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Of mice and men: genetic determinants of iron status

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Fe homeostasis is maintained by regulation of Fe absorption to balance largely unregulated body Fe losses. The majority of human subjects maintain relatively constant Fe stores; however, Fe deficiency and Fe overload are common conditions. Fe overload is frequently associated with mutations in genes of Fe metabolism. The present paper summarises present knowledge of these mutations as well as indicating other genes that animal studies have implicated as candidates for influencing body Fe stores.

Iron overload: Iron deficiency: Iron absorption

Fe is an essential mineral that is required for key biochemical functions such as DNA synthesis and O_2 transport and metabolism. Human subjects possess physiological mechanisms for maintaining relatively constant Fe stores. These mechanisms have only limited capabilities, however, and Fe deficiency and overload are common conditions. Fe stores are maintained by balancing the body Fe losses, which are poorly controlled, with tightly-regulated intestinal Fe absorption (Pietrangelo, 2002; Miret *et al*. 2003). Body Fe losses arise mainly from desquamation of epithelial cells (especially gut cells) and blood losses (Green *et al*. 1968; Hallberg, 2001), the latter being particularly important in menstruating women and in certain pathological conditions. Fe deficiency is mostly associated with high Fe requirements (high growth rates or high body Fe loss rates) in combination with a diet containing insufficient bioavailable Fe to meet these needs. Fe overload is usually found in individuals with low Fe requirements and some other predisposing condition, e.g. inheritance of an Fe-loading mutation or a hereditary anaemia (the latter is not discussed further in the present paper; for further details, see Finch, 1994).

The importance of variations in Fe stores has recently been discussed extensively. For decades there has been a consensus on the importance of identifying and combating Fe deficiency and Fe overload; however, recent advances in molecular genetics have given clinicians powerful new tools for investigating the association between these conditions and any disease. The toxicity of excess Fe suggests that Fe overload is to be avoided (Fuchs *et al*. 2002), and Weinberg (1999) has reviewed many possible pathological consequences of a failure to maintain normal Fe stores. However, specific associations between Fe overload and some diseases remain controversial (Hetet *et al*. 2001; Sullivan & Zacharski, 2001; Halsall *et al*. 2003; Wilson *et al*. 2003).

While human studies have proved informative, Fe metabolism has benefited unusually from parallel studies in rodents and man. Thus, the haemochromatosis gene (*HFE*) was identified in a human study (Feder *et al*. 1996), but mouse experiments are at the forefront of efforts to understand the function of the protein and identify modifier genes (Levy *et al*. 2000; Dupic *et al*. 2002; Simpson *et al*. 2003). On the other hand, several other genes have been identified in other species and subsequently applied to the diagnosis of human genetic Fe-overload conditions (as discussed later; e.g. those coding for transferrin receptor (TFR) 2, duodenal basolateral Fe exporter 1 (IREG1) and hepcidin). Table 1 summarises genes known to affect Fe stores in man and mice.

Variations in Fe stores can be attributed to both dietary and genetic influences (Whitfield *et al*. 2000). Diet has long been known to be important (Hallberg, 2001; Fleming *et al*. 2002; Rossi *et al*. 2001); however, the present paper will focus on genetic factors that influence Fe status in man and experimental animals.

Mutations associated with iron overload in man

Haemochromatosis type 1

C282Y. The genetic cause of hereditary haemochromatosis (HH) eluded scientists for many years, until in 1996 it was reported that the majority of cases of HH

Abbreviations: HH, hereditary haemochromatosis; TFR, transferrin receptor; *hfe*, *HFE*, haemochromatosis gene; hfe, HFE, haemochromatosis gene product; IREG1, duodenal basolateral Fe exporter 1; JH, juvenile haemochromatosis; IRE, Fe-responsive element.

