, TfR, DMT1, IREG1 and several other genes (5). Cytoplasmic iron regulatory proteins (IRPs) bind to cognate iron responsive elements (IREs) in the 5' or 3' UTRs of these genes to either block transcription or stabilize the message. Other modes of regulation exist, but they are not well characterized. For example, at the whole body level iron intake must be tightly controlled since iron excretion is very limited. The HFE protein, which is defective in haemochromatosis, appears to play an important role in this process by regulating intestinal iron transit, but the mechanism has yet to be resolved.

Haemochromatosis

The iron overload disease hereditary haemochromatosis (HHC) is the most common of all autosomally inherited genetic diseases with an incidence in some populations as high as 1 in 250-300 (6). In HHC patients, the normal mechanism by which the body maintains iron balance is disrupted, resulting in continued iron absorption in excess of needs and progressive accretion of body iron stores.

The increased absorption eventually leads to accumulation of iron to toxic



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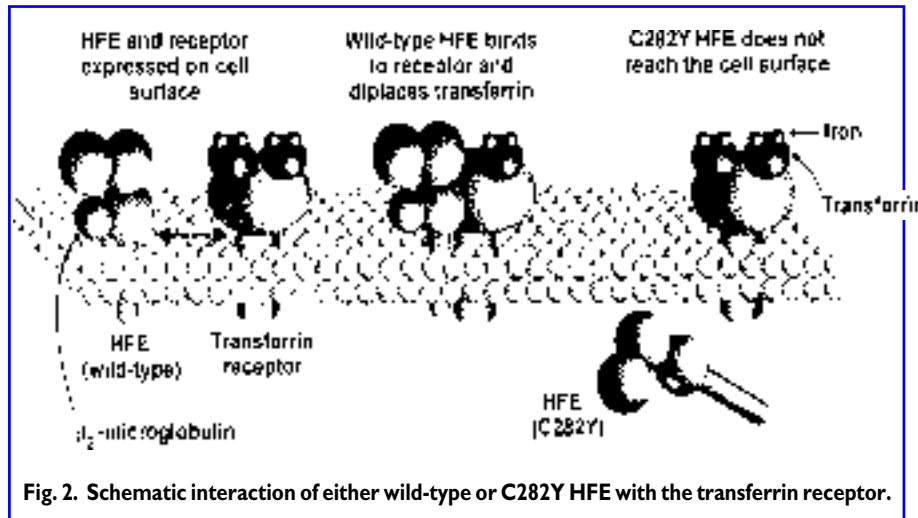


Fig. 2. Schematic interaction of either wild-type or C282Y HFE with the transferrin receptor.

levels, resulting in tissue damage involving the liver, heart, pancreas, joints, and other organs (6).

The gene affected gene in HHC is known as HFE and most patients with haemochromatosis are homozygous for a mutation producing a single amino acid substitution of tyrosine for cysteine at position 282 (C282Y) of the HFE protein (7). However, the mechanism by which a mutation in the HFE gene leads to increased intestinal iron absorption in the face of an elevated body iron content is unknown.

Recent studies have shown that HFE is able to associate with the TfR and thus it may play some role in regulating the entry of iron into cells (8,9) (Fig. 2). This capability is lost in C282Y mutant HFE, and it is tempting to speculate that the ability intestinal crypt cells to take up iron from plasma Tf (a means of signalling the intestine of body iron requirements) may be defective in HHC patients. The resulting paucity of intracellular iron in the intestinal epithelium could explain increased intestinal iron absorption despite body iron overload.

HFE is also expressed in reticulo-endothelial cells and the same defect responsible for increased iron absorption may be responsible for enhanced efflux of iron from the macrophages of HHC patients. The precise role of HFE in cellular and body iron homeostasis remains one of the most fascinating, yet elusive, problems of contemporary trace element metabolism.

Hypotransferrinemia and aceruloplasminaemia

The analysis of these rare recessively inherited disorders of plasma protein synthesis has added substantially to our knowledge of iron trafficking in the body. As their names suggest, they are characterised by very low or undetectable plasma levels of Tf or ceruloplasmin (10,11). For both diseases, it is the accumulation of toxic levels of iron in various tissues that produces the underlying pathology.

It might be expected that individuals lacking Tf would have difficulty in absorbing iron and delivering it to tissues, but this is not the case. They absorb iron very efficiently from the diet and it enters the circulation as non-Tf bound iron which, interestingly, is taken up by cells much more efficiently than Tf-bound iron. Thus Tf is not required for iron delivery to tissues *per se*, but it is required to regulate the delivery of iron to cells. In contrast to most tissues, the erythroid marrow has an absolute requirement for Tf-bound iron and this explains the anaemia which develops in hypotransferrinemia.

