

Genetics of haemochromatosis

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After identification of the hereditary haemochromatosis gene *HFE*, and receipt of confirmation that most patients with the condition were homozygous for a single, founder mutation (C282Y), most assumed that C282Y would be a prevalent, highly penetrant mutation in a gene that plays a key part in the regulation of iron absorption and of whole-body iron homeostasis. With carrier rates of between 10% and 15%, and a homozygote frequency of about one-in-150 in people of northern European descent, C282Y is certainly prevalent. However, it is not highly penetrant. The pronounced variation in phenotype in individuals with the same gene mutation has prompted the search for modifier genes at other loci, and for environmental factors that might affect expression of the condition. Progress in our understanding of how *HFE* regulates the absorption of dietary iron has been slow, but much can be learnt from the study of the rare instances of haemochromatosis that involve mutations in newly-identified iron-metabolism genes, such as *TFR2*—a transferrin receptor isoform—and *ferroportin1/IREG1/MTP1*—an intestinal iron transporter. The availability of definitive information on penetrance and the identity of genetic modifiers will aid the debate on whether population screening for haemochromatosis should be undertaken or whether alternative strategies should be implemented to improve early detection.

Panel 1: Genes mutated in hereditary haemochromatosis

Classification	Mutant gene	Pattern of inheritance	Reference
Type 1 or hereditary haemochromatosis	<i>HFE</i>	Autosomal recessive	1
Type 2 or juvenile haemochromatosis	Unknown	Autosomal recessive	2
Type 3	<i>TFR2</i>	Autosomal recessive	3–5
Type 4	<i>ferroportin1/IREG1/MTP1</i>	Autosomal dominant	6–9
Type 5	H-ferritin	Autosomal dominant	10

Disorders of iron overload have been recognised since the late 19th century, when the term haemochromatosis was used to describe the pathological finding of tissue injury, usually cirrhosis, in association with massive deposits of inorganic iron. Haemochromatosis now refers to the clinical condition of iron overload caused by inherited disorders of iron metabolism. Iron overload is associated with mutations in multiple genes (panel 1),^{1–10} which have different functions in iron metabolism.

Type 1 or hereditary haemochromatosis is an autosomal recessive condition caused by mutations in *HFE*, the first haemochromatosis gene identified, and is HLA-linked. The disorder arises mostly in men and almost exclusively in people of northern European descent.^{1,11} It is the most important subtype of the condition in terms of prevalence and effect on health. Type 2 or juvenile haemochromatosis is a rare, autosomal recessive condition caused by an unidentified locus on chromosome 1q. It presents as an

accelerated form of iron overload in the second and third decades of life, and affects men and women equally.² Type 3 haemochromatosis is an autosomal recessive trait first described in southern Italy.^{3–5} This disorder is caused by mutations in *TFR2*, which encodes a transferrin receptor isoform. Type 4 haemochromatosis is an autosomal dominant form of iron overload, which has been described in three families,^{6–8} and which is associated with mutations in the gene *ferroportin1*,¹² also known as *IREG1*¹³ and *MTP1*,¹⁴ which encodes an intestinal iron transport molecule. Type 5 haemochromatosis is a form of autosomal dominant iron overload, which has so far affected one Japanese family.¹⁰ The mutation for this subtype is in the gene that encodes the H-subunit of the iron-storage molecule ferritin.¹⁰ A further form of familial iron overload with a possible autosomal dominant mode of inheritance was described in 1990,¹⁵ but has not been characterised genetically. Analysis of the iron-loading phenotype in rare forms of haemochromatosis can prove highly informative. In type 4 haemochromatosis, for example, selective iron overload of Kupffer cells coupled with normal transferrin saturation suggests that the normal gene product is important for the release of recycled red-cell iron from the reticuloendothelial system to circulating transferrin.^{7,8} Because of the increasing genetic heterogeneity of haemochromatosis, cases of iron overload are still being diagnosed^{16,17} in which genes known to be associated with the condition (panel 1) have normal sequence, indicating that there are other genes yet to be identified.

Many individuals with type 1 haemochromatosis are homozygous for a single founder mutation in *HFE* that leads to substitution of a tyrosine residue in place of a

Search strategy

PubMed was used to search for published material on haemochromatosis and related topics of iron metabolism, and on proteins of iron transport and storage. Emphasis was placed on papers published since 2000 and those that stressed the importance of the search for genes that modify the phenotype.

