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Molecular basis of iron-loading disorders

Deepak Darshan, David M. Frazer and Gregory J. Anderson*

Iron-loading disorders (haemochromatosis) represent an important class of human diseases. Primary iron loading results from inherited disturbances in the mechanisms regulating intestinal iron absorption, such that excess iron is taken up from the diet. Body iron load can also be increased by repeated blood transfusions (secondary iron loading), usually as part of the treatment for various haematological disorders. In these syndromes, an element of enhanced iron absorption is also often involved. The central regulator of body iron trafficking is the liver-derived peptide hepcidin. Hepcidin limits iron entry into the plasma from macrophages, intestinal enterocytes and other cells by binding to the sole iron-export protein ferroportin, and facilitating its removal from the plasma membrane. Mutations in hepcidin or its upstream regulators (HFE, TFR2, HFE2 and BMP6) lead to reduced or absent hepcidin expression and a concomitant increase in iron absorption. Mutations in ferroportin that prevent hepcidin binding produce a similar result. Increased ineffective erythropoiesis, which often characterises erythrocyte disorders, also leads to reduced hepcidin expression and increased absorption. Recent advances in our understanding of hepcidin and body iron homeostasis provide the potential for a range of new diagnostic and therapeutic tools for haemochromatosis and related conditions.

Iron is an essential element for cell growth and division but is also a strong pro-oxidant, so cells and whole organisms have developed sophisticated mechanisms for keeping iron levels within the physiologically optimum range. Either too little or too much iron can have significant clinical consequences. The main cause of low iron levels in the body is inadequate dietary intake, and iron deficiency is one of the world's most significant nutritional deficiencies (Ref. 1). In contrast, body iron overload usually results from mutations in genes encoding proteins that regulate body iron intake. Although less widespread than iron deficiency, iron-loading syndromes represent an important class of human diseases, particularly in populations where iron nutrition is adequate. One of these disorders, HFE-related hereditary haemochromatosis (HFE refers to the affected protein), is the most common disease of humans

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that shows autosomal recessive inheritance (Ref. 2). There have been important advances in understanding these diseases in recent years, and their analysis has taught us much about the mechanisms of body iron homeostasis.

If the total body iron load increases, the extra iron must come from external sources. There are basically two such sources: the diet and blood transfusions. Iron-loading disorders characterised by defects in proteins directly involved in body iron acquisition are known as primary iron-loading diseases. Under these conditions, intestinal iron absorption is dysregulated such that the body continues to accrete iron in the presence of adequate or even enhanced iron stores (Ref. 3). Secondary iron loading usually results from blood transfusions. Patients who receive transfusions to treat haemoglobinopathies, bone marrow failure or haemolytic syndromes accumulate iron in their tissues after the iron derived from the transfused red cells is released in macrophages following erythrophagocytosis (Ref. 4). Haematological conditions where erythropoiesis is markedly ineffective might also be associated with increased intestinal iron absorption, and this compounds their iron loading (Ref. 5). Thus the distinction between primary and secondary iron loading is often blurred. A summary of the various forms of iron loading is provided in Table 1.

Normal iron metabolism

The adult human male contains approximately 4–5 g of iron (Ref. 6). Most of this iron is used for metabolic functions (in haemoglobin, myoglobin and a myriad of enzymes that require iron as a cofactor), but approximately 1 g is storage iron that is in excess of immediate requirements and can be accessed in times of need. In women the total body iron content is somewhat lower and iron stores average around 300 mg (Ref. 7). In subjects with iron overload, body iron stores can expand enormously and can reach 20–40 g or even higher (Ref. 8).

The body iron content is determined through the regulation of iron transport across enterocytes of the proximal small intestine (Fig. 1). Most dietary iron is in the oxidised or ferric (Fe^{3+}) form and must be reduced to ferrous iron (Fe^{2+}) before it can be utilised by the enterocytes. A candidate ferric reductase is the brush-border enzyme Dcytb (CYBRD1) (Ref. 9), but other reductases undoubtedly exist. The reduced iron is transported across the apical membrane of the enterocyte by divalent-metal-ion transporter 1 [DMT1 (SLC11A2)] (Refs 10, 11). Iron within the enterocyte that is not immediately required by the body is stored within ferritin and is ultimately lost when the enterocyte is sloughed into the lumen at the end of its life. Iron that is needed is transported across the enterocyte basolateral membrane and into the circulation through the iron exporter ferroportin 1 [FPN1 (SLC40A1)] (Refs 12, 13, 14). For this process to occur efficiently, FPN1 must act in conjunction with the iron oxidase hephaestin (HEPH) (Ref. 15). Physiological (Refs 16, 17) and molecular (Refs 18, 19) studies have shown that the basolateral transport of iron is rate limiting for iron absorption.

Iron that enters the bloodstream is bound to circulating plasma transferrin (TF) and is delivered throughout the body to sites where it can be utilised or stored (Ref. 20) (Fig. 1). Transferrin binds to transferrin receptor 1 [TFR1 (TFRC)] on the plasma membrane of most body cells and the iron is delivered internally by receptor-mediated endocytosis (Ref. 20). TFR1 has a particularly high affinity for diferric (or holo) transferrin, but it can also bind monoferric transferrin with lower affinity. Cells that have very high iron requirements express particularly high levels of TFR1. Such cells include immature erythroid cells (which must synthesise large amounts of haemoglobin) and rapidly dividing cells, such as the crypt enterocytes of the intestine. The recycling of haemoglobin-derived iron from senescent red cells is carried out by macrophages (Ref. 21). This is quantitatively the most important iron-trafficking pathway within the body and accounts for approximately 80% of body iron movement (Ref. 7). After iron is released from haem by haem oxygenase, the iron is exported from the macrophages by FPN1 (Ref. 22) (Fig. 1).