Many studies have suggested that ceruloplasmin plays a critical role in iron release from tissues and the phenotype of patients with aceruloplasminaemia has confirmed this. Iron deposition is observed in various tissues, but it is CNS iron accumulation that leads to the neurological abnormalities and retinal degeneration that characterize the disease. When ceruloplasmin is absent, the diminished return of iron to the plasma leads to insufficient iron supply to the erythroid

compartment, which explains the apparent paradox of anaemia in the face of tissue iron loading.

Friedreich ataxia

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder characterized by a variety of nervous system defects including progressive gait and limb ataxia. Non-neurological manifestations of the disease include cardiomyopathy and diabetes mellitus.

The gene affected in FRDA was identified by positional cloning and is known as *frataxin* (12). In most FRDA patients the defect in the *frataxin* gene is an expansion of an intronic trinucleotide repeat, although point mutations have also been found. These gene defects lead to a severe reduction in the levels of *frataxin* protein. Frataxin is a mitochondrial protein and this explains the observation that the tissues affected in FRDA are those showing a high dependence on oxidative metabolism.

The link between FRDA and iron homeostasis was made from the observation that the deletion of the yeast homolog of frataxin (YFH1) leads to mitochondrial iron overload (13) and subsequent oxidative damage to various mitochondrial proteins. There is a growing body of evidence that a similar pathogenesis occurs in FRDA patients. How disruption of frataxin or YFH1 leads to iron accumulation in the mitochondrion has yet to be elucidated. However, these data would suggest that YFH1 and, by inference, frataxin, play critical roles in iron traffic into or out of mitochondria.

Other examples of inherited iron overload

A number of other forms of inherited iron overload have been described for which defective genes have yet to be identified. The most prevalent of these is iron overload among indigenous peoples of sub-Saharan Africa. Although much of this iron overload may result from excessive iron intake, recent data suggest that there is an inherited tendency to absorb excessive amounts of iron from dietary sources (14).

Closer to home, there has been one report of a large Melanesian kindred with a hereditary form of iron overload which

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is histologically very similar to HHC.

Interestingly, this disorder appears to be inherited as an autosomal dominant trait (15). Cases of inherited iron overload have also been found in several populations, particularly those in Mediterranean regions, which are phenotypically similar to HHC but lack mutations in the *HFE* gene. Mutations in the recently described *TFR2* gene may explain at least some of these (16).

While discussing miscellaneous diseases one that should be mentioned is hereditary hyperferritinemia cataract syndrome (17). This is a rare autosomal dominant disorder in the regulation of the ferritin synthesis rather than a disorder of iron metabolism *per se*. Point mutations or deletions in the IRE of the ferritin mRNA abolish IRP binding and ferritin synthesis proceeds unchecked. Affected individuals do not show iron loading and the bilateral cataracts that characterize the disease appear to result from a high concentration of ferritin in the lens.

Inherited iron deficiency syndromes

Each of the disorders of iron metabolism described above is characterised by iron overload of one form or another. Inherited disorders of iron deficiency are rarely observed and there are only occasional reports of refractory anaemias which may fall within this category (18,19). The reason for the relative paucity of inherited iron deficiency disorders is not clear.

It may indicate that the liabilities of iron excess are more severe than the liabilities of iron depletion, or simply that the deleterious effects of iron deficiency are more insidious and difficult to recognise clinically. The human body is well adapted to mount an effective response to iron depletion by increases in intestinal iron absorption and iron uptake by body cells, but it is ill-adapted to disposing of excess iron.

Inherited rodent disorders of iron metabolism

No discussion of inherited disorders of iron metabolism would be complete without considering several inherited iron deficiency anaemias in rodents that have played pivotal roles in helping to understand mechanisms of cellular iron homeostasis. Positional cloning of the

gene affected in the microcytic anaemic (mk) mouse led to the identification of DMT1 as an iron transporter (20) and subsequent studies showed that an anaemic rat strain, the Belgrade rat, also carries a mutation in DMT1.

Extensive phenotypic studies of these animals has provided much of our current knowledge of the role played by DMT1 and in intestinal iron absorption and in transferrin-bound iron uptake. Similarly, phenotypic studies of the sex-linked anaemic (*sla*) mouse delineated the function of hephaestin in iron release from intestinal epithelial cells long before the gene itself was isolated using this strain by positional-candidate analysis (3). None of these strains appears to have a well-defined human counterpart, although some refractory anaemias in humans could be due to mutations in the homologous human genes.

Conclusions

Inherited disorders of iron metabolism (Table I) are generally rare and many remain clinical oddities, but some, such as haemochromatosis, are sufficiently common to be of significance for population health. Over the last five years the application of modern molecular genetics has enabled the genes affected in many of these diseases to be identified with resulting improvements in diagnosis and, in the future, therapy. The identification of these new molecules has also provided essential mechanistic insights into the pathways of normal iron homeostasis. This is an exciting time to be in the field of iron metabolism and there is no

end in sight to the tide of stimulating new discoveries.

