

Role of *HFE* in iron metabolism, hereditary haemochromatosis, anaemia of chronic disease, and secondary iron overload

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Hereditary haemochromatosis is an iron overloading disorder caused by common mutations in the *HFE* gene. However, information with respect to the function of *HFE* protein does not explain how mutations in *HFE* lead to hereditary haemochromatosis. We propose a molecular model in which *HFE* has two mutually exclusive activities in cells: inhibition of uptake or inhibition of release of iron. The balance between serum transferrin saturation and serum transferrin-receptor concentrations determines which of these functions predominates. With this input, *HFE* enables the intestinal crypt cells and reticuloendothelial system to interpret the body's iron requirements and regulate iron absorption and distribution. In our model, mutations in *HFE* result in overabsorption of dietary iron, and patterns of tissue iron deposition in agreement with clinical observations of hereditary haemochromatosis.

Healthy adult human beings accumulate 3–5 g of iron.¹ Most of the iron is distributed between the haemoglobin of red cells, the liver, and in macrophages of the reticuloendothelial system. Iron is recycled continually by breakdown of dying red cells in macrophages and release of iron to serum transferrin. Iron is transported in the circulation bound to transferrin and taken up by transferrin receptors on parenchymal tissues and bone marrow for reincorporation into red cells. About 20–30 mg of iron is recycled each day. By contrast about 1–2 mg of iron is lost from the body each day by processes such as sloughing of skin, and menstruation. This lost iron is replaced by absorption of dietary iron through the mucosa of the duodenum. Iron loss does not seem to be regulated; it is absorption that is responsive to changing iron requirements and is critical to maintaining iron homeostasis.

Research has provided insights into the mechanism of iron absorption by the enterocytes of the duodenal mucosa. Ferric iron (Fe^{3+}) in food is reduced to Fe^{2+} and then absorbed into the enterocyte by an iron transporter protein DMT-1. Iron then crosses the enterocyte and is exported from its basolateral surface by another iron transporter, IREG/ferroportin/mtp-1 (hereafter referred to as ferroportin). In the export process the iron is reoxidised to Fe^{3+} and bound to serum transferrin. The concentrations of DMT-1 and ferroportin expressed in the mature enterocyte affect how much iron is absorbed from the diet. The available evidence suggests that as the cells of the intestinal crypt mature into absorptive enterocytes, the ultimate amount of expression of DMT-1 and ferroportin are programmed with reference to the concentration of iron within the crypt cell. Expression of DMT-1² and ferroportin in the enterocyte increases as the concentration of iron in the crypt cell falls, probably through the action of the intracellular iron response proteins 1 and 2.

The mechanism by which the crypt cells accumulate iron and thus sense body iron needs is not fully understood, but is likely to involve uptake of transferrin bound iron from the circulation³ via the transferrin receptor-1, which is expressed at high concentrations in the crypt. The amount of iron absorbed from the gut is inversely related to the degree of transferrin saturation in serum.³ Mice and human beings that lack transferrin expression become overloaded with iron due to uncontrolled absorption of iron from the gut.⁴ Raja and colleagues reported that intestinal absorption could be reduced in affected animals by repeated injections with transferrin.⁴ These results suggest that the main supply of iron to the intestinal crypt cell is from serum transferrin. In the absence of transferrin the crypt becomes starved of iron leading to maximum activation of the genes involved in iron absorption in the maturing enterocyte. As transferrin saturation rises, the crypt accumulates iron and decreases iron absorption accordingly.

HFE, the protein that is mutated in the common form of hereditary haemochromatosis, is expressed in the crypt cell, and is likely to play a key role in coupling the iron sensing mechanism of crypt cells to iron absorption by the mature enterocyte.⁵ We offer a new hypothesis for the role of *HFE* in the processes of iron sensing, absorption, and storage to explain the abnormalities of hereditary haemochromatosis and some other disorders of iron metabolism.