Lancet 2002; **360**: 1673–81

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cysteine at position 282 (C282Y).¹⁸ An understanding of how this observation relates to the fact that the haemochromatosis phenotype varies greatly in severity between individuals, and that only a small proportion of C282Y homozygotes are iron loaded, is a major challenge and raises questions about the penetrance of the mutation, the presence of genetic modifiers, and how *HFE* interacts with environmental factors. Major questions also remain unanswered about the role of *HFE*, and other genes mutated in haemochromatosis, in iron absorption and maintenance of homeostasis of iron. The function of the wild-type *HFE* protein is not known, even though its crystal structure¹⁹ and the regions that interact with other proteins of iron metabolism have been identified.²⁰ The functions of the normal proteins encoded by *TFR2*²¹ and *ferroportin1* are understood in some detail,¹²⁻¹⁴ but how whole-body iron homeostasis is disturbed when mutations arise in their respective genes is unclear. In this review, I examine these areas of uncertainty from a genetic perspective. Because management issues in haemochromatosis are being continually updated and described in consensus documents²² and management guidelines,^{23,24} they are not considered in detail here.

Clinical features

In haemochromatosis, iron accumulates first in the transferrin pool, manifested by a rise in serum transferrin saturation, and subsequently in tissue stores—especially the hepatic parenchyma—which is accompanied by a progressive increase in concentrations of serum ferritin.²⁵ The clinical features of the disease arise as a result of the progressive accumulation of iron in the parenchymal cells of the liver, pancreas, heart, and anterior pituitary. In the absence of treatment to reduce iron concentrations, a characteristic pattern of tissue injury and organ failure can develop.²⁵

In its most extreme form, the disease manifests as cirrhosis, hepatocellular cancer, diabetes mellitus, sexual dysfunction due to hypogonadotropic hypogonadism, cardiomyopathy, a destructive arthritis, and generalised skin pigmentation. This phenotype is now less frequently seen, however, than previously because of increased awareness of iron overload and early diagnosis.²⁶ Genotyping done for family studies and screening programmes²⁷ have also helped. Nevertheless, there is still an average delay of 10 years between onset of symptoms (lethargy, arthralgia) and diagnosis of haemochromatosis, because of the non-specific nature of the symptoms and because of the unfounded belief among health professionals that haemochromatosis is rare.²⁸

Excess iron damages the liver, and presumably other parenchymal organs, by induction of oxidative stress and expression of cytokines, such as transforming growth factor β (TGF β),²⁹ which promote hepatic fibrosis. The chemical nature of the reactive iron species in cells is not known, but the presence of iron in the circulation in excess of that bound by plasma transferrin is well documented³⁰ and is assumed to promote oxidative damage to the lipid component of cell membranes and intracellular organelles.³¹

Genetic background

The original observation of Simon and colleagues³² of linkage between a haemochromatosis locus and the major histocompatibility complex (MHC), and the presence of an ancestral haplotype¹¹ were of fundamental importance in the search for the haemochromatosis gene. Feder and co-workers¹ finally identified *HFE* in 1996, using a positional cloning strategy to isolate a 250 kb subregion on

	Genotype	
	YY/HH	CY/HD
Country or region		
Australia ³³	100%	0%
Northern France ³⁴	96%	2.7%
UK ³⁵	91%	2.6%
Eire, Ireland ³⁶	90%	3.8%
Germany ³⁷	90%	3.5%
USA ¹	83%	4.5%
Austria ³⁸	77%	7.5%
Southern France ³⁹	72%	4.3%
Italy ¹⁷	64%	5.3%

Frequency of C282Y homozygotes (YY/HH) and compound heterozygotes (CY/HD) in patients with hereditary haemochromatosis

chromosome 6p that was conserved on chromosomes carrying the ancestral haplotype. After all the genes in this region had been identified, the only mutation that segregated with haemochromatosis was a missense mutation (C282Y) in the open reading frame of a new, MHC class I-like gene. A second mutation (H63D) was described, but the relation between this mutation and iron overload was not immediately apparent. Patients with a clinical diagnosis of haemochromatosis were subsequently investigated, many of whom were homozygous for the C282Y mutation (table).^{1,17,33-39} In Europe, the frequency of homozygosity declined from north to south, being lowest in Italy where haemochromatosis is genetically heterogeneous.¹⁷ The opposite was noted for compound heterozygotes, in whom one copy of each of the C282Y and H63D mutations is inherited on separate chromosomes (table).

The ancestral haplotype, carrying C282Y, is about 6 Mb in size, which suggests that the mutation arose in the past 2000 years.⁴⁰ It is absent, or present at very low allele frequency, in non-white populations—eg, African, Asian, or native Australian^{41,42}—and rarely appears through genetic admixture or as a spontaneously arising mutation.⁴⁰ The H63D mutation, by contrast, is an older mutation than C282Y and is carried on a greater diversity of haplotypes. It has an allele frequency of more than 5% in Europe, Mediterranean countries, the Middle East and Far East, and on the Indian subcontinent.^{40,42} H63D seems to be widespread as a result of mutations that arise spontaneously.