Both cellular and body iron trafficking are tightly regulated to ensure an adequate supply of iron during times of increased demand, and to prevent the accumulation of unnecessary and potentially toxic iron. Body iron homeostasis is regulated by a small peptide known as hepcidin (HAMP) (Ref. 23). Hepcidin is synthesised predominantly in the liver and has a crucial role in communicating the body's iron requirements to the intestine (and other cells) (Fig. 1). Its main function is to control the amount of iron

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Table 1. Classification of some iron-loading disorders

Disorder	Gene	OMIM	Hepcidin	Clinical features
Primary iron overload				
HFE-related haemochromatosis (type 1)	*HFE*	235200	Low	Parenchymal iron overload, liver disease
TFR2-related haemochromatosis (type 3)	*TFR2*	604270	Low	Parenchymal iron overload, liver disease
FPN-related haemochromatosis (type 4)	*SLC40A1* (*FPN1*)	606069		
Transport defective			Low to normal[a]	Parenchymal and reticuloendothelial iron overload (liver)
Hepcidin resistance			High	Parenchymal iron overload
Juvenile haemochromatosis (HJV-related) (type 2A)	*HFE2* (*HJV*)	602390	Very low	Severe parenchymal iron overload, cardiac disease, liver
Juvenile haemochromatosis (hepcidin-related) (type 2B)	*HAMP*	613313	Very low to absent	cirrhosis, endocrine failure, diabetes, arthropathy
Secondary iron overload				
Ineffective erythropoiesis				
β-Thalassaemia	*HBB*	141900	Low to normal	Parenchymal and reticuloendothelial iron overload,
Sickle cell anaemia	*HBB*	603903	Low to normal	anaemia, reticulocytosis
X-linked sideroblastic anaemia	*ALAS2*	300751	Low[a]	
Chronic haemolytic anaemias				
Pyruvate kinase deficiency	*PKLR*	266200	Low	Parenchymal iron overload, anaemia
Hereditary spherocytosis	Heterogeneous	182900	Low	Parenchymal iron load, anaemia, jaundice and splenomegaly
Hypoplastic anaemias				
Fanconi anaemia	FANC group	227650	Low[a]	Pancytopaenia, myelodysplastic syndromes
Diamond–Blackfan anaemia	Heterogeneous	105650	Low[a]	Red cell aplasia, predisposition to leukaemia
Chronic renal failure			High	Iron loading and anaemia
Some other iron-loading disorders				
Friedreich ataxia	*FXN*	229300	Unknown[a]	Mitochondrial iron overload, neurological and heart disease
Hereditary atransferrinaemia	*TF*	209300	Low	Severe haemosiderosis of the heart and liver
Hereditary aceruloplasminaemia	*CP*	604290	Low	Iron loading, degeneration of the retina and basal ganglia, diabetes mellitus

[a]Predicted levels based on current knowledge of hepcidin regulation.
Abbreviations: ALAS2, delta aminolevulinate synthase 2; CP, ceruloplasmin; FANC, Fanconi anaemia complementation; FPN, ferroportin; FXN, frataxin; HAMP, hepcidin; HBB, haemoglobin beta; HJV, haemojuvelin; OMIM, Online Mendelian Inheritance in Man (ID); PKLR, pyruvate kinase, liver and red blood cell; SLC40A1, solute carrier family 40 (iron-regulated transporter), member 1; TF, transferrin; TFR2, transferrin receptor 2.

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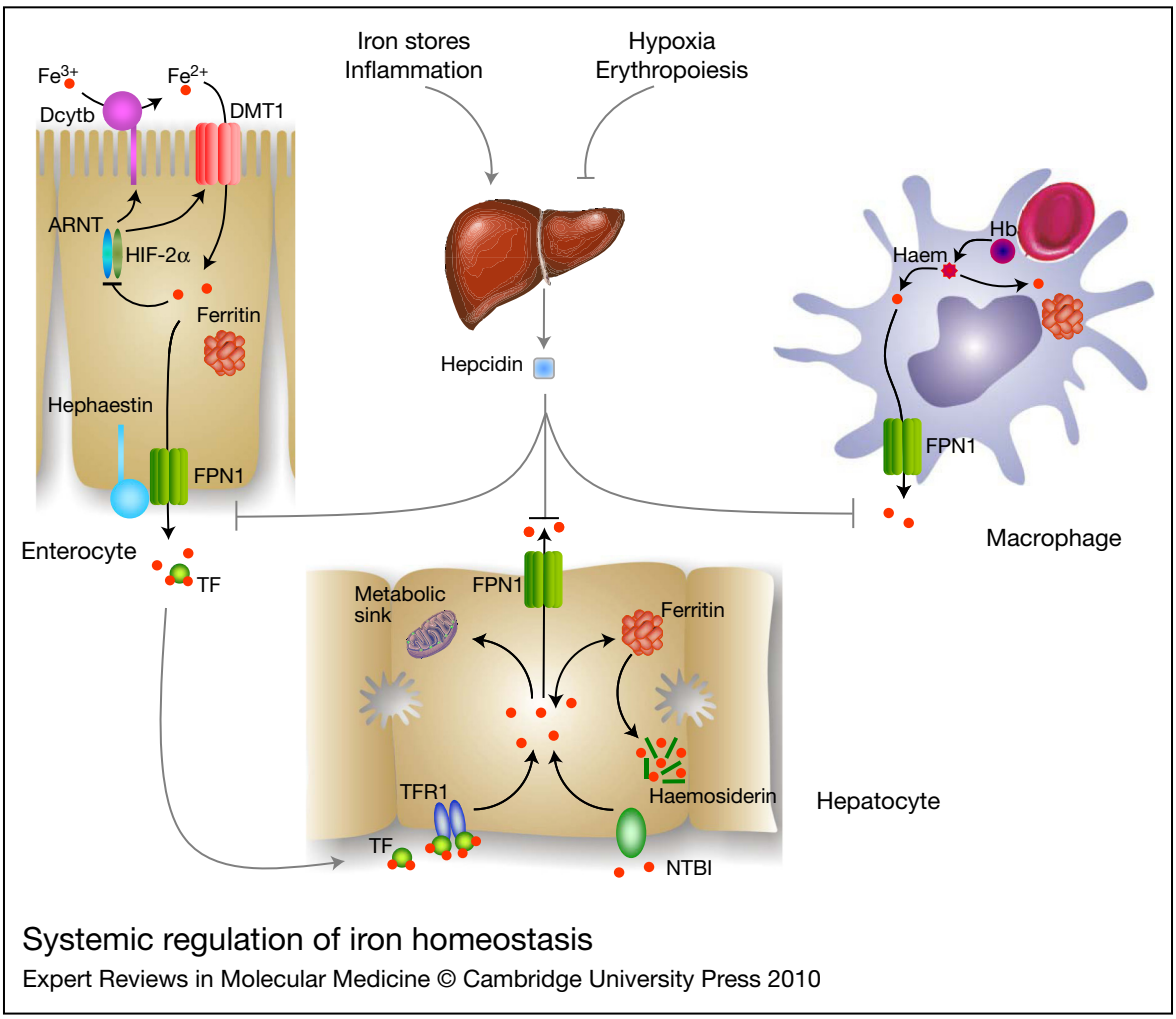