Hereditary haemochromatosis and *HFE*

Hereditary haemochromatosis is a common inherited disorder of iron metabolism.^{1,6} The predominant feature of the condition is excessive absorption of dietary iron,¹ with a relative paucity of iron in reticuloendothelial stores.⁷ Eventually, deposition of iron in parenchymal tissues results in cirrhosis of the liver, diabetes mellitus, skin pigmentation, and testicular failure.^{1,6} An adequate molecular explanation for the disease is still lacking, despite tremendous progress in identifying the defective gene *HFE*, the mutations that result in disease, and some of the biochemical properties of the encoded protein *HFE*.¹

In 1996, Feder and colleagues used positional cloning techniques to isolate the *HFE* gene.⁸ The primary structure of the gene showed homology to class I MHC

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proteins (that are composed of a heavy chain associated with beta-2 microglobulin). Analysis of the crystal structure of recombinant HFE protein confirmed the close structural homology with class I MHC proteins.⁹ This finding lent credence to the earlier observation that mice lacking beta-2 microglobulin expression became overloaded with dietary iron.¹⁰ Two inherited mutations in *HFE* are linked with disease: C282Y and H63D.⁸ The exchange of Y for C at position 282 of the alpha 3 domain of HFE heavy chain disrupts the association with beta-2 microglobulin, causing misfolding of the protein.¹¹ The H63D mutation does not affect assembly or expression of HFE,^{9,12} and the biochemical effect of this mutation is unknown.

HFE binds transferrin receptor 1

The availability of recombinant HFE protein and specific antisera led rapidly to the observation that HFE can be non-covalently bound to transferrin receptor 1 in tissues and cell lines.^{5,13,14} Further work showed that HFE competitively inhibits the binding of iron-loaded transferrin to transferrin receptor 1.¹² The corollary of this observation in several tissue culture cell lines is that overexpression of HFE results in about a 30% reduction in the rate of iron uptake, with commensurate reductions of intracellular iron and ferritin.^{14,15} However, these experiments were done *in vitro* with either low concentrations of human transferrin or with bovine transferrin, which has a low affinity for human transferrin receptor. HFE might effectively inhibit iron uptake in competition with the very high concentrations of human iron-transferrin (about 1 mg/mL, 10 μ M) seen in plasma. Nevertheless, these observations did lead to the hypothesis that the function of HFE *in vivo* is to inhibit transferrin-bound iron uptake by cells. If HFE was defective, cells would tend to take up excess iron, as is seen in many tissues of patients with hereditary haemochromatosis.

This view would predict that those cells noted to express high levels of wild-type HFE protein in normal individuals would be the same ones to become iron loaded in hereditary haemochromatosis patients who express mutated HFE protein. However, the opposite is true. Tissue staining experiments have shown that HFE is reproducibly detected in cells of the intestinal crypt and in reticuloendothelial Kupffer cells in the liver.^{16,17} In patients with hereditary haemochromatosis, and mice that lack either the beta-2 microglobulin gene or *HFE*, the crypt cells behave as though starved of iron.^{1,2,18,19} They upregulate the components required for intestinal iron absorption in the mature enterocytes into which they differentiate.^{2,19,20} Similarly, reticuloendothelial cells from patients with hereditary haemochromatosis (and beta-2 microglobulin or *HFE* knockout mice) tend not to accumulate iron appropriately *in vivo*,^{2,18,19} and release low molecular weight iron at an increased rate *in vitro*.²¹ Expression of wildtype *HFE* in hereditary haemochromatosis monocytes *in vitro* results in greater accumulation of iron and ferritin.²² We have also noted that exposure to HFE results in accumulation of iron and ferritin with downregulation of transferrin receptor 1 in the reticuloendothelial cell line THP-1.²³ These observations are not compatible with the dominant function of HFE as a competitive inhibitor of transferrin binding to transferrin receptor 1. The findings suggest that, in these specialised cells, HFE either enhances uptake of iron, as suggested by Wahed and colleagues,⁵ or inhibits the release of iron.¹⁷ It is this second possibility that we focus on in our hypothesis. The inhibited iron

