

## Iron deficiency and iron overload: effects of diet and genes

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Like most essential nutrients, Fe needs to be maintained in the body at a defined level for optimal health, with appropriate adaptation to varying Fe needs and supply. The primary mechanism for controlling Fe level is the regulation of Fe absorption. Several different proteins have been identified as contributors to the process. Despite a complex regulatory system, Fe disorders (both Fe deficiency and Fe overload) occur. Fe deficiency is a common problem worldwide, resulting from inadequate dietary Fe and blood loss. Complications include pre-term labour, developmental delay, and impaired work efficiency. No specific genetic syndromes causing isolated Fe deficiency have been described, but animal studies and clinical observations suggest that such a relationship may be a possibility. Conversely, the known causes of Fe overload are genetic. Fe overload is less common than Fe deficiency, but can result in serious medical complications, including cirrhosis, primary liver cancer, diabetes, cardiomyopathy and arthritis. The most common and best characterized syndrome of Fe overload is hereditary haemochromatosis (HHC), an autosomal recessive disorder. Mutations in the HFE protein cause HHC, but the clinical presentation is variable. Of particular interest is the factor that some HFE genotypes appear to be associated with protection from Fe deficiency. Other genetic variants in the regulatory pathway may influence the likelihood of Fe deficiency and Fe overload. Studies of genetic variants in HFE and other regulatory proteins provide important tools for studying the biological processes in Fe regulation. This work is likely to lead to new insights into Fe disorders and potentially to new therapeutic approaches. It will not be complete, however, until coordinated study of both genetic and nutritional factors is undertaken.

### Iron deficiency: Iron overload: Hereditary haemochromatosis: Iron regulatory pathway

Fe is required for many life functions and is present in every cell. Its most important role is in O<sub>2</sub> transport: 90 % of the body's Fe is contained in erythrocytes as a component of the haemoglobin molecule. Fe also facilitates O<sub>2</sub> use and storage in muscles, interacts with cytochromes in cellular metabolism, and serves as a cofactor for the function of several tissue enzymes (Yip *et al.* 1998). Like most essential nutrients, Fe needs to be maintained in the body at a defined level for optimal health. However, sources of dietary Fe are variable, and many disorders cause excessive loss of Fe. In addition, requirements for Fe differ at different stages of life. To maintain the optimal Fe level the body needs to adjust to varying Fe needs and supply.

The primary mechanism for controlling Fe level is the regulation of Fe absorption by the gastrointestinal tract (Yip *et al.* 1998; Andrews, 1999). Once Fe is absorbed the body has no known physiological mechanisms to control Fe

excretion. The regulatory mechanism for Fe uptake is still under investigation, but several different proteins, and thus several genes, have been identified as contributors to the process (Feder *et al.* 1996; Andrews, 1999; Levy *et al.* 2000).

Despite a complex regulatory system, Fe disorders (both Fe deficiency and Fe overload) are important health problems. In the present review we consider current knowledge about the dietary and genetic contributors to Fe disorders, with special attention to the new insights that are emerging from the study of genetic disorders of Fe overload and their implications for public health. Our review addresses two questions: (1) to what extent do Fe disorders reflect problems in dietary Fe supply *v.* failures in the regulatory mechanism controlling Fe uptake; (2) how can knowledge about diet and genes help to reduce the problems of Fe deficiency and Fe overload?

**Abbreviations:** DMT1, divalent metal transporter; HHC, hereditary haemochromatosis; JH, juvenile haemochromatosis; RDA, recommended dietary allowance.

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### Iron deficiency

Fe deficiency is a common problem worldwide (Galan *et al.* 1998; Lawson *et al.* 1998; Yip *et al.* 1998), and includes a spectrum of conditions from mild Fe depletion without physiological impairment to Fe-deficiency anaemia. In the USA Fe deficiency is most common in young children, the elderly and women (Looker *et al.* 1997). Approximately 9 % of children aged 1–2 years are Fe deficient, and 2–3 % remain Fe deficient in later childhood. Men have low rates of Fe deficiency until middle age (<1 %), but 4 % of men aged 70–80 years are Fe deficient. Approximately 10 % of women are Fe deficient during their reproductive years, and at all ages beyond infancy Fe deficiency is higher in women than in men (Looker *et al.* 1997).