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Table 1. Genes causing altered iron metabolism

Gene	Function	Mutations causing disease in man	Disease and symptoms	Gene locus	References
HFE	Senses Fe status. Interacts with TFR1 and, perhaps, TFR2. Reduces affinity of TFR1 for TF	C282Y, H63D, S65C and other rare mutations (see pp. 11–13)	Hereditary haemochromatosis (type 1): Fe hyperabsorption, liver cirrhosis and fibrosis, hyperferritinaemia, Fe deposits in the heart, pancreas and anterior pituitary	6p21.3	Feder *et al.* (1996), Waheed *et al.* (1997)
HFE2	Unknown	Unknown in HFE2, hepcidin (rare, see below and p. 14)	Juvenile haemochromatosis (type 2): liver cirrhosis, diabetes, arthritis, endocrine disease, cardiomyopathy	1q	Montes-Cano *et al.* (2002), Roetto *et al.* (1999, 2003)
HFE3–transferrin (TF) receptor (*TFR*) 2	May associate with HFE in duodenal crypt cells (TFR1 homologue) or in liver	Y250X, E60X, M172K, AVAQ594-597δ, Q690P	Hereditary haemochromatosis (type 3): see HFE	7q22	Camaschella *et al.* (2000*a*), Roetto *et al.* (2001), Girelli *et al.* (2002), Mattman *et al.* (2002), Hattori *et al.* (2003)
HFE4–*IREG*1	Transports Fe out of enterocytes and reticulo-endothelial (RE) cells	N144H, A77D, Val162δ, D157G, Q182H, G323V, G490D	Autosomal dominant haemochromatosis (type 4): Fe accumulation in liver and RE cells, hyperferritinaemia	2q32	Njajou *et al.* (2001), Montosi *et al.* (2001), Cazzola *et al.* (2002), Devalia *et al.* (2002), Wallace *et al.* (2002*a*), Hetet *et al.* (2003), Jouanolle *et al.* (2003)
L-ferritin	Light chain of ferritin: essential for core formation of ferritin (cellular Fe storage protein)	Any mutation in 5′IRE structure of L-ferritin mRNA	Hereditary haemochromatosis cataracts syndrome: hyperferritinaemia, early onset of bilateral cataracts	19q13	Camaschella *et al.* (2000*b*), Hetet *et al.* (2003), Cremonisi *et al.* (2003)
H-ferritin	Heavy chain of ferritin: provides ferroxidase activity of ferritin	ntA49U in 5′IRE of H-ferritin mRNA	Hereditary haemochromatosis (type 5): Fe overload in liver and heart	11q13	Kato *et al.* (2001)
TF	Plasma Fe transporter: delivers Fe to cells	A477P, G277S, other polymorphisms modifying Fe homeostasis (see pp. 15–16)	Atransferrinaemia: microcytic anaemia and Fe loading	3q21	Beutler *et al.* (2000), Lee *et al.* (2001*c*), Kasvosve *et al.* (2000)
TFR1	Binds Fe–TF at cell surface and transports Fe into cells by endocytosis of the Fe–TF. Interacts with HFE	Unknown	Unknown	3q26.2-qter	Parkkila *et al.* (1997*a*), Waheed *et al.* (1999), Davies *et al.* (2003)
Caeruloplasmin	Cu-binding plasma ferroxidase	Splice site variant, 5 bp insertion in exon 7, W858X, Q146E, ntAins2917	Acaeruloplasminaemia: absence of plasma ferroxidase activity, neurodegeneracy, diabetes	3q23-25	Harris *et al.* (1995), Yoshida *et al.* (1995), Takahashi *et al.* (1996), Bosio *et al.* (2002)
Hepcidin	Antimicrobial peptide synthesized in the liver, putative Fe ‘signaling’ molecule	nt93δG, R56X	Juvenile haemochromatosis: see above	19q13	Roetto *et al.* (2003)
β2-Microglobulin	Binds MHC class I proteins to transport proteins to the cell surface. Binds HFE	Unknown	Unknown	15q21	Waheed *et al.* (1999)
DMT1	Transports divalent metals into duodenal enterocytes and endocytic compartments of phagocytes	Unknown	Unknown	12q13	Gunshin *et al.* (1997)
Dcytb	Apical membrane ferric reductase	Unknown	Unknown	2q31	McKie *et al.* (2001)
Hephaestin	Basolateral membrane-bound ferrioxidase (caeruloplasmin homologue)	Unknown	Unknown	Xq11-q12	Vulpe *et al.* (1999)
IRP1	Regulates translation of ferritin and transferrin by binding to IRE	Unknown	Unknown	9p13-22	Ponka *et al.* (1998)
Haemoxygenase1	Degrades haem	Unknown	Unknown	22q12	Yoshida *et al.* (1988)