Figure 1. Systemic regulation of iron homeostasis. Iron is reduced at the brush-border membrane of duodenal enterocytes (Dcytb is a candidate ferric reductase) and transported across the membrane by DMT1. Excess iron is stored in ferritin and the remainder is transported across the basolateral membrane and into the circulation by the iron exporter FPN1. Excess iron can also suppress the ARNT–HIF-2α complex, which normally induces DMT1 and Dcytb expression. Iron that enters the bloodstream is bound to circulating plasma TF. TF delivers iron to various tissues, including hepatocytes where it is either stored in ferritin or utilised for cellular metabolism. Iron that is not bound to TF (NTBI) can also enter hepatocytes and contributes to the labile iron pool. Excess iron is stored in ferritin and its degradation product haemosiderin. When required, iron can be removed from ferritin and exported from hepatocytes. Senescent red blood cells are engulfed by reticuloendothelial macrophages. Hb is broken down, and iron is either released into the circulation or stored in ferritin. The liver-derived peptide hepcidin blocks cellular iron release by binding to FPN and facilitating its removal from the membrane, thereby regulating iron entry into the plasma. Iron stores and inflammation positively regulate hepcidin levels, whereas hypoxia and increased erythropoiesis downregulate it. Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; DMT1, divalent-metal-ion transporter 1; FPN1, ferroportin; Hb, haemoglobin; HIF-2α, hypoxia-inducible factor 2α; NTBI, non-transferrin-bound iron; TF, transferrin; TFR1, transferrin receptor 1.

released into the circulation and it does this by binding to the iron exporter FPN1 and facilitating its internalisation and degradation (Ref. 24). When body iron requirements are high (e.g. under iron-deficient conditions or when erythropoiesis is elevated), hepcidin levels are reduced and enterocytes, macrophages and other cells are free to donate iron to the plasma. In contrast, when iron demands are reduced, high levels of hepcidin mean a reduction in iron

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transport to the plasma. Thus there is a close inverse relationship between hepcidin levels and iron absorption. An understanding of the biology of hepcidin is critical for understanding the pathogenesis of iron-loading disorders, and is considered in detail below.

Primary iron-loading disorders

Primary iron loading in most cases results from increased absorption of dietary iron by the small intestine, although in some cases there might also be a redistribution of iron between tissues. These diseases are collectively referred to as haemochromatosis.

HFE-related (type 1) haemochromatosis

The most common type of primary iron overload is HFE-related haemochromatosis (Ref. 2). This autosomal recessive disorder is usually caused by a homozygous mutation within the *HFE* gene on chromosome 6, leading to the C282Y substitution in the membrane protein HFE, although several other mutations have also been described (Ref. 3). HFE-related haemochromatosis is essentially restricted to populations of northern European origin, in which the prevalence of homozygosity for the main mutation that predisposes to iron loading can be 1 in 200 or even higher (Ref. 2). However, although the great majority of such individuals accumulate excess iron in their body, only a minority progress to significant disease. A recent population study demonstrated that approximately 30% of males but only 1% of females develop clinical disease (Ref. 25). The reasons for this are not entirely resolved, but a range of environmental and genetic factors are likely to be involved. In the case of women, menstrual blood loss and childbirth are significant contributors to the loss of body iron and probably the main reason why the disease is far less common in women. However, studies in *Hfe*-knockout mice demonstrate that multiple pregnancies do not reduce iron stores (Ref. 26). Recently, genome-wide association studies have shown that polymorphisms in the transferrin gene in combination with C282Y mutation in the *HFE* gene can explain 40% of the genetic variation in serum transferrin levels (Ref. 27). Polymorphisms in other genes that contribute to genetic variation of the iron status have been reported extensively; however, they are beyond the scope of this review.

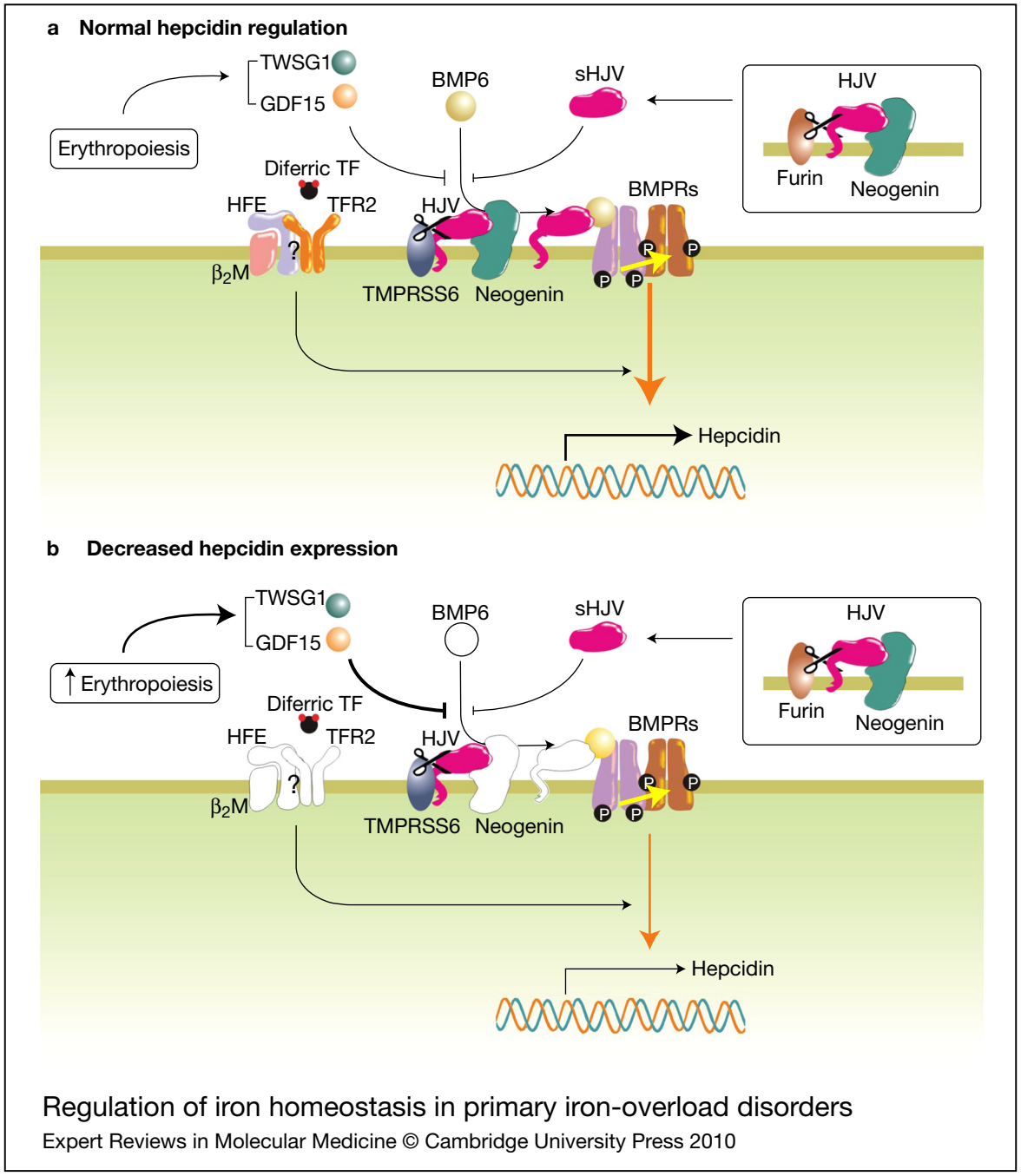
Like other primary iron-loading disorders, HFE-related haemochromatosis is characterised by reduced hepcidin levels (Fig. 2), increased intestinal iron absorption and the subsequent deposition of iron in a range of tissues, including the liver, heart and pancreas (Ref. 2). Iron acts as a strong pro-oxidant and is able to catalyse reactions leading to the production of reactive oxygen species (Ref. 28). These in turn are able to damage a wide range of biological macromolecules, and this underlies the tissue damage that is associated with body iron loading (Ref. 29). Many of the clinical manifestations of the disease reflect hepatic iron accumulation. Iron initially deposits in the hepatocytes surrounding the portal tracts, but in advanced cases iron deposition can be panlobular. Resident macrophages of the liver (Kupffer cells) are characteristically low in iron in haemochromatosis patients but might contain iron in advanced cases.