The prevalence of the C282Y mutation in European populations is estimated at 0.4% and 9.2% for the homozygous and heterozygous states, respectively.⁴³ Similar figures have been reported for white populations in North America,⁴⁴ New Zealand,⁴⁵ and Australia,⁴⁶ where migration from Europe over the past 400 years has introduced the *HFE* mutation. In Europe, there are pronounced regional variations in the frequency of the Y allele, which decreases in both north-to-south and west-to-east directions.⁴⁷ The highest frequency has been noted in Ireland (14.2%),³⁶ western France (9.4%),⁴⁸ and Wales (8.3%),⁴⁹ and the lowest in Italy (1.6%)¹⁶ to the south, and Hungary (3.4%)⁵⁰ to the east. That the frequency of the Y allele and of clinical haemochromatosis decline in all directions from regions of north-western Europe, which were inhabited by residual populations of Celtic origin,⁴⁷ suggests that the founder mutation originated in this group and spread by population migration.^{11,42}

Many mutations to *HFE*, in addition to C282Y and H63D, have been described.⁵¹ Clinically, the most significant of these is S65C, which arises in 2–3% of white people and is implicated in mild iron overload when inherited in the compound heterozygous state with

either C282Y or H63D.^{52,53} Because this mutation is rare, however, it is not generally included in routine screening tests.

Penetrance of mutations

The variability in clinical symptoms and signs in haemochromatosis, and the absence of a consensus on what phenotypic features should be used in studies to investigate penetrance, has led to highly polarised views. The initial erroneous impression, that the homozygous C282Y genotype was highly penetrant, was based on results of genotyping in cross-sectional case series. However, patients in these studies were diagnosed in the pregenotyping era on the basis of iron overload and features of haemochromatosis. These studies were, therefore, subject to great ascertainment bias. Nevertheless, even in these highly selected patients, 5–10% of men and up to 30% of women homozygous for C282Y did not express the iron-loaded phenotype,⁵⁴ indicating that the genotype is not fully penetrant.

Results of other studies have since confirmed this conclusion. Findings of large-scale genotyping surveys of blood donors from south Wales⁴⁹ and Canada⁵⁵ indicated that the prevalence of homozygosity for C282Y was one in 147 and one in 327, respectively. Biochemical evidence of iron overload, however, was noted in only 20% of homozygous individuals (in whom iron overload was mild), and in neither of the surveys did homozygotes have evidence of tissue damage. Generally though, because of the regular loss of iron in blood-donors, expression of the phenotype is mild.²² Results of other screening studies, in which individuals were selected for genotyping on the basis of biochemical indices of iron overload^{56,57} or liver damage,⁵⁸ or on the basis of a striking discrepancy between the C282Y homozygotes detected and the number of patients diagnosed with haemochromatosis,^{59,60} also suggest that penetrance is low. The studies do not, however, allow this notion to be quantified.

A direct estimate of penetrance was possible in one study,⁶¹ in which all individuals were genotyped, rather than just those with abnormal serum iron variables. Of about 41 000 individuals who attended a health appraisal clinic in California, USA, 152 were homozygous for the C282Y mutation. Symptoms and signs of haemochromatosis were no more frequent in the homozygotes than in matched controls and only one homozygous patient had clinical features of haemochromatosis, leading the authors to suggest that penetrance of the genotype was less than 1%. In a longitudinal study,⁶² only half the individuals homozygous for C282Y had clinical features of haemochromatosis, and a quarter had serum ferritin concentrations that remained in the normal range for 4 years. These individuals were all women, however, confirming that penetrance is low in women.

The penetrance of the compound heterozygote genotype C282Y/H63D is low in men and women. Iron stores estimated by phlebotomy range from 0.5–5 g, compared with a normal range of 0.5–1.0 g, and clinical features of haemochromatosis, especially liver disease, are rare.^{17,52,63} The homozygous H63D genotype is also of low penetrance,⁶⁴ although there seem to be regional variations. In a case series of 50 individuals from southern France, about 25% were significantly iron loaded and three individuals had cirrhosis.⁶⁵ Individuals heterozygous for the C282Y mutation could have partial biochemical expression but no significant iron overload. Less than 10% have raised transferrin saturation or serum ferritin, and liver biopsy abnormalities, rather than being caused by