HFE, HFE, haemochromatosis gene and gene product respectively; *IREG1*, duodenal basolateral Fe exporter 1 gene; H-ferritin, L-ferritin, heavy and light ferritin chain subunits; DMT1, divalent metal transporter 1; IRP1, Fe-regulatory protein; IRE, Fe-responsive element.

were caused by a missense mutation in a most unlikely candidate, *HFE* (Feder *et al*. 1996). *HFE* is a class I MHC-related gene located at chromosome 6p21.3. Feder *et al*. (1996) reported that a homozygous mutation at nucleotide position 845 was the cause in 85% of patients suffering from HH. The G→A nucleotide substitution replaced a cysteine with a tyrosine residue at position 282 on the protein (C282Y), preventing the formation of an essential disulphide bridge and causing the protein to become conformationally unstable and unable to form a complex with the peptide β_2-microglobulin. An association between HFE and β_2-microglobulin is required for HFE to be expressed at the cell surface (Waheed *et al*. 1999); however, in the C282Y mutation, in HFE this transport process is impaired and HFE accumulates in the endoplasmic reticulum. Biochemical data has shown that normal HFE interacts with TFR1 at the cell surface (Parkkila *et al*. 1997*a*; Waheed *et al*. 1999) and in endocytic vesicles (Davies *et al*. 2003) and reduces its binding affinity for transferrin (Feder *et al*. 1998). The precise mechanism by which HFE modulates Fe metabolism is still under debate, as is how the C282Y mutation in HFE leads to Fe overload. It has been demonstrated that HFE is highly expressed in the crypt enterocytes of the duodenum but not in the villus (Parkkila *et al*. 1997*b*), a pattern of expression also observed for TFR1 (Waheed *et al*. 1999). HFE has also been shown to be closely associated with TFR2 within crypt cells (Griffiths & Cox, 2003). HFE is also expressed in the Kupffer cells of the liver. Thus, it is thought by some researchers that HFE is involved in the 'sensing' of body Fe status, although this idea is still controversial (Frazer & Anderson, 2003). The C282Y mutation in HFE disrupts the HFE–TFR1 interaction and somehow alters the way in which transferrin is taken up into cells (Waheed *et al*. 1997), but again the exact basis of how such a mutation disturbs Fe homeostasis remains to be solved.

There is also some dispute about the clinical penetrance of the C282Y mutation in the general population, where homozygosity ranges from about 1 in 400 in the USA, to 1 in 100 in Northern Ireland (Murphy *et al*. 1998; Steinberg *et al*. 2001). The mutation is, however, very rare in countries in which the population is of non-Northern European or Celtic descent, e.g. in Greece the homozygosity rate of the C282Y mutation is <1 in 100 000 (Papanikolaou *et al*. 2000). A global allele frequency of the C282Y mutation of 1·9% has been reported after the analysis of 2978 normal subjects from forty-two different populations (Merryweather-Clarke *et al*. 1997). The highest frequency was found in Northern Europe and recent evidence suggests that the mutation occurred within the Germanic Iron Age population and migrated with the Vikings (Milman & Pedersen, 2003).

H63D. The second-most-common *HFE* mutation found to cause HH is a C→G substitution at nucleotide position 187, which brings about an amino acid change of histidine residue 63 to an aspartic acid residue (H63D). This mutation is in fact more common in the general population than the C282Y mutation and has a global distribution, with the highest frequencies in Spain (Merryweather-Clarke *et al*. 2000). The homozygous H63D state rarely has a profound effect on Fe homeostasis. Clinical consequences can manifest themselves when a patient is a compound heterozygote for C282Y and H63D, but in these cases the effects of Fe loading are less severe than the C282Y homozygotes (Risch, 1997).

In vitro studies have suggested that the H63D mutation decreases the ability of HFE to reduce the binding affinity of the TFR1 to Fe-loaded transferrin at the cell surface (Waheed *et al*. 1997), but the precise mechanism by which this mutation can alter Fe homeostasis *in vivo* remains unclear. It seems that the H63D mutation in HFE acts as a modifier of Fe metabolism when inherited with some additional factor to cause penetrance of disease. One example is that subjects with the β-thalassaemia trait who are homozygous for H63D tend to have higher ferritin levels than β-thalassaemia carriers with normal HFE (Melis *et al*. 2002).