TFR2-related (type 3) haemochromatosis

This is a rare form of primary iron loading associated with mutations in the transferrin receptor 2 (*TFR2*) gene (Ref. 30). TFR2 is a homologue of TFR1, but it has a much more restricted tissue distribution with predominant expression in the parenchymal cells of the liver (hepatocytes) where it is expressed at levels several times higher than TFR1 (Ref. 31). Like TFR1, TFR2 can bind diferric transferrin and facilitate its internalisation (Ref. 32). However, the primary role of TFR2 does not appear to be iron acquisition because the livers of patients with TFR2 mutations (or *Tfr2*-knockout mice) readily accumulate iron (Refs 30, 33). Rather, TFR2 has an important role in regulating the expression of the iron-regulatory peptide hepcidin (Refs 34, 35) (Fig. 2). This is considered in more detail below. Patients with TFR2 mutations show a histological pattern of iron deposition similar to that seen in HFE-related disease, and the consequences of iron loading are essentially the same (Ref. 36). Examination of the small number of clinical cases suggests that the rate of iron accumulation in type 3 haemochromatosis may be somewhat greater than HFE-related iron loading, and hence the disease is of slightly greater severity (Ref. 36). This has been borne out in studies using knockout mice where animals lacking TFR2 accumulate greater

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Regulation of iron homeostasis in primary iron-overload disorders

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Figure 2. Regulation of iron homeostasis in primary iron-overload disorders. (a) Normal hepcidin regulation. The presence of HFE, TFR2, HJV and neogenin on the hepatocyte surface is required for normal hepcidin expression and regulation. Neogenin interacts with and stabilises HJV, preventing its cleavage by TMPRSS6 and furin. TMPRSS6 cleaves HJV and thus reduces hepcidin expression in hepatocytes. TWSG1, GDF15 and soluble HJV inhibit binding of BMP6 to BMPRs and regulate hepcidin negatively. The nature of the interaction between HFE and TFR2 remains unclear. (b) Decreased hepcidin expression. Mutations in, or the absence of, HFE, TFR2, HJV, neogenin or BMP6 result in a decrease in hepcidin expression, increased intestinal iron absorption and body iron overload. Abbreviations: BMP6, bone morphogenetic protein 6; BMPRs, BMP receptors; GDF, growth differentiation factor 15; HJV, haemojuvelin; β_2M, β_2-microglobulin; TF, transferrin; TFR2, transferrin receptor 2; TMPRSS6, transmembrane protease, serine 6; TWSG1, twisted gastrulation 1.

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amounts of iron than those lacking HFE (Ref. 37).

Juvenile (type 2) haemochromatosis

Severe, early-onset iron loading is known as juvenile haemochromatosis (Ref. 38). Symptoms in affected patients are usually seen in the second decade of life, and by the third decade organ damage may occur. Cardiac dysfunction is far more prevalent in juvenile haemochromatosis than in patients with mutations in *HFE* or *TFR2* (Ref. 38). Overall, however, the pattern of iron deposition is similar to that seen in HFE-related haemochromatosis and is consistent with greatly increased dietary iron absorption. Almost all cases of juvenile haemochromatosis can be attributed to mutations in one of two genes, those encoding haemojuvelin [HJV (HFE2)] (type 2a haemochromatosis) (Ref. 39) or hepcidin (type 2b haemochromatosis) (Ref. 40). Mutations in HJV are more common, but both conditions are considered rare. Like subjects with mutations in HFE or TFR2, hepcidin levels are reduced in juvenile haemochromatosis (Fig. 2), but in this condition the levels are negligible or undetectable (Refs 39, 40). This explains the uninhibited iron absorption under these conditions and the rapid iron deposition in the tissues.

