

Of metals, mice, and men: what animal models can teach us about body iron loading

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J Clin Invest. 2000;**105**(9):1185-1186. <https://doi.org/10.1172/JCI9972>.

Commentary

Hemochromatosis is a common inherited disease of iron overload that results from mutations in the HFE gene (1). It has long been known that basic processes of body iron homeostasis, and in particular intestinal iron absorption, are regulated aberrantly in hemochromatosis, so an understanding of how HFE functions in cellular and body iron homeostasis may hold the key to dissecting these essential physiological processes. However, despite a growing body of elegant experimentation, the recalcitrant HFE molecule has yet to reveal all its secrets. The clinical features of hemochromatosis are almost exclusively due to increased iron storage in the parenchymal cells of many tissues, particularly the liver (2). However, the iron accumulation and the associated clinical presentation can be highly variable, even within one family, and this remains one of the most puzzling aspects of the disease. A recent population study (3) found that only half of those who were homozygous for the major HFE mutation had clinical features of hemochromatosis and one quarter had serum ferritin levels (a marker of body iron stores) that remained normal over a 4-year period. Environmental factors such as physiological and pathological blood loss sometimes explain these differences, but often they do not, and it seems more likely that genetic factors are involved. Furthermore, there are clear cases, particularly in Mediterranean populations (4), of iron [...]

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Hemochromatosis is a common inherited disease of iron overload that results from mutations in the *HFE* gene (1). It has long been known that basic processes of body iron homeostasis, and in particular intestinal iron absorption, are regulated aberrantly in hemochromatosis, so an understanding of how HFE functions in cellular and body iron homeostasis may hold the key to dissecting these essential physiological processes. However, despite a growing body of elegant experimentation, the recalcitrant HFE molecule has yet to reveal all its secrets.

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the liver (2). However, the iron accumulation and the associated clinical presentation can be highly variable, even within one family, and this remains one of the most puzzling aspects of the disease. A recent population study (3) found that only half of those who were homozygous for the major *HFE* mutation had clinical features of hemochromatosis and one quarter had serum ferritin levels (a marker of body iron stores) that remained normal over a 4-year period. Environmental factors such as physiological and pathological blood loss sometimes explain these differences, but often they do not, and it seems more likely that genetic factors are involved. Furthermore, there are clear cases, particularly in Mediterranean populations

(4), of iron overload in which *HFE* coding sequences are normal. Thus, genes other than *HFE* and/or *HFE* mutations in introns or in flanking regulatory sequences may be responsible.

One might expect that the gene defective in a disorder with a variable phenotype would contain a spectrum of mutations with varying functional consequences, but this is not the case for hemochromatosis. A single missense mutation in *HFE* has been found to be responsible for the vast majority of clinical disease, so mutations in other genes must be invoked to explain the differences in iron loading. Three recent clinical studies (5–7) have provided evidence for hemochromatosis-modifying genes, but the identity of these genes is not known. The obvious candidate genes are those encoding proteins known to be important in iron transport. In this issue, Levy et al. (8) have examined the effects of several of these genes on iron loading by crossing *HFE* knockout mice (which show a phenotype very similar to human hemochromatosis) with other strains of mice with natural or engineered defects in other genes affecting iron homeostasis. While some of the results were predictable, others were not.

A major pathophysiological feature of hemochromatosis is an increase in intestinal iron absorption, so one might predict that mutations in genes important for intestinal iron transit would ameliorate the hemochromatosis phenotype. This is indeed the case, and *HFE* knockout mice carrying mutations in the gene for the brush border iron transporter DMT1 show reduced levels of hepatic iron accumulation. DMT1 is the major molecule facilitating the uptake of dietary iron by the epithelial cells of the proximal small intestine (9, 10), and mice defective in DMT1 (*mk* mice) have a profound microcytic, hypochromic anemia. The demonstra-