Fe deficiency represents an imbalance between the body's need for Fe and the amount of Fe absorbed. In developed countries this imbalance is most commonly caused by an inadequate amount of Fe in the diet (Yip *et al.* 1998). In developing countries, by contrast, parasitic infections or blood loss are a common cause of Fe deficiency. Under normal conditions the absorption of Fe is sufficient to replace Fe losses, and to maintain both Fe stores and an adequate serum Fe level. A small amount of Fe is lost on a daily basis through faeces and desquamation of skin and mucosal cells (approximately 1 mg/d). Gastro-intestinal absorption normally varies with Fe status, but cannot always compensate for dietary insufficiency. During their reproductive years women lose additional Fe through menstruation (approximately 0.3–0.5 mg/d) and in child-birth (Yip *et al.* 1998; Andrews, 1999). Normally only about 5–15 % of the dietary Fe is absorbed; as a result, adult men require on average about 10 mg dietary Fe/d and women of reproductive age about 15 mg/d (Yip *et al.* 1998). When the body's Fe needs increase for physiological or pathological reasons, Fe absorption increases (Andrews, 1999). Fe content of the diet and its bioavailability also play a critical role in the amount of Fe absorbed (Yip *et al.* 1998; Andrews, 1999).

The body conserves Fe once it has been absorbed. When erythrocytes are destroyed, the Fe in them is recycled. About 95 % of the Fe used in production of new erythrocytes in men, for example, is estimated to come from recycled Fe (Yip *et al.* 1998). Fe is stored in soluble form as a component of serum ferritin and in insoluble form as a component of haemosiderin. A small amount of ferritin is present in the serum, but under normal circumstances most stores are found in the liver, bone marrow, spleen and skeletal muscles. The majority of Fe (60–70 %) is stored as ferritin (Yip *et al.* 1998; Andrews, 1999). These stores are progressively depleted when inadequate amounts of Fe are absorbed.

In a 1994–6 survey by the US Department of Agriculture, more than half the children aged 1–2 years were found to have dietary Fe below the recommended dietary allowance (RDA; US Department of Agriculture, Agricultural Research Service, 1997). Similarly, a substantial proportion of women at all ages had Fe-poor diets. During their reproductive years only one-quarter of the women surveyed met the RDA for Fe (US Department of Agriculture, Agricultural Research Service, 1997). Data from other

developed countries present a similar picture. In a British survey 12 % of children aged 2 years had Fe-deficiency anaemia (Lawson *et al.* 1998). A French survey reported that 23 % of menstruating women and 5 % of post-menopausal women had Fe depletion (serum ferritin <15 µg/l; Galan *et al.* 1998). In addition, 93 % of menstruating women had dietary Fe intakes lower than the RDA and 53 % consumed less than two-thirds of the RDA (Galan *et al.* 1998). Rates of Fe deficiency are even higher in undeveloped countries and among vulnerable groups such as immigrants, racial and ethnic minorities and the economically disadvantaged (Lawson *et al.* 1998; Yip *et al.* 1998; Wharton, 1999).

Variation in Fe status may reflect the form as well as the amount of Fe in the diet. Bioavailability of Fe varies with several factors. Haem-Fe (Fe bound to haemoglobin) has the greatest bioavailability (Yip *et al.* 1998), and is found in meat, poultry and fish. It constitutes 5–10 % of the Fe in Western diets (Lawson *et al.* 1998; Yip *et al.* 1998) and is absorbed separately from other forms of Fe, in an efficient uptake mechanism that may involve cellular endocytosis (Andrews, 1999). Absorption of non-haem-Fe is mediated by a cell-membrane protein (divalent metal transporter-1; DMT1) and is subject to a variety of modifying influences that may enhance or inhibit uptake, as shown in Table 1 (Yip *et al.* 1998; Andrews, 1999). The bioavailability of Fe may vary considerably in diets poor in haem-Fe, depending on both the food source containing the Fe and the other foods eaten with it. In addition Fe status influences the efficiency of Fe absorption (Yip *et al.* 1998; Andrews, 1999).