Ferroportin disease (type 4 haemochromatosis)

Mutations in the gene encoding FPN1 are also able to lead to body iron loading (Ref. 41). This form of iron overload, which is inherited in an autosomal dominant manner, is often known as ferroportin disease and shows a relatively complex pathogenesis. In some cases, plasma iron levels are increased and iron accumulates predominantly in hepatic parenchymal cells, consistent with increased intestinal iron absorption; however, in most cases reticuloendothelial iron deposition is more prominent and plasma iron levels are low (Ref. 41). The difference in the iron-loading mechanisms between these two variants of ferroportin disease depends on the nature of the underlying mutation. Most mutations lead to an FPN1 protein that is defective in iron transport. In these cases the total body iron load is not increased as intestinal iron transport is reduced. In contrast, iron recycling through macrophages is also defective, and this means that the normal internal recycling of iron is impeded. Consequently, iron accumulates in reticuloendothelial macrophages and plasma iron levels are low. However, some mutations do not impair the iron-transport capacity of FPN1 but affect its ability to bind hepcidin. Hepcidin 'resistance' mutations mean that the body is unable to limit its iron absorption effectively and an iron-loading phenotype results, which is similar to that of patients with HFE, TFR2, HJV or hepcidin mutations. Many mutations in FPN1 have been described, and ferroportin disease is the most frequently reported form of non-HFE primary iron overload (Ref. 3). Unlike HFE-related haemochromatosis, ferroportin disease does not show a strong founder effect and cases have been identified worldwide.

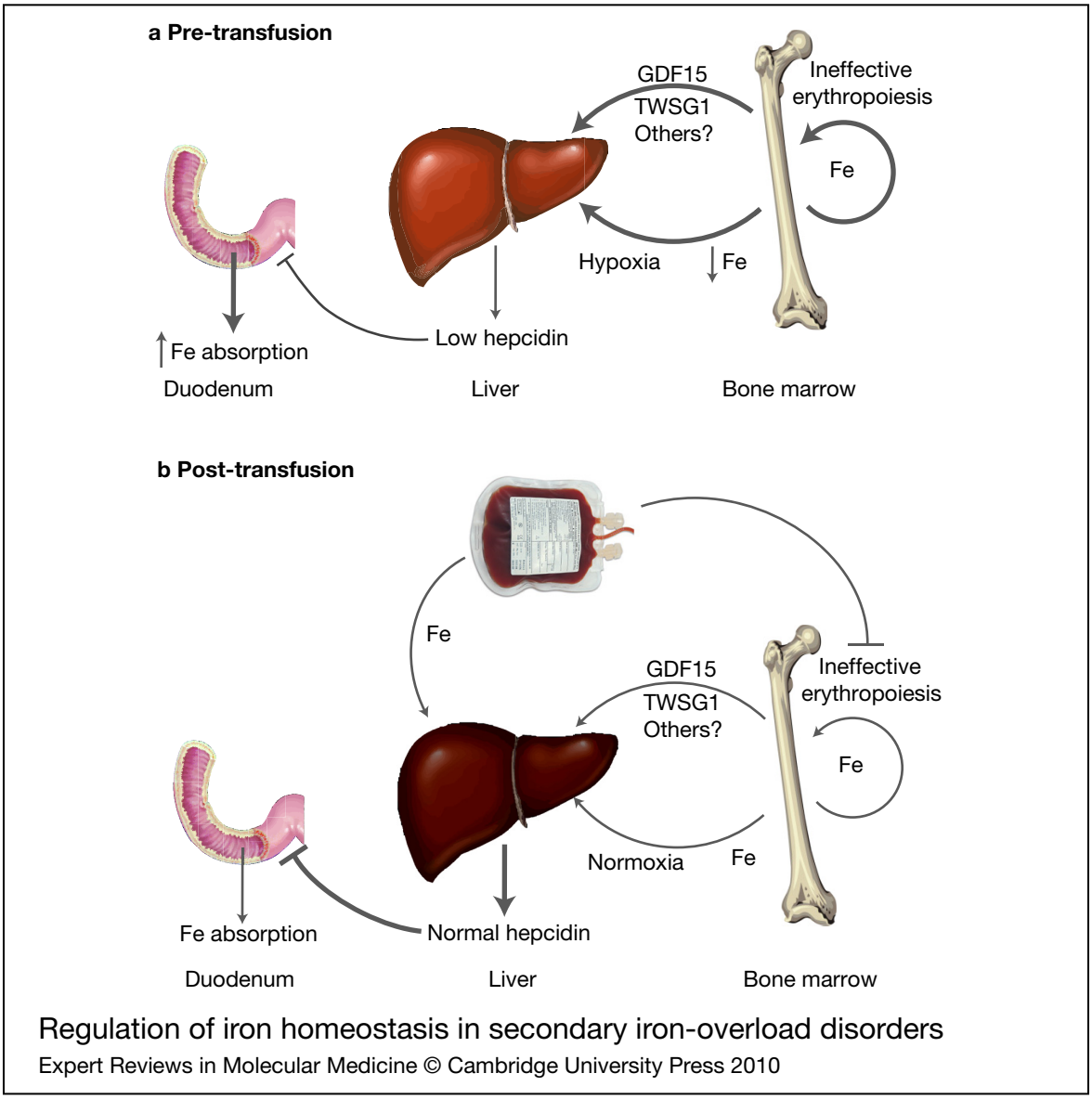
Secondary iron-loading disorders

The term secondary iron loading can be applied to a range of iron-overload disorders where the pathway regulating intestinal iron absorption is intact (Ref. 4). The excess iron in these conditions comes from two main sources: either from increased intestinal iron absorption as a consequence of some underlying pathology or from repeated blood transfusions (transfusional siderosis). Often both routes are involved. The best-characterised disorders of this class are certain diseases of the red blood cell (Ref. 4). These include those associated with ineffective erythropoiesis (e.g. β-thalassaemia, sideroblastic anaemia), chronic haemolytic anaemias (e.g. pyruvate kinase deficiency, sickle cell anaemia) or hypoplastic anaemias (e.g. chronic renal failure, aplastic anaemias). Repeated blood transfusions are usually the main source of the iron in these conditions, but in those associated with ineffective erythropoiesis, iron absorption is often markedly elevated (Ref. 5) (Fig. 3). Indeed, in some disorders, such as β-thalassaemia, heavy iron loading can occur even in the absence of transfusion.