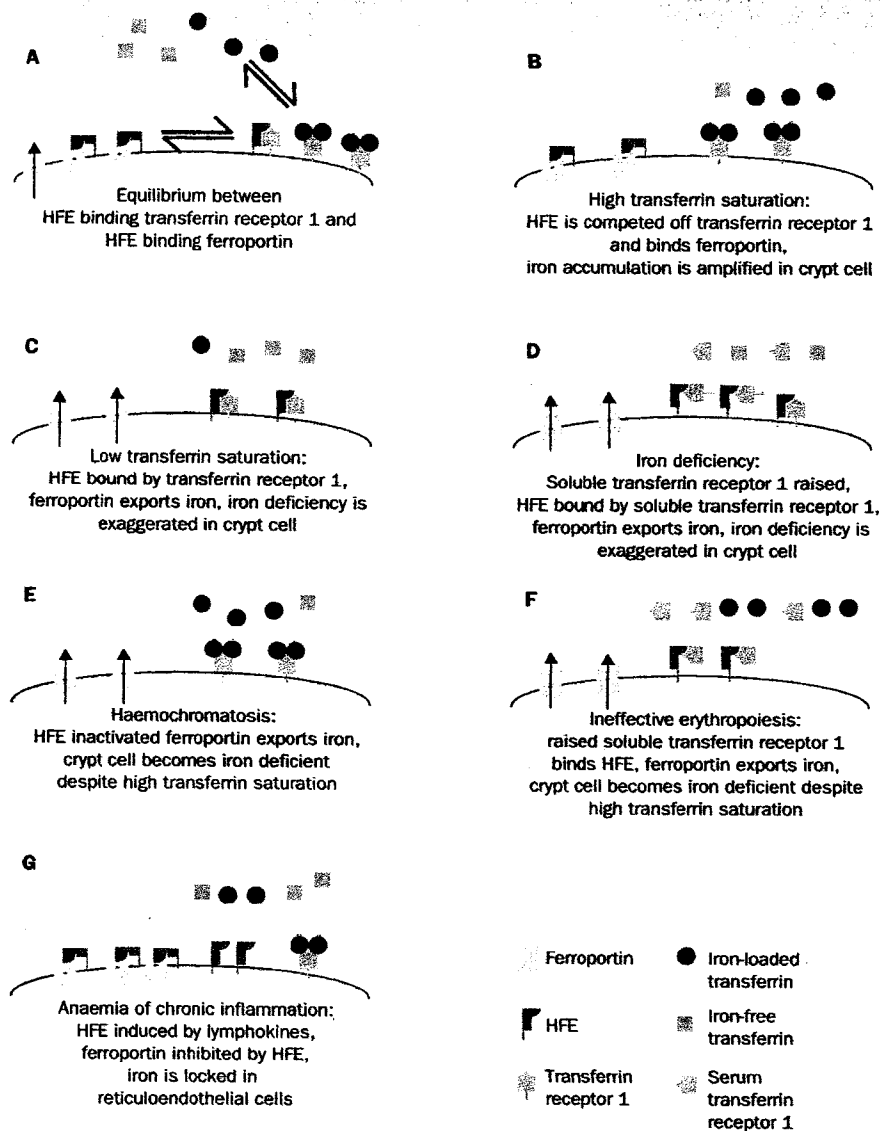
exporter could be ferroportin, which like HFE is expressed in liver Kupffer cells,²⁴ and at low concentrations in the intestinal crypt.²⁰

Hypothesis: model for HFE activity

The advantage of the notion that HFE can inhibit iron export is that it naturally suggests a signal enhancing function for HFE through a positive feedback loop. Consider a crypt cell that expresses ferroportin, HFE, and transferrin receptor 1 (figure). Suppose that HFE binding to transferrin receptor 1 is competed for by iron-loaded transferrin,¹² so that the proportion of HFE molecules on the cell surface bound by transferrin receptor 1 is inversely related to the degree of transferrin saturation in serum. HFE not bound to transferrin receptor 1 is available to inhibit ferroportin, so that an equilibrium exists between HFE bound to transferrin receptor 1 and HFE that is available to inhibit ferroportin (figure). When transferrin saturation is high, HFE dissociates from transferrin receptor 1, binds ferroportin, inhibits iron export, and enhances iron accumulation within the cell (figure). The iron-loaded crypt cells then mature into enterocytes programmed to absorb less dietary iron. When transferrin saturation is low, HFE is predominantly bound by transferrin receptor 1, ferroportin becomes active, and intracellular iron is further reduced (figure). The iron-deficient crypt cell then matures into an enterocyte with an increased capacity for iron absorption. In both instances the presence of HFE amplifies the signal from transferrin saturation to the crypt cell. In the reticuloendothelial cell the same positive feedback loop would enhance iron storage in conditions of high transferrin saturation, and stimulate iron release in response to low serum transferrin saturation. Because the degree of transferrin saturation is a reflection of body iron stores, this mechanism would allow direct sensing of iron stores by the crypt cells and reticuloendothelium.