Fe deficiency is more likely to occur when Fe needs are increased. Thus, Fe deficiency is more common in women of child-bearing age, especially in pregnancy and in periods of rapid growth as in early childhood and adolescence, when Fe needs exceed what is usually supplied in the diet. Excess loss of Fe, usually from blood loss, is also a factor (Yip *et al.* 1998; Andrews, 1999; Wharton, 1999). Fe loss is usually due to pathological conditions such as peptic ulcer disease, hookworm or other conditions causing bleeding. In infancy blood loss from the gastrointestinal tract can occur as a result of an allergic response to cow's milk (Wharton, 1999). Fe loss can also occur with menstrual bleeding, and with disorders that result in problems in gastrointestinal absorption (Provan, 1999).

About one-third to half the Americans with Fe deficiency have Fe-deficiency anaemia (Yip *et al.* 1998), with multiple clinical effects (Yip *et al.* 1998; Andrews, 1999). In infants Fe-deficiency anaemia can cause developmental delay and behavioural problems. In adults Fe-deficiency anaemia can impair work efficiency and cause symptoms of fatigue and

**Table 1.** Dietary factors that affect uptake of non-haem-iron (Yip *et al.* 1998; Skikne, 2000)

Enhancers of Fe absorption	Inhibitors of Fe absorption
Vitamin C	Polyphenols (in some vegetables) Tannins (in tea)
Low pH	Phytates (in bran)
High protein intake	Ca

malaise. Fe deficiency during pregnancy can result in preterm labour and low-birth-weight babies.

The prevalence of dietary intake below the RDA is substantially higher than the prevalence of Fe deficiency in both men and women (Yip *et al.* 1998). This observation suggests that many individuals can maintain adequate Fe levels in the face of suboptimal dietary Fe, and may be an indicator of varying efficiency of Fe uptake. If such variation occurs, both nutritional and genetic factors are likely contributors. No specific genetic syndromes causing isolated Fe deficiency have been described, but some anaemia syndromes suggest this possibility. For example, a congenital Fe-deficiency anaemia has been described that fails to respond to oral Fe supplements but is resolved with parenteral Fe; an inherited disorder of gastrointestinal absorption of Fe could explain this clinical picture (Andrews, 1999). Mouse studies also provide support for this possibility. Differences in response to Fe-deficient diets have been observed in genetically-distinct mice (Morse *et al.* 1999), suggesting that genetic variation contributes to Fe status. In addition, mutations in two proteins involved in Fe metabolism have been shown to cause Fe deficiency in mice (Levy *et al.* 2000).

### Iron overload

When excess Fe is absorbed for a prolonged period it exceeds the storage capacity provided by ferritin and haemosiderin. The result is the accumulation of catalytically-active Fe that causes tissue damage (Brown & Bacon, 2000). Fe overload is less common than Fe deficiency, but can result in serious medical complications, including cirrhosis, primary liver cancer, diabetes, cardiomyopathy and arthritis (Bothwell *et al.* 1995). Most Fe overload occurs on a genetic basis. Data on the prevalence of Fe overload have derived primarily from studies in Europe, Australia and North America. In these studies biochemical evidence of Fe overload, i.e. serum Fe measures or tissue biopsies indicating excess Fe stores, occurs in up to 0.5 % of the population (Bradley *et al.* 1998; Burt *et al.* 1998). The proportion of individuals with abnormal Fe measures who will ultimately develop clinical complications is unknown, however, and may be substantially lower (Cogswell *et al.* 1998). Fe overload is more common in men than in women. Symptoms typically occur at about 45 years or later. The prevalence of Fe overload among individuals of non-European ethnicity has not been systematically studied, but the condition has been described in sub-Saharan Africans and in African-Americans (Gorduek *et al.* 1992; Barton *et al.* 1995). One study of Hispanic Americans found a prevalence of Fe overload similar to that of European Americans (Centers for Disease Control and Prevention, 1996).

### *Hereditary haemochromatosis*

Hereditary haemochromatosis (HHC) is the best characterized genetic disorder of Fe metabolism, and accounts for most of the Fe overload occurring in individuals of European descent. HHC is an autosomal recessive condition resulting in increased absorption of Fe from the gastro-

intestinal tract, leading to a slow accumulation of excess Fe and the complications of Fe overload (Bothwell *et al.* 1995). Regulation of non-haem-Fe absorption still occurs in HHC (i.e. non-haem-Fe absorption increases or decreases based on Fe stores), but the regulatory system is impaired (Conrad *et al.* 2000). Further, absorption of haem-Fe appears to be insensitive to Fe stores; as a result, a net accumulation of Fe occurs with time (Conrad *et al.* 2000). Linkage of HHC to the human leucocyte antigen A3 complex on chromosome 6 was described more than 20 years ago (Simon *et al.* 1976), and the gene, now termed HFE (Bodmer *et al.* 1997), was identified in 1996 (Feder *et al.* 1996). With gene discovery, two missense mutations of the HFE gene, C282Y and H63D, were described, accounting for 88 % of the 178 patients with HHC in the initial study. Studies of HHC and of these two HFE mutations provide several insights into Fe metabolism and the aetiology of Fe disorders.