Panel 2: Potential modifiers of the haemochromatosis phenotype

Iron absorption genes	Regulatory genes	Others
<i>DCYTB</i> ⁷²	<i>HFE</i> ⁷⁵	Caeruloplasmin ⁷⁴
<i>DMT1</i> ⁷³	<i>B2M</i> ⁷⁶	Haptoglobin ⁷⁴
Ferritin heavy and light chain ⁷⁴	Transferrin ⁷⁷	Hepcidin ⁷⁴
<i>ferroportin1/IREG1/MPT1</i> ⁷⁴	<i>TFR1</i> ⁶⁹	
Hephaestin ⁶⁹	<i>TFR2</i> ⁷⁸	
Juvenile haemochromatosis gene ²	<i>IRP1</i> ⁷⁴	
	<i>IRP2</i> ⁷⁴	

excess iron, are associated with intake of excess alcohol, hepatitis, or porphyria cutanea tarda.⁶⁶

In patients with iron overload, who are homozygous for C282Y, the degree to which the phenotype is manifest is variable, as suggested by the observation that iron stores can vary as much as ten-fold between individuals.⁶⁷ The expression of the phenotype depends on a complex interplay of genetic and epigenetic factors, age and sex, and environmental aspects, such as diet, alcohol,⁶⁸ and blood loss.²⁵

Several genes of iron metabolism act as modifiers of phenotype, as shown in *Hfe* knockout mice. In these compound mutants, the additional inactivation of genes involved in iron absorption, such as *DMT1* (apical transport) and *Heph* (basolateral transport), decrease iron loading.⁶⁹ By contrast, inactivation of *B2m* and *Tfr1*, genes that encode proteins that interact with Hfe and that could have a regulatory role in iron absorption, increase iron loading.⁶⁹ Experiments of similar design indicate that in mice deficient for *B2m* and *Hfe*, the background strain⁷⁰ and sex⁷¹ of the animals also affect the degree of iron overload, confirming that there are multiple, as yet unidentified, genetic loci that modify phenotype.

Despite clear indications that genetic modifiers exist, surveys of multiple genes of iron metabolism done in patients homozygous for the C282Y mutation and in controls (panel 2)^{2,69,72–79} have not revealed polymorphisms or mutations associated with increased iron loading. Iron load is, however, affected by polymorphisms of haptoglobin, a haemoglobin-binding plasma protein that permits the hepatic recycling of free-circulating haemoglobin; men carrying the polymorphism Hp 2-2 had higher plasma iron indices and higher iron stores than those with Hp 1-1 and 1-2 polymorphisms.⁷⁹ Reports from several centres^{80–82} suggest that, in individuals homozygous for C282Y, inheritance of two copies of the ancestral haplotype rather than one or none leads to higher concentrations of stored iron, implying that genetic loci in the region of the MHC act as genetic modifiers of the iron-loading phenotype. A detailed analysis of an extended haplotype of 6p21.3 and polymorphisms in *HFE* did not identify candidate modifiers.⁷⁴ However, evidence of the relation between *HFE* genotype and biochemical indices of iron status in patients recruited from the Australian Twin Registry shows a significant effect of genetic loci, other than *HFE*, on iron stores and transferrin saturation.⁴⁶

Other aspects of the phenotype—eg, the extent of liver damage—could be also be affected by genetic factors. Hepatic fibrosis, for example, is related to polymorphisms in the promoter region of the tumour necrosis factor α gene.⁸³

HFE and its role in iron metabolism

The haemochromatosis gene encodes HFE, a 343 amino acid protein¹ that is homologous to MHC class I

molecules, a family of transmembrane glycoproteins that function in the immune system by presenting peptide antigen to T cells. The protein is organised in three extracellular domains— $\alpha 1$, $\alpha 2$, and $\alpha 3$ —a membrane spanning domain, and a short cytoplasmic tail; the $\alpha 1$ and $\alpha 2$ domain helices form the peptide-binding groove and interact with the T-cell receptor, and the highly conserved immunoglobulin-like $\alpha 3$ domain binds non-covalently to $\beta 2$ microglobulin, the light chain of class I, to maintain correct orientation in the cell membrane.⁸⁴

HFE retains many of the overall structural features of classical class I molecules, but inward displacement of the $\alpha 1$ domain helix narrows the peptide-binding groove and hence prevents binding. Consequently, the $\alpha 1$ and $\alpha 2$ helices of HFE do not bind to the T-cell receptor but interact with the helical domain of the transferrin receptor (TFR1),²⁰ a molecule that plays a central part in iron metabolism and homeostasis.⁸⁵ HFE/TFR1 complexes have been isolated from tissues of the duodenum⁸⁶ and placenta,⁸⁷ which have important roles in iron metabolism. HFE is not unique among MHC class I in binding to cell-surface receptors, since peptides derived from the $\alpha 1$ domain of classical class I molecules have been reported by Olsson and colleagues⁸⁸ to bind to insulin receptors, preventing internalisation and increasing the steady-state number of active receptors on the surface. Before HFE was cloned, these authors⁸⁸ suggested that the internalisation of transferrin could be inhibited, presumably as a consequence of interaction between class I and the transferrin receptor.