S65C. This mutation has been reported to have an allele frequency of 1·6–5·5% in Caucasians (Rochette *et al*. 1999). Like the H63D mutation, the S65C mutation appears to produce a mild Fe overload phenotype when inherited with the C282Y mutation, but again penetrance of the compound heterozygotes is low. Nevertheless, it has been suggested that screening for S65C–C282Y compound heterozygosity is important, as these individuals may have an increased risk of Fe overload, which may become augmented by other factors such as excessive alcohol intake and various dietary factors (Wallace *et al*. 2002*b*).

Other haemochromatosis gene mutations. As well as the more common C282Y, H63D and S65C mutations, several other rare *HFE* mutations have been reported in the literature. The nature of these uncommon *HFE* mutations vary from missense (G93R, I105T, Barton *et al*. 1999; Q127H, de Villiers *et al*. 1999; V272L, Worwood *et al*. 1999; Q283P, Le Gac *et al*. 2003) to nonsense (E168X and W169X; Piperno *et al*. 2000), frameshift (V68δT and P160δC; Pointon *et al*. 2000) to splice variants (IV53 IG-T; Wallace *et al*. 1999).

Most causal mutations arise in conjunction with heterozygosity for C282Y or H63D, but it is not known how these mutations result in altered Fe metabolism. It is, however, interesting to note that the G93R and I105T mutations are in a domain of HFE that interacts with TFR1 (Barton *et al*. 1999) and both the E168X and W169X mutants result in truncated HFE that are non-functional (Piperno *et al*. 2000).

Although rare, these mutations may provide exciting clues into how Fe homeostasis is maintained at a molecular level and how communication is achieved between Fe absorption at the duodenum and Fe stores in the liver. A very recent case study has reported a unique case of a liver-transplant patient heterozygous for an unidentified R6S mutation in *HFE* receiving a liver from a C282Y heterozygous donor. The recipient had no previous history of Fe loading, but 4 years after the transplant developed severe Fe overload. This report has rekindled the almost forgotten idea that the duodenum and liver are not mutually exclusive factors when considering regulation of Fe absorption in response to Fe stores, and further complicates the role of HFE in the liver and gut in controlling Fe homeostasis (Adams, 2003; Wigg *et al*. 2003).

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Non-haemochromatosis gene-related haemochromatosis

Haemochromatosis type 2 or juvenile haemochromatosis. Haemochromatosis type 2 has been termed juvenile haemochromatosis (JH) because symptoms appear in the second and third decades of life rather than in the fourth or fifth decades as seen in C282Y homozygotes or compound C282Y–H63D heterozygotes. JH is a rare autosomal recessive disease with clinical consequences much more severe than classical HH (Camaschella, 1998). Symptoms of JH include early Fe deposition in the liver, diabetes, joint disease, skin hyperpigmentation and endocrine disease (hypogonadatropic hypogonadism), and most patients die (if untreated) from cardiomyopathy. In addition, JH affects both sexes equally, whereas classical *HFE*-related HH manifests itself mainly in males.

The gene that causes this disease remains to be identified, but the locus has been mapped to chromosome 1q21 (Roetto *et al*. 1999; Montes-Cano *et al*. 2002). The rapid presentation of Fe overload in haemochromatosis type 2 indicates that the gene responsible is a major player in maintaining Fe homeostasis and may provide essential clues as to the signalling molecules involved in regulating Fe absorption. In addition, there have also been reports of JH unlinked to 1q (Papanikolaou *et al*. 2002). There has been a recent report of two probands with JH found to be caused by two separate homozygous mutations in the hepcidin gene, a new molecule implicated in Fe metabolism, which will be discussed in more detail below.