In patients with various iron-loading anaemias, iron enters the reticuloendothelial system as defective erythrocytes are cleared from the marrow or from the circulation. The same fate befalls transfused red cells as they age and are processed by tissue macrophages. In the early stages of the disease, the iron released from these erythrocytes is recycled back to the bloodstream and is reutilised as part of the

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Regulation of iron homeostasis in secondary iron-overload disorders

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Figure 3. Regulation of iron homeostasis in secondary iron-overload disorders. (a) Pre-transfusion. Ineffective erythropoiesis in various red blood cell disorders leads to an increase in iron recycling in the bone marrow, an increase in the expression of the erythropoiesis-related factors TWSG1 and GDF15, and a decrease in serum iron and oxygenation. These factors combine to reduce hepcidin expression in the liver and increase iron absorption in the duodenum. (b) Post-transfusion. Transfusion of blood leads to suppression of ineffective erythropoiesis, lower levels of TWSG1 and GDF15, and relative normoxia and serum iron levels. It also contributes to higher iron stores in the liver. These factors combine to increase hepcidin levels and restore iron absorption to normal levels. Abbreviations: GDF15, growth differentiation factor 15; TWSG1, twisted gastrulation 1.

enhanced erythropoiesis associated with these conditions. However, because humans do not possess a mechanism for the active secretion of iron (Ref. 42), the extra iron derived from transfusions accumulates. Initially, iron accumulation is observed in macrophages, but over time deposition in parenchymal cells develops as well. In secondary iron-loading disorders, cardiac toxicity tends to be more prominent compared with the predominantly hepatic manifestations associated with primary iron loading (Ref. 4). Nevertheless, hepatic and

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endocrine dysfunction can also occur in transfusional siderosis. Patients with secondary iron loading cannot be treated with phlebotomy therapy, so the excess iron is removed by chelation therapy (Ref. 4). For many years the most commonly used chelator was desferrioxamine, which required prolonged subcutaneous infusion. However, in recent years the orally active and highly effective chelator deferasirox has come into widespread use (Refs 43, 44). An intriguing recent finding is that administration of transferrin (either apo or holo protein) was able to ameliorate many of the pathological features of β-thalassaemia in a murine model (Ref. 45). How transferrin exerts these effects is unclear, but alterations in marrow iron supply and effects on hepcidin synthesis could both be involved.

In addition to the disorders described in this and the previous section, there are several miscellaneous forms of iron overload of varying aetiologies, but most of these are uncommon to rare. These include medicinal iron overload (a classical, but rare, form of secondary iron loading) (Ref. 46), African iron overload (which appears to have both genetic and environmental components) (Ref. 47), rare diseases of plasma protein synthesis, such as congenital atransferrinaemia (Ref. 48) and aceruloplasminaemia (Ref. 49), and neonatal haemochromatosis (Ref. 50). In each of these situations severe iron loading can develop. Less substantial iron loading is associated with chronic liver disease (e.g. alcoholic liver disease, fatty liver disease, dysmetabolic iron-loading syndrome) (Ref. 51) and porphyria cutanea tarda (Ref. 52). A full discussion of each of these disorders is beyond the scope of this review, but disturbances in hepcidin regulation have an important role in a number of them.

Mechanisms of body iron loading: central role of hepcidin

A common feature of primary iron-loading disorders is increased intestinal iron absorption. Under these conditions, the normal mechanism that limits the absorption of excess iron when the body is iron replete is dysfunctional and absorption continues in the face of adequate or even elevated iron stores (Ref. 53). In subjects with inherited erythroid disorders characterised by iron loading, iron absorption is also increased (Ref. 5). In these cases, however, the high absorption is a normal physiological response to the accelerated erythropoiesis associated with these conditions. Despite the importance of this process, few studies have examined iron absorption per se in primary haemochromatosis and essentially all the work that has been done in humans has been carried out in HFE-related haemochromatosis. An important study by McLaren et al. showed that for a given level of body iron, the amount of iron absorbed by the small intestine is several times higher in patients with HFE-related haemochromatosis than in normal individuals (Ref. 54). Interestingly, the normal reciprocal relationship between body iron stores and iron absorption (i.e. higher iron stores means lower iron absorption) was still observed in such patients, and the slope of the correlation line was very similar to that of controls. However, the entire curve was shifted to the right, such that a given amount of iron repressed iron absorption less in subjects with HFE-related haemochromatosis than in normal controls. This shows that such patients are able to respond to increases in body iron load, but that the sensitivity of the intestinal response to increased body iron is reduced. Although it has not been examined directly, a similar response might be expected in patients with TFR2 mutations. However, in patients with much more severe iron loading, such as subjects with HJV or HAMP mutations (juvenile haemochromatosis), it is unlikely that significant regulation of iron absorption remains (Ref. 55).

As described above, the primary systemic regulator of intestinal iron absorption is the liver-derived peptide hepcidin (Ref. 23). This is best demonstrated in primary iron-loading disorders. Human subjects with *HAMP* mutations have very low or no circulating hepcidin (Ref. 40), and hence iron absorption continues unimpeded. In a normal individual, hepcidin levels would increase as body iron accumulates, and this would feed back to the gut to limit further iron absorption. It comes as no surprise that other forms of primary iron loading are also characterised by disruption of the hepcidin–FPN axis (Fig. 2). Patients with mutations in HFE, TFR2 and HJV also have low or negligible hepcidin expression (Refs 34, 39, 56), implicating these molecules in the regulation of hepcidin and explaining why they cannot limit their iron intake. Further evidence

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has recently come from studies in mice: targeted ablation of HFE in liver recapitulated the haemochromatosis phenotype, whereas ablation of HFE in intestine did not (Refs 57, 58). This, in conjunction with the lower hepcidin levels seen in *Hfe*-knockout mice, clearly demonstrates that HFE has a role in the regulation of hepcidin expression in the liver rather than directly regulating iron absorption in the intestine. In those subjects with ferroportin disease resembling classical primary iron overload, the mutations in FPN interfere with hepcidin binding to the exporter and effectively render hepcidin inactive (Ref. 3).