The C282Y mutation prevents the formation of a disulfide bond in the $\alpha 3$ domain, abrogating the binding of $\beta 2$ microglobulin and leading to retention of the mutant protein in the endoplasmic reticulum, where it undergoes accelerated degradation and does not reach the cell surface.⁸⁹ By contrast, the H63D mutation, which affects the $\alpha 1$ domain, does not seem to affect the function of HFE in terms of TFR1 binding and cell-surface expression.⁸⁹

TFR1 functions as an iron transport molecule⁸⁵ by binding diferric transferrin at the cell surface (pH 7.4), an event that triggers internalisation of the receptor/ligand complex to an acidified endosome (pH 6.0). At low pH, iron is released from transferrin, which remains bound to the receptor and returns to the cell surface where, on encountering neutral pH, it is released into the cellular microenvironment.⁸⁵ Once bound to TFR1, HFE also undergoes endocytosis but, unlike transferrin, dissociates from the receptor when it encounters low pH and returns to the cell surface in uncomplexed form.⁹⁰ TFR1 thus binds both HFE and diferric transferrin and although both molecules compete for binding to the same or overlapping sites on the receptor, the stoichiometry of a TFR1/HFE complex on a cell membrane, and how this is affected by the binding of transferrin, remains to be elucidated.²⁰

The functional consequences of the binding between TFR1 and HFE were first investigated in cultured cells engineered to express high concentrations of wild-type or mutant protein. Results of these experiments showed that wild-type HFE was a negative regulator of TFR1 activity, leading to lowered concentrations of intracellular iron and ferritin and increased numbers of transferrin receptors.^{91–95} The mechanism by which this regulatory mechanism works is not known, although it is dependent on the receptor and HFE being associated in the endosome, since an HFE mutant with impaired TFR1 binding but normal endosomal targeting does not lower concentrations of intracellular iron.⁹⁰

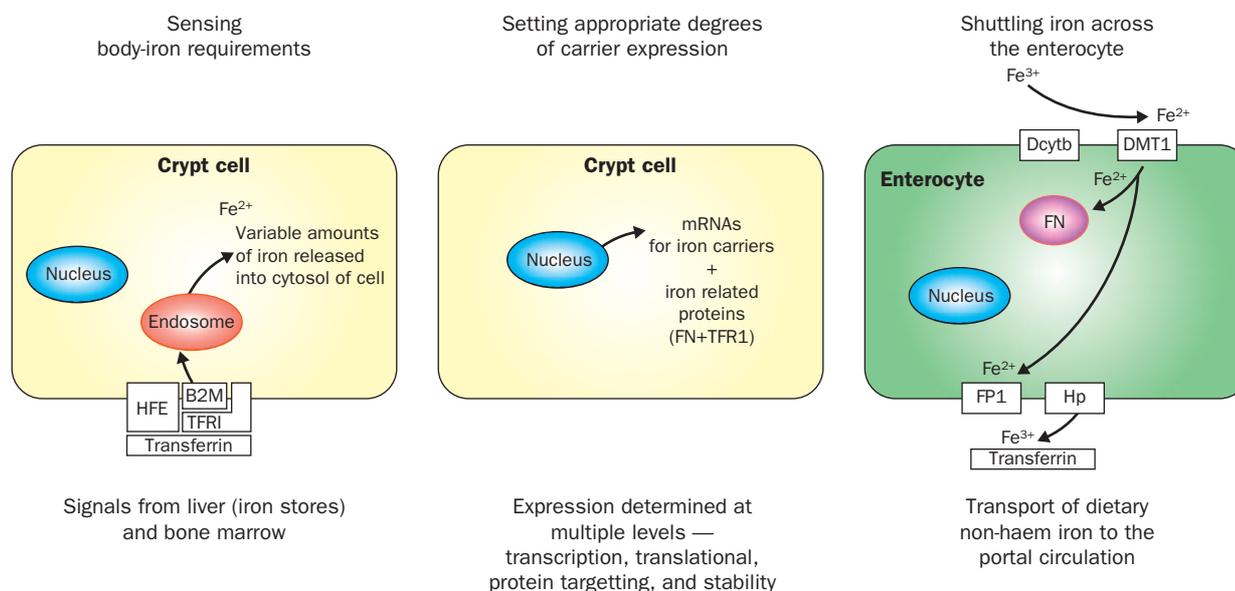
The physiological relevance of these experiments is questionable because only HFE was transfected, and the normal functioning of HFE is dependent on the non-covalent binding of $\beta 2$ microglobulin. When both HFE and $\beta 2$ microglobulin are overexpressed, TFR1 recycling is enhanced and more receptors are expressed at the cell surface, the net result being increased iron uptake by the cells.⁹⁶ In these circumstances, the C282Y mutation in HFE, which disrupts binding of $\beta 2$ microglobulin, would be expected to lead to an iron-deficient phenotype. As discussed in the next section, duodenal crypt cells and macrophages have indeed been found to be iron poor in HFE-related haemochromatosis.^{97,98}