Hepcidin. The advent of hepcidin into the world of Fe metabolism has provided one of the most promising candidates for the signalling molecule linking Fe stores in the liver and Fe absorption rates in the intestine. Expressed in hepatocytes, hepcidin was originally identified as an antimicrobial peptide found in abundance in urine (Park *et al*. 2001), but was fortuitously found to be involved in Fe metabolism when the hepcidin gene was accidentally knocked out in mice that subsequently developed a haemochromatosis phenotype (Nicolas *et al*. 2001). In addition, severe Fe deficiency was found in mice over-expressing hepcidin (Nicolas *et al*. 2002). Such mouse models indicated that poor regulation of hepcidin or mutations within the gene could cause an inappropriate imbalance in Fe homeostasis in man. Indeed, as mentioned earlier, there has been a recent report of two hepcidin mutations in two unrelated probands with JH: a homozygous mutation in which a guanine base was deleted in exon 2 at nucleotide position 93 resulting in a frameshift, and a nonsense C→T substitution at position 166 in exon 3 resulting in an R56X mutation (Roetto *et al*. 2003). These mutations seem to be isolated causes of JH, and most cases of JH are linked to 1q. It could be that the common gene responsible for JH encodes the elusive hepcidin receptor or some component of its signalling pathway. Nevertheless, the severity of the phenotype seen in patients with JH and the fact that levels of hepcidin are inappropriately low in patients with HFE-related HH (Bridle *et al*. 2003) enhances the idea that hepcidin plays a central role in Fe metabolism.

Haemochromatosis type 3 – transferrin receptor 2. Located on chromosome 7q22, *TFR2*, a *TFR1* homologue (Kawabata *et al*. 1999), has been implicated as yet another key player in Fe homeostasis. Unlike TFR1, TFR2 mRNA expression does not appear to be regulated by cellular Fe levels (Fleming *et al*. 2000), although it does interact with transferrin *in vitro* but at a lower affinity than TFR1 (West *et al*. 2000). Mutations in *TFR2*, however, have a large impact on Fe homeostasis, causing Fe overload similar to that caused by *HFE*-related HH. The first mutation described, in an Italian family with Fe overload, was a homozygous Y250X nonsense mutation in exon 6 of *TFR2* (C→G substitution), encoding a truncated protein (Camaschella *et al*. 2000*a*). There have been several other homozygous mutations in *TFR2* reported in patients who were Fe loaded. These mutations include E60X, in which a C base insertion causes a premature stop codon (Roetto *et al*. 2001), a missense M172K mutation (T→A substitution; Roetto *et al*. 2001), an AVAQ 594–597 del mutation in which a 12 bp deletion in exon 16 brings about the deletion of four residues in the protein sequence (Girelli *et al*. 2002) and a Q690P missense mutation in exon 17 (Mattman *et al*. 2002).

The majority of the *TFR2* mutations have been confined to Southern Europe, mainly Italy; however, the AVAQ 594–597 del mutation has also been reported in a Japanese family with severe Fe loading in the hepatocytes and bile ducts (Hattori *et al*. 2003). Several other screening studies of the *TFR2* gene in patients with Fe overload have revealed many other polymorphisms, but none have proven to be the cause of the disease (Aguilar-Martinez *et al*. 2001; Barton *et al*. 2001; Lee *et al*. 2001*c*; Hofmann *et al*. 2002).

Tissue distribution and regulatory features of TFR2 and TFR1 are distinct, and the precise role of TFR2 in Fe homeostasis still needs to be addressed. Recently, it has been demonstrated that wild-type HFE co-localises with TFR2 in the crypt cells of the small intestine, and both proteins interact in a specialised early endosome compartment involved in the transport of Fe-loaded transferrin (Griffiths & Cox, 2003). However, other researchers have found no evidence in favour of a physical interaction between HFE and TFR2 (West *et al*. 2000).

African iron overload (Bantu siderosis). Clinically distinct from classical HH, Fe overload occurring in sub-Sahara Africa was originally believed to be the result of excessive dietary Fe intake, in particular the consumption of home-made beer brewed in non-galvanised steel drums. The symptoms usually present themselves in middle-aged men with Fe loading in hepatic parenchymal cells and in macrophages. In addition, serum ferritin levels are often elevated, but transferrin saturation levels vary. Mutations in the *HFE* gene have been ruled out as a cause of Fe loading in Africans (McNamara *et al*. 1998). It is now believed that heterozygosity for an unknown gene leads to a predisposition for Fe loading in Africans, which is augmented by excessive Fe intake, and homozygosity may lead to a more severe phenotype (Moyo *et al*. 1998). Non-*HFE* HH has also been described in Americans of African descent (Monaghan *et al*. 1998), but again the responsible genetic factor is unknown.