BMP–SMAD signalling and hepcidin regulation

The low hepcidin levels in patients with HFE, TFR2 and HJV mutations show that these proteins are upstream regulators of hepcidin. The dissection of how HJV alters hepcidin expression has been the most revealing to date. HJV is a glycosylphosphatidylinositol (GPI)-linked membrane-associated protein that acts as a co-receptor for the bone morphogenetic protein (BMP) signalling pathway (Fig. 4), and when signalling through this pathway is increased, hepcidin expression also increases (Ref. 59). The BMPs are members of the transforming growth factor β (TGF-β) superfamily of proteins, and they bind to a complex of type I and type II BMP receptors on the plasma membrane (Ref. 60). The type II receptors then phosphorylate the type I receptors, and this activated complex in turn phosphorylates the transcriptional regulators SMAD1, -5 and -8 (small mothers against decapentaplegic homologues) in the cytoplasm. HJV binds to the BMPs and enhances their effectiveness in activating the SMAD signalling pathway (Ref. 59). In hepatic cells, BMPs 2, 4 and 6 appear to be the endogenous ligands for HJV (Ref. 61). Interestingly, the absence of HJV leads to a reduction in basal hepcidin expression rather than a change in the responsiveness of *HAMP* to BMPs (Ref. 62). Activated SMAD1/5/8 binds to SMAD4, and this complex translocates to the nucleus where it stimulates the expression of a range of target genes, including *HAMP* (Refs 59, 60) (Fig. 4). The finding that mice lacking SMAD4 have negligible hepcidin expression and develop body iron loading (Ref. 63) provides very strong evidence that the BMP–SMAD signalling system has a central role in hepcidin regulation. Two other SMADS, SMAD6 and SMAD7, are able to antagonise SMAD1/5/8 binding to SMAD4, and recent data have shown that SMAD7 inhibits *HAMP* gene expression (Ref. 64). Thus the SMAD–BMP pathway has emerged as a central player in the regulation of hepcidin.

Signalling through the BMP–SMAD pathway can be modulated through a range of upstream events, and thus the regulation of hepcidin is potentially quite complex. BMPs 2, 4, 5, 6, 7 and 9 have all been shown to be able to stimulate *HAMP* expression (Refs 59, 63, 65), but recent studies have suggested that BMP6 is physiologically the most important (Figs 2 and 4). In the absence of BMP6, mice develop systemic iron loading and hepcidin is barely detectable (Refs 66, 67), suggesting that the other BMPs cannot compensate for the loss of BMP6. In addition, the administration of exogenous BMP6 increases hepcidin expression (Ref. 66). BMP6 itself is regulated by iron (Ref. 68), and this provides at least one mechanism by which body iron stores may be communicated to the hepcidin-regulatory machinery. When the body iron load is high, BMP6 levels increase, leading to enhanced BMP–SMAD signalling and increased hepcidin expression. Intriguingly, BMP6 does not appear to be regulated by HFE, TFR2 or HJV because knockout mouse studies show that *Bmp6* expression correlates with iron stores in these animals as normal (Refs 69, 70, 71). This implies that HFE and TFR2 regulate hepcidin expression in a BMP6-independent manner. Because HJV acts as a BMP co-receptor, it would not be expected to affect *Bmp6* expression, but instead potentiates the signal produced when BMP6 binds to its receptor (Ref. 71).

Of additional interest is the potential role of BMP9 in the regulation of hepcidin. Tissue culture studies have shown it to be the most potent stimulator of hepcidin expression of all the BMPs tested and it is almost exclusively expressed in the nonparenchymal cells of the liver (Refs 68, 72). However, it does not appear to bind HJV and is not regulated by iron stores (Ref. 66), implying that BMP9 induces hepcidin expression by a mechanism different from that of BMP6. Whether BMP9 has a role in regulating hepcidin expression in vivo remains to be determined. It is likely that other iron-related

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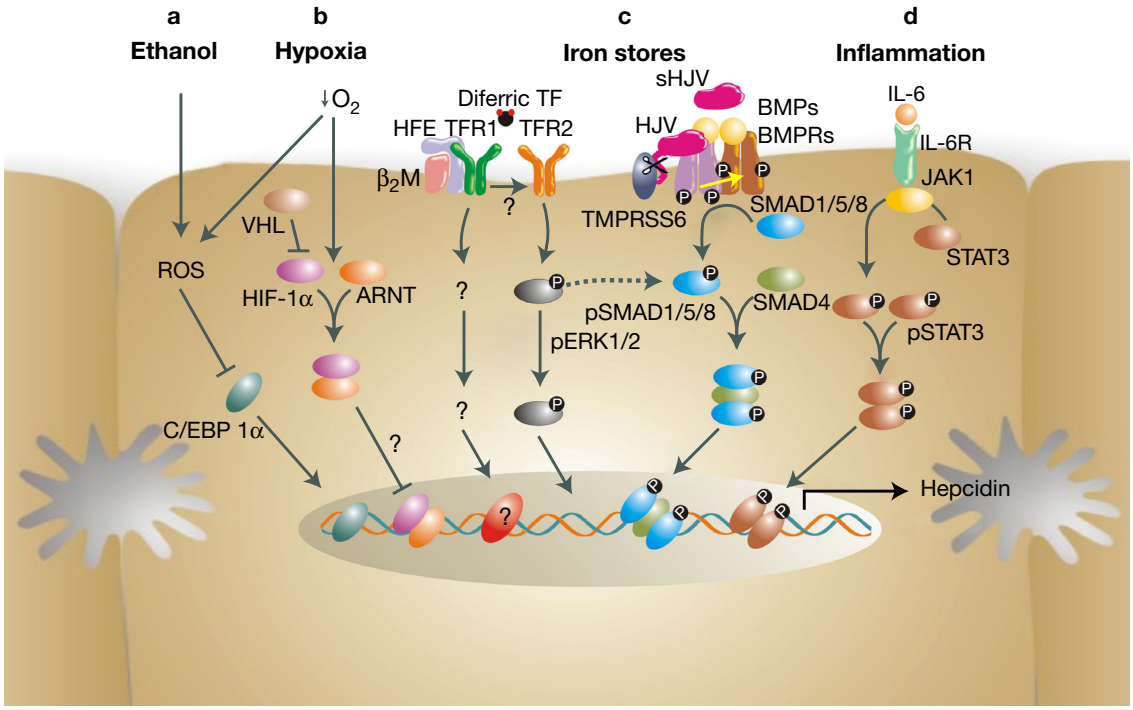