Regulation of function of TFR1 in a given tissue should be dependent on the co-expression of HFE. Feder and colleagues¹ investigated concentrations of HFE mRNA in healthy human tissues and found highest amounts in the liver, where transcripts are localised to Kupffer cells,⁹⁹ and intermediate expression in small intestine. In the rat gastrointestinal tract, HFE mRNA is present at low concentrations in the duodenum, the site of iron absorption, and high concentrations in the caecum and colon, regions where TFR1 is also abundant but where iron absorption does not take place.¹⁰⁰ The investigation of expression of HFE protein in the duodenum in individuals with and without haemochromatosis has produced results that vary between laboratories. In normal duodenum, HFE immunolocalises to crypt cells in a perinuclear distribution,¹⁰¹ but in some studies antibody staining was not uniform and only scattered cells seemed to express the protein.^{99,102} In individuals homozygous for the C282Y mutation, HFE is reported to either accumulate in supra-nuclear granules in villus enterocytes¹⁰³ or to be down-regulated and localised to crypt cells.¹⁰² The lack of a consistent picture of HFE expression makes it difficult to develop a coherent model of function.

Iron absorption

Absorption of dietary iron takes place in the duodenum, where specific carrier molecules are expressed by the villus enterocyte (figure). Briefly, duodenal crypt cells are thought to receive signals about iron requirements in the body, in part through the binding of transferrin to the HFE/ $\beta 2$ microglobulin/TFR1 complex. Other less well defined signals are also thought to be involved.⁹⁷ Iron release from the endosome leads to variable degrees of cytosolic iron in the crypt cell, which in turn dictates or sets levels of carrier expression at multiple sites of control. Dietary non-haem is shuttled across the enterocyte by the action of divalent metal transporter 1 (DMT1;¹⁰⁴ apical) and ferroportin (basolateral).^{12–14} There is also a requirement for accessory proteins that change the oxidation state of iron to facilitate transport; duodenal cytochrome b (Dcytb)⁷² is a ferric reductase that provides soluble, ferrous ions at the brush border membrane for transport by DMT1, while at the basolateral membrane, hephaestin¹⁰⁵ (a copper-binding ferroxidase with strong homology to caeruloplasmin) oxidises ferrous ions as they are transported by ferroportin1 to the portal circulation. Enterocyte ferritin can also act as a pump for expulsion of excess iron (figure). Mutations of HFE or $\beta 2$ microglobulin could, hence, lead to defective sensing, iron-poor crypt cells, and inappropriate expression of enterocyte carriers.⁹⁶

There is growing evidence to suggest that the degree of expression of carrier molecules could play an important part in iron absorption and, by inference, whole-body iron homeostasis. Duodenal expression of DMT1 and ferroportin1 is raised in mice in which iron absorption is increased,^{12–14,104} whereas in naturally anaemic mice,



Regulation of expression of iron carriers in the duodenal enterocyte

B2M=β2 microglobulin; FN=Enterocyte ferritin; FP1=ferroportin1; Hp=hephaestin. Adapted from reference 97 by permission of W B Saunders.

disabling mutations in genes encoding DMT1 and hephaestin lead to defective iron absorption.^{69,105} Increased expression of apical and basolateral carriers has also been reported in people^{13,106,107} and in mouse models of haemochromatosis,^{108,109} and Dupic and colleagues¹¹⁰ have made the important observation that duodenal expression of Dcytb, DMT1, and ferroportin1 is significantly increased in a strain of *Hfe*-deficient mouse that has a severe iron-loading phenotype, but is normal in a second strain in which there is only a small increase in stores of liver iron.¹¹⁰

Tight linkage between carrier expression and iron absorption is difficult to validate though; increased expression is seen in untreated patients in whom stores of iron are greatly increased¹⁰⁶ and in treated patients with normal iron stores.¹⁰⁷ Absorption of radioactive iron from a test meal differs in the two groups,¹¹¹ being suppressed before treatment by high concentrations of hepatic iron and increased after treatment by low stores of iron and increased erythroid activity, a powerful stimulus to absorption.¹¹² Furthermore, the absorption of dietary haem iron, which is highly bioavailable and contributes up to 30% of absorbed iron, is an unregulated process that uses an alternative pathway that does not involve DMT1.¹¹³ Additionally, the regulation of DMT1 is strain-specific, and iron loading clearly takes place in some strains of *Hfe*-deficient mice in which DMT1 expression is not increased.¹¹⁴ Although there is uncertainty over the strength of the evidence, linking increased carrier expression and increased iron absorption in haemochromatosis, compound heterozygous *Hfe*-deficient mice in which DMT1 or hephaestin are additionally disabled do not become iron loaded, confirming that iron transport in haemochromatosis does involve flux by these carriers.⁶⁹

Progress in understanding how mutations in HFE lead to inappropriate iron absorption has been slow because normal mechanisms of control have not been defined. In the absence of clearly identified signals, physiological regulators have been proposed that would transmit information about body iron requirements to the absorptive epithelium.¹¹² A so-called stores regulator is thought to act to increase iron absorption when iron stores are low and conversely limit absorption when stores are replete.