Neonatal haemochromatosis. Neonatal haemochromatosis is a unique and rare form of Fe overload,

characterised by early liver failure in association with Fe deposition in a variety of organs (Knisely, 1992). The onset of the disorder normally presents itself in the third trimester of pregnancy, perinatally or in early infancy. As with JH and African Fe overload, neonatal haemochromatosis is thought to be a consequence of an autosomal recessive inheritance. Candidate genes such as *HFE*, β_2-microglobulin, haem oxygenase 1 and 2 (the latter two genes are important in neonatal Fe metabolism) have been excluded as the cause of neonatal haemochromatosis (Kelly *et al*. 2001). The sporadic and rare occurrences of neonatal haemochromatosis and the lack of a genetic marker makes prediction of predisposition for the disease an almost impossible task.

Haemochromatosis type 4: autosomal dominant hereditary haemochromatosis. The identification of IREG1 (McKie *et al*. 2000; also known as ferroportin1 (Donovan *et al*. 2000) and metal transporter protein 1 (Abboud & Haile, 2000), presented a new and exciting candidate for non-*HFE*-related autosomal dominant haemochromatosis. This transmembrane protein is expressed at high levels on the basolateral membrane of duodenal enterocytes, reticulo-endothelial cells of the spleen, Kupffer cells of the liver, placenta and kidney. In 2001 independent reports from two countries identified mutations in *IREG1* found to be associated with haemochromatosis type 4 (Montosi *et al*. 2001; Njajou *et al*. 2001). An A→C substitution brought about an N144H amino acid change in a Dutch family with HH (Njajou *et al*. 2001), whereas a C→A change resulting in an A77D substitution was described in Italy (Montosi *et al*. 2001). At the time, two opposing hypotheses were proposed to explain how the two mutations brought about a HH phenotype. Njajou *et al*. (2001) suggested that the N144H mutation led to a gain of function for IREG1 and so would enhance Fe absorption, whereas Montosi *et al*. (2001) postulated a loss of function. This latter suggestion would result in retention of Fe in the Kupffer cells and reticulo-endothelial macrophages, a typical phenotype of haemochromatosis type 4. Since 2001 several other heterozygous *IREG1* mutations have been reported to result in haemochromatosis type 4. The most-frequently-reported change in sequence has been the Val162δ (Cazzola *et al*. 2002; Devalia *et al*. 2002; Wallace *et al*. 2002*a*). This mutation is brought about by the deletion of any three sequential base pairs of a four GTT repeat, causing the loss of one of three conserved valine residues. The phenotype of patients heterozygous for Val162δ presents itself as hyperferritinaemia with Fe loading in the Kupffer cells and reticulo-endothelial macrophages. These clinical findings support the loss of function hypothesis, but further studies are required to verify this hypothesis.

Four further mutations have recently been reported in patients with unexplained hyperferritinaemia: D157G, Q182H, G323V (Hetet *et al*. 2003) and G490D (Jouanolle *et al*. 2003). Mutations N144H, D157G, V162δ and Q182H all lie on a predicted intertransmembrane loop. The zebrafish mutation L169F (corresponding to L170F in the human sequence), responsible for a hypochromic anaemia phenotype, is also found in this region, suggesting that this part of the protein is important in the efficiency of Fe release from the cell. A77D and G490D are also located on loops between transmembrane helices, but on opposite ends of the protein sequences; however, these residues may be in close proximity spatially, defining another potentially-important region of the protein. G323V is located in a predicted transmembrane domain and may alter the conformation of the protein.

Other rare disorders of iron metabolism

Hereditary hyperferritinaemia-cataract syndrome. Ferritin is the main Fe-storage protein and is found in various isoforms related to the relative proportions of heavy and light ferritin chain subunits (Harrison & Arosio, 1996). The synthesis of ferritin is regulated at the translational level by Fe through a conserved Fe-responsive element (IRE) in the 5′ untranslated region of all ferritin mRNA. In the absence of Fe Fe-regulatory proteins bind to IRE and repress the translation of ferritin mRNA. When Fe is in abundance it binds to Fe-regulatory proteins, releasing them from IRE and stimulating translation of ferritin (Ponka *et al*. 1998).