First, the relationship between HFE genotype and the presence of clinical disease is complex. The majority of patients with HHC carry two copies of the C282Y mutation; this genotype carries the highest risk of Fe-overload disease (Burke *et al.* 2000). However, an increased risk of Fe overload is associated with other HFE genotypes as well, including genotypes that contain the less-severe H63D mutations. While most patients with HHC carry two HFE mutations, disease also occurs rarely in carriers, that is, individuals who carry only one HFE mutation (Burke *et al.* 2000). Fe-overload disease in individuals thought to be carriers could be due to additional mutations in the HFE gene. For example, a third HFE missense mutation, S65C, has been implicated in a mild form of HHC in one study (Mura *et al.* 1999). Seven other allelic variants of the HFE gene have now been reported (National Center for Biotechnology Information, 2000), but their clinical significance is still unknown. Conceivably these variants could contribute to disease in some carriers of C282Y or H63D mutations, and could lead to variation in Fe uptake that is not sufficient to cause Fe overload.

More importantly, a high-risk genotype does not inevitably lead to Fe overload. Elderly individuals have been reported who have the C282Y/C282Y genotype but are without Fe overload (Adams *et al.* 1997a; Bacon & Sadiq, 1997; Olynyk *et al.* 1999). In addition, the severity of disease in symptomatic patients is variable (Adams, 1992; Niederau *et al.* 1996). These data indicate that the clinical effects of HFE deficiency may be either exacerbated or minimized by modifying factors.

One important modifier is gender. In family studies of haemochromatosis, equal numbers of brothers and sisters carry haemochromatosis genotypes, as would be expected for an autosomal recessive disorder. Among patients with clinical symptoms, however, the proportion of females is much lower, ranging from 11 to 35 % in three large reported series of studies (Fargion *et al.* 1992; Niederau *et al.* 1996; Adams *et al.* 1997b). In a screening trial the prevalence of Fe overload ascertained by liver biopsy or phlebotomy was twice as frequent in males as females (Niederau *et al.* 1998). This gender difference has been attributed to Fe loss from menstruation, pregnancy and lactation, but could also be influenced by gender differences in Fe intake.

Dietary factors are assumed to play a role in HHC disease expression, although this issue has not been studied systematically (Halliday, 1998). For example, Fe and vitamin C supplements and high intake of dietary Fe, particularly in the form of haem-Fe, would be expected to increase the rate of Fe accumulation, and therefore the likelihood of symptomatic disease in HHC. Studies with rats have shown that hepatic Fe deposits similar to those seen in haemochromatosis can be produced with excess dietary Fe, although in this animal model the dietary Fe was forty to ninety times the control level, well beyond the variation expected in human dietary intake (Ramm, 2000). Excessive alcohol consumption has been shown to increase the likelihood of liver disease in individuals with HHC (Adams *et al.* 1997b); chronic hepatitis may have a similar effect (Fargion *et al.* 1992).

Finally, HFE mutations are common among individuals of European descent; about 10 % carry the C282Y mutation and more than 20 % carry the H63D mutation (Table 2). These high carrier rates suggest that carriers might have a selective advantage, possibly protection from Fe deficiency. Several studies demonstrate higher Fe levels in HHC carriers compared with non-carriers (Bulaj *et al.* 1996; Edwards *et al.* 2000; Whitfield *et al.* 2000). A population-based twin study in Australia involving 3375 adult twins found that Fe status in both C282Y and H63D heterozygotes differed from that of those with normal genotypes (Whitfield *et al.* 2000). The difference was small; less than 5 % of phenotypic differences in Fe status were accounted for by these genes. Of equal importance, the twin study provided evidence for additional genetic factors that have a substantial effect on Fe status.