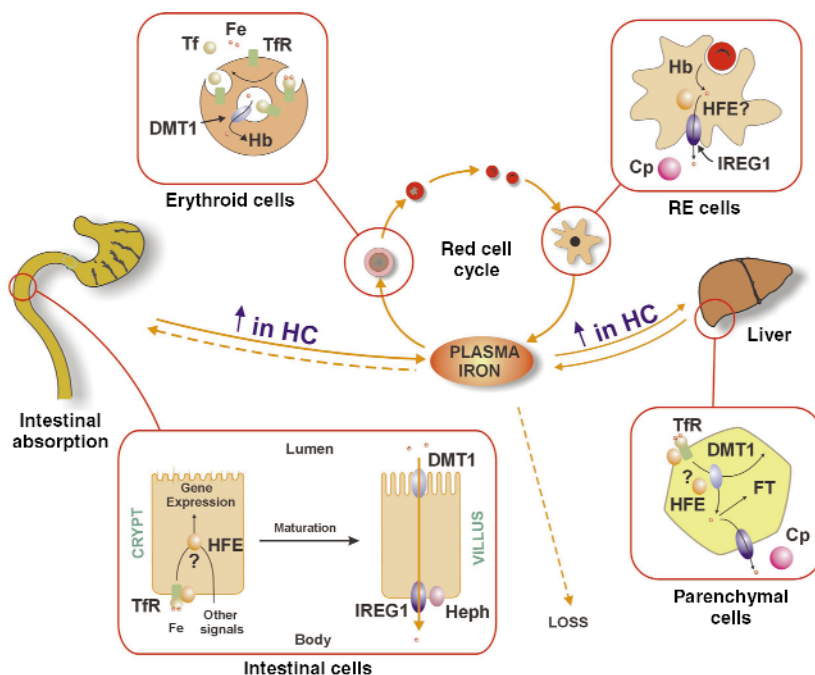


Figure 1

Pathways of iron metabolism showing sites where HFE may play a role. The basic components of iron influx and efflux are shown in intestinal, erythroid, reticuloendothelial (RE), and parenchymal cells. Tf, transferrin; Cp, ceruloplasmin; Fe, iron; Heph, hephaestin; FT, ferritin; Hb, hemoglobin; HC, hemochromatosis.

tion by Levy and colleagues (8) that deletion of the *HFE* gene cannot correct the *mk* phenotype shows that *DMT1* is the major route of iron entry into intestinal epithelial cells in hemochromatosis.

Genetic interactions between *HFE* and *DMT1* are only part of the story. The intestinal epithelial cell is a polarized cell and iron must traverse not only the apical membrane but the basolateral membrane as well. Insights into this iron efflux step have come from the cloning of the gene mutated in *sla* mice (11), another mouse strain with an inherited anemia. The affected gene has been named *Hephaestin* and it bears significant homology to the copper-containing plasma protein ceruloplasmin, which has long been known to play a role in iron release from tissues. *HFE* knockout mice carrying the *sla* mutation show considerably reduced hepatic iron levels, indicating that hephaestin plays an important role in iron loading. However, since the block to absorption is not complete, alternative basolateral iron transfer pathways appear to be operating. We would expect that disruption of the newly identified basolateral iron transporter gene *IREG1* (or *ferroportin1*) (12, 13) would also ameliorate the iron overloading phenotype of *HFE* knockout mice.

Somewhat more unexpected was the finding that *HFE* knockout mice also lacking the β_2 -microglobulin (*$\beta 2m$*) gene showed significantly more iron loading than *HFE* knockout mice with wild-type *$\beta 2m$* . Since *HFE* is a member of the MHC class I family, an interaction with *$\beta 2m$* is essential for its correct cellular processing and function, and deletion of the *$\beta 2m$* gene results in an iron overload phenotype (14). However, the findings of Levy et al. (8) suggest that *HFE* is not the only *$\beta 2m$* -associated protein that participates in body iron loading and support the evidence that another gene in the distal MHC is involved in this pathway (7).

The most intriguing finding of this study comes from a cross between *HFE* knockout mice (or mice carrying the major hemochromatosis mutation in their *HFE* gene) and mice lacking one transferrin receptor (*Trfr*) allele. Mice lacking both *Trfr* alleles die in utero and

cannot be studied, but mice with defects in *HFE* and with haploinsufficiency for the *Trfr* show a higher degree of hepatic iron loading than do other animals deficient in *HFE*. A decreased concentration of transferrin receptors in the erythroid compartment would be expected to promote iron-deficient erythropoiesis, which in turn would stimulate intestinal iron absorption. Decreased iron delivery to the intestinal crypt cells would exacerbate this effect and could lead to increased body iron content. More puzzling, however, is the situation when *HFE* is normal and one *Trfr* allele is missing. Animals with this genotype are either mildly iron-deficient (15) or normal with respect to iron status (8), indicating that haploinsufficiency for *Trfr* leads to increased iron loading only in the presence of defective *HFE*. The interaction between *HFE* and *Trfr* has been well documented (16), but the physiological relevance of this interaction is still unclear. Levy et al. suggest that the two gene products together establish a "set point" for body iron homeostasis, although no mechanism is proposed for how this might affect iron absorption and other iron trafficking within the body.

Are these studies relevant to iron homeostasis in humans? The answer is almost certainly yes, but to what degree variations in the known iron transport genes contribute to the clinical presentation of hemochromatosis and other iron disorders is not known. Several groups have sought mutations in *DMT1*, *$\beta 2m$* , and *Trfr* in individuals with iron overload, but these small studies have not yet demonstrated a clear association between polymorphisms in these genes and iron loading. The field of iron homeostasis is in a state of rapid flux. The last four years has seen the identification of several important genes of iron transport, and we are only just beginning to understand how they function to control iron uptake and release. Furthermore, it is likely that there are other genes that play important roles in determining iron status that have yet to be described. Under-

standing how these molecules operate in vivo is even more challenging. Iron homeostasis results from a complex interplay of signals and responses, and it is difficult to study such a complicated physiological process in vitro or in cell culture models. Consequently, in vivo approaches, such as the present one by Levy and colleagues, hold our best hope for elucidating the causes of human diseases of iron metabolism such as hemochromatosis.

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