Heterozygous mutations in the 5′IRE of the ferritin light chain have been found to cause a disease known as hereditary hyperferritinaemia-cataract syndrome. This disease is characterised by high serum ferritin as a result of uncontrolled synthesis of the ferritin light chain in the face of high Fe, early presentation of bilateral cataracts and normal to low serum Fe and transferrin saturation (Camaschella *et al*. 2000*b*). The development of cataracts in hereditary hyperferritinaemia-cataract syndrome is a direct result of mutations in the ferritin light chain IRE, as ferritin accumulates and crystallises in the lens of the eye.

One recent report, in which two new mutations of the ferritin light chain have been identified in patients with hereditary hyperferritinaemia-cataract syndrome (a U34→C substitution and a G47→A substitution), highlighted the phenotypic variability seen in patients with this disease (Hetet *et al*. 2003). Serum ferritin levels, although elevated, can vary enormously (800–3000 μg/l), as can the age of onset of cataracts. Another two previously-unidentified mutations in the IRE of the ferritin light chain have recently been found in hereditary hyperferritinaemia-cataract syndrome: C36→G and A37→G (Cremonisi *et al*. 2003).

Interestingly, there has been one isolated incidence of autosomal dominant hyperferritinaemia in a Japanese family that has been linked to a mutation in the 5′ untranslated region of the ferritin heavy chain (Kato *et al*. 2001). Other studies have analysed cohorts of patients with Fe overload for mutations in the ferritin heavy chain (Lee *et al*. 2001*a*), but this Japanese case appears to be the only mutation in the ferritin heavy chain affecting Fe metabolism reported so far.

Atransferrinaemia (hypotransferrinaemia). Atransferrinaemia is a rare disorder characterised by severe microcytic anaemia and Fe loading. The plasma transferrin is diminished to the point of absence, but the condition can be successfully treated by parenteral administration of transferrin to avoid fatality. One case has been characterised at the gene level (Beutler *et al*. 2000). The proband

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was a compound heterozygote for mutations consisting of a 10 bp deletion followed by a 9 bp insertion of a duplicated sequence, and a G→C transversion at position 1429 causing an A477P substitution at a highly-conserved site.

Mutations associated with iron deficiency in man

Transferrin

Polymorphisms in the transferrin gene appear to subtly modify Fe metabolism. It has been shown that a G→A mutation at position 829 on the cDNA of transferrin (a G277S mutation) reduces total Fe-binding capacity, and it has been suggested that this polymorphism may put menstruating Caucasian women at an increased risk of Fe deficiency (Lee *et al*. 2001*b*). In addition, a report has suggested that heterozygosity for wild-type transferrin and cathodal transferrin may provide protection from Fe overload in black Africans (Kasvosve *et al*. 2000).

Genes involved in altered iron metabolism in experimental animals or fish

Studies in mutant experimental animals have been essential to the discovery of five key genes of mammalian Fe metabolism, i.e. divalent metal transporter 1 (nramp2, SLC11A1), *IREG1* (or ferroportin or metal transporter protein 1; SLC40A1), hephaestin, Dcytb and hepcidin. The first known mammalian Fe transporter, divalent metal transporter 1, was discovered in studies of Fe-deficient rats. The contemporary finding that this gene was mutated in microcytic anaemia mice (*mk*) and Belgrade rats (for review, see Andrews, 2000) was a major contribution to understanding the importance of this gene for Fe absorption. Similarly, IREG1, or metal transporter protein 1, was discovered in studies with genetic hypotransferrinaemia mice (McKie *et al*. 2000), with rats (Abboud & Haile, 2000) and with anaemic zebrafish (*Danio rerio*; Donovan *et al*. 2000). Hephaestin was discovered by studying the sex-linked anaemia in mice (Vulpe *et al*. 1999) and Dcytb identified with the aid of the hypotransferrinaemic mice (McKie *et al*. 2001). Most recently, the identification of the role of hepcidin as an Fe-absorption regulatory hormone was greatly aided by studies of upstream stimulatory factor 2 knock-out mice (Nicolas *et al*. 2001, see earlier, p. 14).