Two other studies failed to observe a difference in Fe status between C282Y carriers and those with a normal HFE genotype, and found no evidence for reduced rates of Fe-deficiency anaemia in carriers (Boulton *et al.* 2000; Rossi *et al.* 2000). However, these studies found reduced Fe deficiency among individuals with C282Y/H63D and H63D/H63D genotypes. These genotypes are associated with a low risk of Fe overload (Fe-overload disease is expected to develop in 1 % or fewer of those with a C282Y/H62D genotype and in fewer than 1 % of those with the H63D/H63D genotype; Burke *et al.* 2000), and both occur in approximately 2 % of individuals of European descent (Table 2). Thus, at minimum, these studies indicate that at least 4 % of northern Europeans carry genotypes offering protection from Fe deficiency. Differences between studies could be accounted for by differences in Fe intake or other modifying influences. Taken together, the studies are

consistent with improved Fe status in at least some HHC carriers.

#### *Other iron-overload syndromes*

Genetically-distinct syndromes of Fe overload other than HHC have been described. The additional syndromes further illustrate the complexity of the Fe regulatory system. Two of these conditions (non-human leucocyte antigen-linked haemochromatosis and juvenile haemochromatosis (JH)) are autosomal recessive, and thus could represent other genetic conditions associated with a selective advantage for carriers.

*Hereditary haemochromatosis-like syndrome, unlinked to the chromosome 6 human leucocyte antigen region.* Families have been described with an inherited Fe-overload syndrome that is clinically indistinguishable from HHC but is unlinked to chromosome 6 (Pietrangelo *et al.* 1999). A recent Italian study reported the identification of a new gene that is linked to Fe-overload disease in two Sicilian families with this disorder (Camaschella *et al.* 2000). The gene, TFR2, codes for a protein which has a 66 % homology to the transferrin receptor, binds transferrin and is presumed to play a role in cellular Fe uptake.

*Juvenile haemochromatosis.* Juvenile haemochromatosis (JH) differs from typical HCC by its severity. While clinical expression of HHC is more common in males, JH affects both sexes equally. Fe accumulation begins early in life and causes clinical symptoms before the age of 30 years (Kaltwasser, 2000). If untreated, the disease is lethal due to cardiac complications. Although the organ damage in JH is more severe, Fe distribution is similar to that in HHC, as inferred by liver biopsies and autopsy findings (Kaltwasser, 2000). In patients with JH the rate of Fe absorption is two- to fivefold higher than that in patients with HHC. The gene for JH has recently been mapped to chromosome 1 (Roetto *et al.* 1999); the function of the protein for which it codes is unknown.

*Iron overload in Africans and African-Americans.* Fe overload has been observed among Bantus in sub-Saharan Africa and among African-Americans. Fe overload in African-Americans differs clinically from HHC in that transferrin saturation levels are often lower and HFE mutations have not been found in affected individuals (Barton *et al.* 1995). In addition, the pattern of Fe deposition in the liver differs. In Fe overload in Africans Fe accumulates primarily in reticulo-endothelial cells. In Africa Fe overload was originally observed in association with alcoholic beverages made in iron pots, and was assumed to be caused by high Fe intake. However, Fe overload was also found among individuals who were not heavy drinkers, and not all drinkers developed Fe overload. Family studies suggest genetics as a causative factor, but the genetics of these Fe-overload conditions remain poorly understood (Gorduek *et al.* 1992; Bloom *et al.* 2000).

*Other iron-overload syndromes.* Rare families have been reported with atransferrinaemia and Fe overload; the cause of the disorder has not been defined (Beutler, 2000). In addition, Fe overload in neural tissue, and to a lesser extent in the liver, has been observed in rare patients with ceruloplasmin deficiency secondary to mutations in the

**Table 2.** Prevalence of HFE genotypes in individuals of European descent (Hanson *et al.* 2000)

Genotype	Prevalence (%: average from published studies)
C282Y/C282Y	0.4
C282Y/H63D	1.8
H63D/H63D	2.0
C282Y/+	9.2
H63D/+	21.6

caeruloplasmin gene; these patients also demonstrate Fe-deficiency anaemia (Andrews, 1999; Beutler, 2000).