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Table I. Summary of some inherited disorders of iron metabolism

Disease	Iron-related phenotype	Chromosomal location	Gene affected	Population frequency ^a	Main Clinical features
Human					
Haemochromatosis	Iron overload	6	HFE	1:250	Liver disease
African iron overload	Iron overload	Unknown	Unknown	Common ^b	Liver disease
Atransferrinemia	Iron overload	3	Transferrin	Rare ^c	Liver and heart disease
Aceruloplasminemia	Iron overload	3	Ceruloplasmin	Rare ^c	Neurological disease
Hereditary hyperferritinemia cataract syndrome	Lenticular ferritin deposition	19	L-ferritin	Rare ^d	Bilateral cataracts
Friedreich Ataxia	Iron overload (mitochondria)	9	Frataxin	1:50,000	Neurological and heart disease
Mouse					
Microcytic anaemia	Iron deficiency	15	DMT1	-	Anaemia
Sex-linked anaemia	Iron deficiency	X	hephaestin	-	Anaemia

^a The incidences reported are generally for populations in which the disease is most prevalent.

^b The proportion due to inherited factors has yet to be determined.

^c These disorders are extremely rare.

^d There is insufficient experience with this disorder for an accurate incidence to be given.

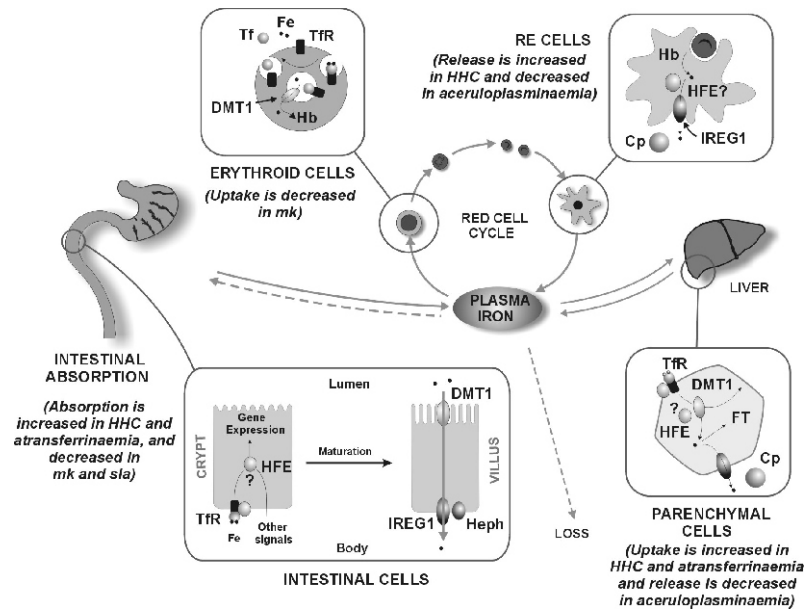


Fig. 1

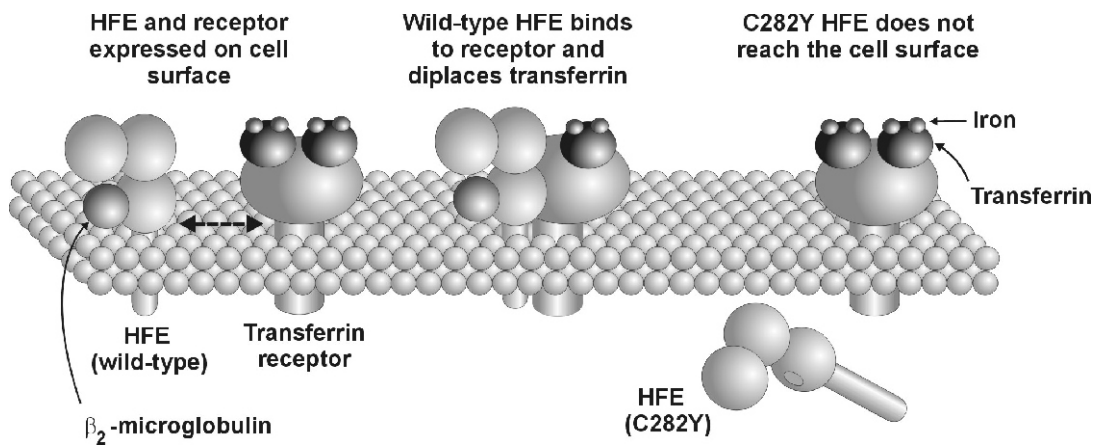


Fig. 2