Experimental evidence suggests that this regulator is fairly limited in its ability to affect iron absorption,¹¹² although an erythroid regulator, provides a more powerful stimulus to iron absorption especially when erythropoiesis is coupled with iron deficiency. HFE has only a limited effect on the function of the two regulators; *Hfe*-deficient mice, for instance, retain the ability to regulate iron stores and erythroid activity.¹¹⁵ Regulation of iron absorption, though less efficient, also occurs in *B2m*-deficient mice,¹¹⁶ and it seems that HFE/B2m could be part of a signalling complex that responds to iron status. When B2m is mutated, regulation of iron absorption is attenuated but not lost altogether.

Although the identity of regulatory signals is not known, the stores regulator is thought to respond to the degree of circulating transferrin that is saturated with iron.¹¹⁷ One of the key components of the signalling system seems to be the concentration of intracellular iron within crypt enterocytes,¹¹⁷ which is ascertained by the binding of transferrin to abundantly expressed TFR1 to establish a set point that determines the subsequent expression of carriers in mature enterocytes.¹¹⁸ The finding that HFE associated with β2 microglobulin binds to TFR1 in the duodenum⁸⁶ has led to the suggestion that HFE could participate in the iron-sensing function of crypt cells, and that the C282Y mutant form, in failing to interact with β2 microglobulin and reach the basolateral membrane, is defective in this function.¹¹⁹ Two further pieces of evidence support this notion. First, cultured cells engineered to overexpress HFE and β2 microglobulin show enhanced TFR1-dependent iron uptake, indicating that the mutant form of HFE could lead to cellular iron deficiency.⁹⁶ Second, in the *Hfe*-deficient mouse, iron uptake from plasma transferrin by the duodenum is significantly impaired compared with in the wild-type strain.¹²⁰ Confirmation that crypt enterocytes from iron loaded homozygous C282Y patients are paradoxically iron deficient comes from the many reports that these cells have low concentrations of ferritin expression^{121,122} and increased concentrations of TFR1¹²³ and iron regulatory protein 1,¹²² all of which are surrogate markers of cellular iron deficiency.

The link between iron-poor crypt cells and increased expression of iron carriers by mature enterocytes in haemochromatosis requires an understanding of the molecular regulation of these molecules. The mRNAs for ferroportin1¹²⁻¹⁴ and one of two DMT1 isoforms¹⁰⁴ contain iron responsive elements (IREs) in their 5' and 3' untranslated regions, respectively. This provides the potential for regulation at the post-transcriptional level, with concentrations of ferroportin1 decreased and DMT1 increased in iron-deficient cells through the binding of iron regulatory proteins to IREs.¹²⁴ However, this pattern of expression is not seen, and the concentration of the two carriers seems always to be tightly coupled—ie, upregulation in iron deficiency.^{12-14,104} Furthermore, the site of maximum expression (mature enterocyte) is separated in both time and place from the site at which iron sensing occurs (crypt cell). Presumably, multiple levels of control are required to establish and maintain appropriate expression as enterocytes migrate from crypt to functional regions of the villus.^{97,125}

Mutations in several other genes of iron metabolism cause iron overload (panel 1), but the role, if any, of these genes in the processes of iron absorption and regulation is unclear. Mutations in ferroportin1 are associated with iron loading in Kupffer cells,^{7,8} and venesection is poorly tolerated, suggesting a defect in the release of iron to circulating transferrin. Such a loss-of-function mutation in ferroportin could have multiple effects on iron metabolism, including less efficient duodenal iron transport or an enhanced stimulus to iron absorption as a result of impaired release of iron from hepatic non-parenchymal cells. Mutations in TFR2 can be associated with severe parenchymal iron overload, but it is difficult to envisage a regulatory role in iron absorption for this protein because expression in the small intestine is low²¹ and the gene product does not interact with HFE.¹²⁶ TFR2 is most highly expressed in the parenchymal cells of the liver, and it is conceivable that mutations could disrupt signalling between the liver and gut. Hence, there could be multiple regulatory pathways or a single pathway, containing multiple components, involved in the control of iron absorption. HFE plays an important part but is not the prime regulator, since iron absorption also responds to the stores of iron and erythroid regulators when HFE is either mutated or absent.¹¹⁵