### The regulatory mechanism

Growing knowledge about HHC and other genetic conditions leading to Fe overload provides important tools for the study of the Fe regulatory system. Mapping of the HFE gene led to identification of a previously-unrecognized function, and provided a basis for cellular studies to clarify the biological processes involving the HFE protein. The HFE example also illustrates the contribution of animal models to the understanding of the genetic components of a complex biological system. Mouse models lacking specific protein functions, including a 'knockout' mouse that lacks the HFE protein function, have been employed to investigate the effect of the loss of specific proteins in the regulatory pathway and interactions between different proteins (Levy *et al.* 2000). *In vitro* studies and clinical observations of Fe-overloaded patients, particularly those with HHC, also contribute to a greater understanding of the regulatory process, which is reviewed in detail elsewhere (Elsenstein & Blemings, 1998; Andrews, 1999; Conrad *et al.* 2000; Skikne, 2000).

The most well-defined proteins involved in the Fe regulatory process are summarized in Table 3. From studies of these proteins in human and animal models, several interactions related to Fe overload and Fe deficiency can now be described. DMT1, the membrane transporter, mediates absorption of non-haem-Fe. It is assumed to interact with intestinal brush-border enzymes that reduce Fe to its Fe<sup>3+</sup> form to facilitate Fe uptake (Andrews, 1999). Loss of DMT1 causes Fe deficiency in mice and prevents Fe loading in

HFE-deficient mice (Levy *et al.* 2000). However, when the HFE protein is absent DMT1-deficient mice absorb marginally more Fe than when it is present (Levy *et al.* 2000), suggesting a small amount of absorption by an alternate uptake pathway that might be regulated by HFE.

HFE binds to  $\beta_2$ -microglobulin, a protein that also binds to other major histocompatibility class I proteins (Feder *et al.* 1997). In mouse models loss of  $\beta_2$ -microglobulin results in both Fe loading and immunological abnormalities (Levy *et al.* 2000). In addition, mice lacking either HFE or  $\beta_2$ -microglobulin develop Fe overload (Zhou *et al.* 1998; Levy *et al.* 2000), and a more severe Fe overload is observed if both proteins are lacking (Levy *et al.* 2000). The HFE protein product also binds to the transferrin receptor and reduces its affinity for Fe-loaded transferrin by five- to ten-fold (Feder *et al.* 1998). The C282Y mutation alters the HFE protein structure, disrupting  $\beta_2$ -microglobulin association and its transport to and presentation on the cell surface (Lebron *et al.* 1998). The H63D mutation does not prevent the  $\beta_2$ -microglobulin association or cell surface expression, consistent with its milder clinical effect (Waheed *et al.* 1997). The localization of the HFE protein in the crypt cells of the duodenal site of dietary Fe absorption, and its association with transferrin receptor in these cells are consistent with its presumed role in the regulation of Fe absorption (Parkilla *et al.* 1997; Waheed *et al.* 1999).

The delineation of the role of these and other proteins in the regulatory pathway achieved thus far by genetic analysis still leaves many questions unanswered. Clinical and animal studies indicate that the rate of Fe absorption is influenced by three factors: the amount of Fe absorbed from the diet; the amount of Fe stored in the body; the rate of erythrocyte production (Andrews, 1999). The feedback mechanisms that

**Table 3.** Well-defined proteins in iron regulation

Protein	Function	Effect of loss of function (mouse and human studies)
Divalent metal transporter-1 (DMT1)	Major transmembrane importer molecule for non-haem-Fe; also involved in transport of other metals	Human: no mutations known Mouse: mice homozygous for the <i>mk</i> mutation have marked reduction in DMT1 function and intestinal absorption of Fe and have microcytic anaemia (Edwards & Hoke, 1972; Levy <i>et al.</i> 2000)
HFE	HFE has a role in Fe absorption that is not yet well defined. It binds to $\beta_2$ -microglobulin; this complex interacts with the transferrin receptor, reducing affinity of the receptor for Fe-loaded transferrin	Human: mutations in HFE account for the majority of patients with hereditary haemochromatosis (HHC; Feder <i>et al.</i> 1996) Mouse: A 'knockout' mouse lacking HFE function develops Fe overload similar to that seen in HHC (Zhou <i>et al.</i> 1998)
Ferritin	Ferritin subunits form an intracellular storage structure for Fe	Human: no mutations known Mouse: no mutations known
Transferrin	Transport molecule for Fe	Human: rare families have been described with atransferrinaemia and Fe overload (Beutler, 2000) Mouse: no mutations known
Transferrin receptor	Binds Fe-laden transferrin and facilitates cellular uptake. As already noted transferrin receptor complexes with HFE- $\beta_2$ -microglobulin	Human: no mutations known Mouse: mice lacking transferrin receptor die during embryogenesis of severe Fe deficiency (Levy <i>et al.</i> 2000)
Caeruloplasmin and homologue hephaestin	Appears to mediate movement of Fe out of body tissues	Human: acaeruloplasminaemia due to a caeruloplasmin gene mutation results in Fe-deficiency anaemia and neural damage related to excess Fe (Andrews, 1999) Mouse: mice deficient in hephaestin have inefficient transfer of Fe into the circulation (Levy <i>et al.</i> 2000)