Understanding of the function of any gene of Fe metabolism is further aided by studies with mice that are deliberately created with targeted mutations in the genes of interest. Fig. 1 shows how destruction of the *hfe* gene protects mice against Fe-deficiency anaemia when they are fed diets that are not Fe-rich (destruction of the *hfe* gene has a similar effect to C282Y mutation). These findings are an experimental verification of the hypothesis, developed from human studies, that *HFE* mutations that prevent the HFE protein from functioning can protect against Fe deficiency when a Fe-poor diet is being consumed (Datz *et al*. 1998; Beutler *et al*. 2003). Understanding of hfe function was greatly enhanced by studies with β_2-microglobulin knock-out mice. These mice develop Fe overload

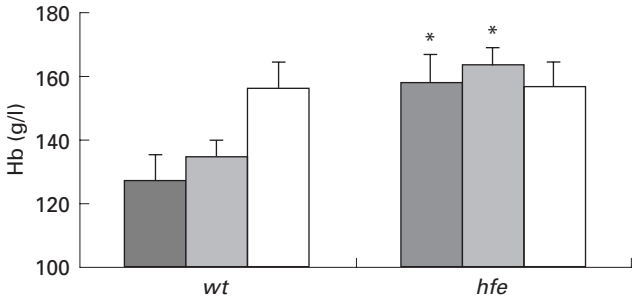

Fig. 1. Mice were fed purified diets of varying iron content for 5 weeks from 7 weeks of age: iron-poor (6 mg/kg; ■), normal (180 mg/kg; ■), iron-rich (20 g/kg; □). For further details of diets, see Simpson *et al.* (2003). *wt*, Wild type (i.e. genetically normal); *hfe*, homozygotes for the haemochromatosis gene deletion. Values are means with their standard errors represented by vertical bars for eight to twenty-six mice. Mean values were significantly different from those for the corresponding *wt* mice: $^*P < 0{\cdot}05$. Mean value was significantly different from those for *wt* mice fed normal or iron-poor: $\dagger P < 0{\cdot}05$. Statistical analysis was by two-way ANOVA followed by subsidiary *t* tests. The results indicate that haemochromatosis gene deletion leads to increased Hb levels except in mice fed the iron-rich diet and that *wt* mice have higher Hb levels when fed the iron-rich diet.

similar to haemochromatosis (Santos *et al*. 1996), and this finding was explained by the interaction between hfe protein and β_2-microglobulin within cells (Waheed *et al*. 1997). Levy *et al*. (2000) showed that loss of a *tfr1* allele can influence Fe metabolism in *hfe* knock-out mice, presumably as a result of the interaction between the two proteins.

Other genes

In addition to the previously mentioned genes, studies in both man and mouse have shown that other, yet to be identified, genes influence Fe status. As discussed earlier, JH, a rapid-onset hereditary Fe overload, is attributable to an unidentified gene on chromosome 1q. Linkage disequilibrium studies with *HFE* suggest that another MHC-related gene affects Fe stores (Pratiwi *et al*. 1999). Twin studies in man show that genes other than *HFE* are important in determining Fe status (Whitfield *et al*. 2003), while studies with mouse strains have confirmed this finding (Dupic *et al*. 2002). It is also apparent from knowledge of Fe metabolism that other genes are likely to be identified, e.g. those coding for hepcidin receptor(s) or proteins that sense hepcidin levels, haem receptors or transporters (Miret *et al*. 2003) and also proteins involved in the regulation of hepcidin levels (Nemeth *et al*. 2003). Any or all the genes for these proteins may prove to have variants in man that influence Fe stores. As noted in Table 1, variants of genes not directly related to Fe metabolism, such as caeruloplasmin, can lead to altered Fe stores (for references, see Bosio *et al*. 2002). Analogous with the discovery of hepcidin, it is likely that genes not presently associated with Fe metabolism will prove to influence Fe status.

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Conclusions

Fe lies at the heart of cellular energy and O_2 metabolism, and it is not surprising that failure to correctly maintain Fe levels leads to complex and diverse consequences. Alterations in Fe stores can be a result of genetic or dietary variation. Genetic variation can be a consequence of known Fe-metabolism genes or other genes not previously implicated in this process. The new tools of molecular genetics are rapidly being applied to testing whether altered Fe-metabolism genes may be a factor in a variety of disorders. It is expected that new genes and variants that influence Fe stores will be found in the near future. Since the preparation of this paper, a gene responsible for 1q-linked juvenile haemochromatosis has been proposed and named *HFE2*, and a causal mutation identified (Papanikoloau *et al*. 2004), while a new mutation (Q248H) in *IREG1* has been found to be associated with iron overload in Africans and African-Americans (Beutler *et al*. 2003; Gordeuk *et al*. 2003).

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