Screening in haemochromatosis

Screening for iron overload with biochemical methods and with genotyping in patients who present with chronic liver disease or with symptoms and signs that could be caused by iron overload is good medical practice. Patients with type 2 diabetes mellitus, atypical cardiac failure, early onset impotence, and early or atypical arthritis have also been identified as target populations.²⁴ Additionally, the practice of screening the families of patients with iron overload is now well established, having been simplified by the introduction of genotyping after the cloning of *HFE*. Krawczak and colleagues¹²⁷ have calculated that, when applied to a frequent recessive mutation, such as C282Y in *HFE*, screening of previously identified index cases—so-called cascade genetic screening—is highly efficient and cost effective. They estimate that up to 40% of individuals at risk could be identified through screening of first-degree to third-degree relatives of patients with iron overload, and that the efficiency of this strategy can be about 50 times greater than that of population-wide screening, although this differential will be eroded as the cost of population screening falls.

Whether or not the general population should be screened for haemochromatosis is a source of continuing debate,²⁷ even though the condition fulfils many of the criteria for screening proposed by the WHO.¹²⁸ Proponents of screening^{24,62,129} point out that haemochromatosis is a genetic condition of high prevalence in selected populations; there is a recognised presymptomatic or latent phase; a reliable and safe diagnostic test is available; and, after diagnosis, an effective and inexpensive treatment exists that prevents morbidity and mortality. Most health-care professionals, however, emphasise the uncertainty that surrounds disease penetrance,¹³⁰⁻¹³² natural history of iron overload,^{133,134} and burden of disease in the population,^{135,136} and argue that the introduction of large-scale population screening would be premature. If the penetrance in some populations is as low as 1%,⁶¹ unmanageably large numbers would need to be screened to prevent one case of haemochromatosis from progressing to the stage of severe tissue damage.¹³⁰ All agree, however, on the importance of raising the awareness of haemochromatosis among the public and medical community through education programmes, and on ensuring that there is ready access to screening facilities.

Methods used in screening studies include those to ascertain phenotype, genotype, or a combination of the two. There is agreement that transferrin saturation is the standard test of haemochromatosis phenotype by which other assays should be judged,¹³⁷ although there is argument over which laboratory method should be used to measure saturation.^{138,139} Direct analysis of transferrin saturation requires the immunological measurement of transferrin in serum, and colourimetric measurement of serum iron. Immunological measurement is not an automated method and is more expensive than simpler, less robust methods in which transferrin saturation is derived after having estimated serum iron concentrations and total iron binding capacity (transferrin saturation=serum iron/total iron binding capacity), or unbound iron binding capacity (transferrin saturation=serum iron/serum iron+unbound iron binding capacity). Further studies should be done, focussing particularly on the sensitivity of these different laboratory methods.

Screening by genotype alone would be fraught with difficulties, especially if done at birth as part of a neonatal screening programme in a region where mutations in *HFE* were prevalent. Screening the population for H63D is not indicated, because this mutation is of low penetrance. There would be issues over storage and security of the large amount of data that would be generated, since up to one in 150 births would be expected to be homozygous for the C282Y mutation. Both penetrance and expressivity would be major considerations because there is currently no means of knowing who would go on to develop iron overload (mild or severe). In view of these difficulties, expert panels do not recommend genetic testing of children for adult onset diseases,²² and there is agreement that the optimum age for screening for haemochromatosis is 30 years,^{22,27,128} even though compliance in this age group is a concern.¹²⁸ There are further difficulties re the use of genotyping in areas where a large and growing proportion of iron overload is caused by mutations in genes other than *HFE* and, here, screening by phenotype would be more applicable.

There have been many attempts to quantify the cost of screening, both in the pregenotyping era^{140,141} and since the cloning of *HFE*.^{55,56,142} The cost of the combination of phenotypic screening and *HFE* genotyping is estimated to be in the range US\$1500–2700 per individual. Screening

becomes less cost effective as penetrance decreases. However, estimates suggest that the approach would remain cost effective to a penetrance as low as 20%. These estimates suggest that screening for haemochromatosis is several times more cost effective than other programmes, such as those screening average risk populations for colorectal cancer and breast cancer.¹⁴³

Debate on the ethical and social implications of screening for genetic conditions, including haemochromatosis,^{22,27,128} has led to the widespread introduction of legislation¹⁴⁴ to prevent exploitation of an individual on account of their personal genetic information. In the UK, for example, the government has imposed a 5-year moratorium on the use of genetic tests to determine insurance policies,¹⁴⁵ and has made a commitment that compliance by the industry will be closely monitored.

Conflict of interest statement

None declared.

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