The second part of our model is the effect of soluble transferrin receptor 1. The concentration of soluble transferrin receptor 1 in serum reflects the overall rate of synthesis of transferrin receptor 1, most of which is expressed in red cell precursors in bone marrow (the "erythron"). Concentrations rise in iron deficiency states once iron stores have been depleted and in conditions associated with ineffective erythropoiesis (such as the thalassaemia syndromes). In thalassaemia, concentrations can rise by more than ten fold.²⁵ In experiments by Lebron and colleagues,⁹ the conditions revealing the highest binding affinity of transferrin receptor 1 for HFE arose when soluble transferrin receptor 1 was exposed to fixed HFE, as would be the case here. In our model, soluble transferrin receptor would bind HFE in the membranes of the crypt and reticuloendothelial cells, and thus release ferroportin to export intracellular iron (figure), resulting in a reduction in the concentration of iron in the cell. The effect in the crypt would be to programme the mature enterocyte for increased dietary iron absorption. Reticuloendothelial cells would respond by increased iron export. Thus, the effect of soluble transferrin receptor would allow direct sensing of the activity of the "erythron" by the crypt and reticuloendothelium.

These two parts of our model could account for both the "stores regulator" and the "erythron regulator"²¹ through the common final path of the inhibitory action of HFE on iron export. However, we point out that the model does not exclude other components that could interact with, or alter the concentrations of, the three main interacting molecules: HFE, transferrin receptor 1, and ferroportin. Furthermore, the model does not exclude



Model for the function of HFE in iron metabolism

The cell depicted expresses HFE, transferrin receptor 1, and ferroportin, and represents either an intestinal crypt cell or a Kupffer or other reticuloendothelial cell. A: Transferrin saturation affects the equilibrium between HFE binding either transferrin receptor 1 or ferroportin. B: High transferrin saturation drives the equilibrium towards HFE binding ferroportin. C: Low transferrin saturation drives the equilibrium towards HFE binding transferrin receptor 1. D: Raised serum transferrin receptor 1 in iron deficiency prevents HFE binding ferroportin. E: Loss of HFE in hereditary haemochromatosis uncouples ferroportin from the effect of transferrin saturation. F: Serum transferrin receptor 1 released from bone marrow due to ineffective erythropoiesis prevents HFE binding ferroportin. G: HFE induced by inflammatory cytokines inhibits ferroportin in reticuloendothelial cells.

alternatives to these three main components that would have the same functions (for instance, an additional HFE like molecule associated with beta-2 microglobulin,²⁶ or an alternative to ferroportin that is expressed in crypt and Kupffer cells).

Explanatory power of the model for disease states and animal models

Hereditary haemochromatosis

The common mutation in the *HFE* gene associated with hereditary haemochromatosis is C282Y, which is known to abolish the interaction between HFE and beta-2 microglobulin,¹¹ and with transferrin receptor-1. We suggest that the inhibitory interaction with ferroportin is also lost. Our model predicts that in cells bearing the C282Y mutation iron export would continue unabated with the result that intracellular iron concentrations in the crypt and reticuloendothelial cells would fall to inappropriately low concentrations for the degree of

serum transferrin saturation (figure). The crypt would continually underestimate the degree of transferrin saturation and maintain the mature enterocytes in a programme for iron absorption, by enhancing expression of the iron importer DMT1 and the ferroportin exporter.^{2,19,20} Reticuloendothelial cells would export their iron, and store an inappropriately low proportion of total body iron. These are the predominant biochemical features of the common form of haemochromatosis.

Genes that modify the haemochromatosis phenotype in animals

Knockout of a single copy of the transferrin receptor-1 gene (that presumably results in reduced transferrin receptor-1 protein expression), results in reduced iron accumulation in mice.^{26,27} Our model would predict that lowering the availability of transferrin receptor 1 to the crypt cell would free up HFE to inhibit ferroportin. The net result would be enhanced accumulation of iron by the

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