control absorption are only poorly understood. At least two Fe regulatory proteins have been identified. Their function occurs at least in part through regulation of the rate of transcription of key proteins in the absorption pathway, e.g. proteins like DMT1, transferrin, ferritin and HFE (Elsenstein & Blemings, 1998). This regulatory function is mediated by Fe responsive elements, structural elements within mRNA molecules that control the rate of mRNA transcription (Elsenstein & Blemings, 1998). The same feedback mechanisms are also presumed to influence cellular uptake of Fe.

### Implications for public health

In developed countries Fe deficiency appears to be largely the result of inadequate nutrition whereas Fe overload is largely the result of genetic disorders. However, clinical observations and the complexity of the Fe regulatory system suggest that neither disorder has a simple causative mechanism. Genetic variation in the multiple proteins that contribute to Fe regulation could influence Fe status in a variety of ways that either increase or decrease the likelihood of different Fe disorders.

Common variants in the genes coding for DMT1 or other regulatory proteins might, for example, interact with the HFE protein in ways that influence the clinical expression of HHC. Study of other genetic disorders of Fe overload, such as JH-type African Fe overload and non-human leucocyte antigen-linked haemochromatosis, will help to clarify this possibility through the identification and characterization of other proteins in the Fe regulatory system. Non-genetic factors also appear to play a role in Fe overload, as indicated by the variation in clinical outcome among individuals with the C282Y/C282Y HFE genotype, including the marked difference between men and women in the prevalence of clinical symptoms of HHC. The role of diet and nutrition in Fe overload disease, especially in gene expression, remains to be determined.

Genetic variants in the Fe regulatory systems are also likely to influence the likelihood of Fe deficiency, as is seen in mouse models (Levy *et al.* 2000). Some variants may predispose to Fe deficiency, while others may provide protection; the C282Y and H63D mutation in the HFE gene appear to be examples of the latter. From an evolutionary perspective the existence of common mutations that enhance Fe status is of great interest, given the high prevalence of Fe deficiency. If carriers of C282Y and H63D mutations have an advantage in Fe retention, HHC (and by extension, other autosomal recessive syndromes leading to Fe overload) might be the result of a selection process favouring carrier genotypes. The adverse consequences of such genotypes may be apparent only in developed societies that offer access to protein-rich diets and longer lifespans; in previous eras even those with highest-risk genotypes might have had a survival advantage. The physiological advantage of mutation carriers is likely to remain important until medical science offers better solutions to the problem of Fe deficiency.

The analytical work made possible by genetics will not be complete until the interaction between dietary and genetic

factors is fully understood. The genetic factors that influence Fe absorption are likely to respond variably to dietary factors. Thus, polymorphisms in proteins involved in non-haem-Fe absorption, e.g. ferredoxinases and DMT1, could influence the efficacy of Fe supplements. Variation in proteins involved in the separate uptake pathway for haem-Fe could have important implications for the importance of haem *v.* non-haem sources of Fe in the diet, and might modify outcome in HHC. Dietary Fe may have other effects on the feedback mechanisms within the regulatory pathway.

As data accumulate in the future, it may become possible to adjust diet to genotype, to minimize risks of both Fe deficiency and Fe overload. In addition, delineation of the regulatory pathway may lead to drug treatments that block known protein functions, either to enhance or reduce Fe uptake. Solving the dual problems of Fe deficiency and Fe overload will require coordinated study of genes and diet, and may ultimately lead to innovative strategies to prevent Fe disorders.

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