Figure 4. Molecular regulation of hepcidin expression. In hepatocytes, hepcidin can be regulated by ethanol, hypoxia, iron stores and inflammation. (a, b) C/EBPα binds to the promoter and stimulates hepcidin expression. Ethanol and hypoxia induce ROS, downregulate C/EBPα binding and thus lead to suppression of hepcidin. (b) Under hypoxic conditions, HIF-1α is stabilised, forms a heterodimer with ARNT, translocates to the nucleus and downregulates hepcidin expression. VHL destabilises HIF-1α by marking it for ubiquitination and degradation. (c) Diferric transferrin stimulates hepcidin expression, probably through its interactions with TFR2 and the HFE–TFR1 complex on the cell surface. Signalling through the ERK pathway appears to be involved. BMPs, notably BMP6, bind to BMPRs and the co-receptor HJV to induce signalling through the SMAD pathway to modulate hepcidin expression. TMPRSS6 downregulates hepcidin expression by cleaving HJV. (d) Inflammatory cytokines, such as IL-6, bind to their receptors (IL-6R) and stimulate STAT3 to induce hepcidin expression. Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; BMPRs, BMP receptors; BMPs, bone morphogenetic proteins; C/EBPα, CCAAT/enhancer-binding protein alpha; pERK1/2, phosphorylated extracellular-signal-regulated kinase 1/2; HIF-1α, hypoxia-inducible factor 1α; HJV, haemojuvelin; IL-6, interleukin-6; JAK1, Janus kinase 1; β_2M, β_2-microglobulin; ROS, reactive oxygen species; SMAD, small mothers against decapentaplegic homologue; STAT, signal transducer and activator of transcription; TF, transferrin; TFR, transferrin receptor; TMPRSS6, transmembrane protease, serine 6; VHL, von Hippel–Lindau.

signals also influence hepcidin expression, and these are considered in more detail below.

It is clear that the GPI-linked, membrane-associated form of HJV acts as a BMP co-receptor to stimulate hepcidin expression. However, a soluble form of HJV (sHJV) has been identified in the circulation and it has been shown to antagonise signalling through the BMP–SMAD pathway, presumably by competitively inhibiting the binding of membrane-bound HJV to BMPs (Ref. 72) (Fig. 2). The source of sHJV is unclear. Although HJV is expressed at reasonable levels in the hepatocyte, the site of expression of most components of the hepcidin-regulatory pathway, it is much more strongly expressed in skeletal and cardiac muscle (Ref. 39). Muscle has a particularly high iron requirement for the production of myoglobin; hence it is possible that muscle-derived sHJV reflects the iron

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requirements of this tissue and communicates it to the liver. However, this remains speculation at present. The release of sHJV requires the protease furin (Ref. 73) (Fig. 2). Furin levels can be increased by iron depletion and hypoxia and this can explain why levels of sHJV increase under iron-deficient conditions (Ref. 73), a situation conducive to reduced *HAMP* expression. It has been reported that the release of sHJV also requires the membrane protein neogenin (Ref. 74), but more recent studies have suggested that neogenin stabilises GPI-linked HJV and inhibits its secretion (Ref. 69) (Fig. 2). This is consistent with the observation that neogenin is required for HJV-mediated BMP signalling (Ref. 70) and that neogenin-knockout mice have low hepcidin levels and develop iron overload (Ref. 69).

The most recent member of the hepcidin-regulatory network to be identified is a membrane-bound serine protease known as matriptase-2 (TMPRSS6). The link between this enzyme and iron homeostasis was first made through two mouse models of anaemia with impaired matriptase-2 function (Refs 71, 75), and has been consolidated with the finding that many cases of refractory iron deficiency in humans can be ascribed to mutations in the *TMPRSS6* gene (Refs 76, 77). Significantly, several recent genome-wide association studies in humans seeking genetic determinants of iron status have all identified matriptase-2 as a strong candidate (Refs 78, 79, 80). The physiological substrate of matriptase-2 appears to be membrane-associated HJV (Ref. 81) (Fig. 2). When HJV is cleaved, it can no longer act as a BMP co-receptor and hepcidin levels are reduced (Ref. 82). This implies that the normal function of this protease is to repress hepcidin production through the BMP–SMAD pathway. Overall, these data suggest that matriptase-2 has a crucial role in maintaining basal hepcidin expression.

Hepcidin regulation by erythropoiesis and inflammation

Strongly enhanced erythropoiesis is a major stimulus for iron absorption and an important suppressor of hepcidin expression (Ref. 83) (Fig. 3). This is particularly relevant to understanding the iron loading associated with a range of haematological disturbances where erythropoiesis is ineffective and the erythropoietic drive is very strong. There are several potential mechanisms for the suppression of *HAMP* by erythropoiesis. First, increased erythropoiesis creates a high demand for iron and reduced systemic iron levels could feed back to alter *HAMP* transcription (Ref. 23). The iron-related regulation of hepcidin could occur through the modulation of *BMP6* or *FURIN* expression, or through HFE and TFR2 as discussed in the following section. Second, a reduction in the level of circulating haemoglobin can lead to systemic hypoxia, and this could also reduce hepcidin expression (Ref. 84). The *HAMP* gene contains two hypoxia-response elements, and it has been demonstrated that HIF-1α (HIF1A) signalling is able to reduce *HAMP* transcription (Ref. 84) (Fig. 4). In addition to iron- and oxygen-related signals, there is evidence that the erythroid-specific factors growth differentiation factor 15 (GDF15) and twisted gastrulation (TWSG1) are also involved in hepcidin regulation. Their expression is increased when erythropoiesis is stimulated, and each has been shown to act on hepatic cells to reduce hepcidin expression (Refs 85, 86) (Fig. 2). However, one study failed to find a correlation between GDF15 and hepcidin during the recovery of erythropoiesis following bone marrow transplantation (Ref. 87). The expression of *GDF15* and *TWSG1* is particularly high in β-thalassaemia where hepcidin levels are low and iron absorption is strongly upregulated (Refs 85, 86). Precisely how these proteins exert their effects has yet to be determined, but it is likely that they interfere with BMP signalling (Refs 88, 89).

Inflammation is a strong inducer of hepcidin (Ref. 90), and the hypoferraemia that accompanies inflammation can be explained by increased plasma hepcidin levels. The anaemia associated with a range of chronic inflammatory conditions, known as the anaemia of chronic disease (ACD), can be explained by increased hepcidin levels, and therefore antagonism of the hepcidin–ferroportin axis (Refs 23, 91) represents an important therapeutic avenue for these conditions. Inflammation stimulates the synthesis of a range of pro-inflammatory cytokines such as interleukin-6 (IL-6), and it is these that enhance hepcidin expression by signalling through the JAK–STAT pathway (Ref. 92) (Fig. 4). There appears to be crosstalk between this pathway and the BMP–SMAD signalling pathway as a functional SMAD-binding site in the *HAMP* promoter is necessary for the IL-6 stimulation of hepcidin expression (Ref. 63).

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Role of HFE and TFR2 in sensing body iron levels

How the iron-regulatory system, and specifically hepcidin, responds to changes in body iron status remains unclear. As noted above, BMP6 and furin are regulated by body iron levels and provide some potential mechanisms for relaying body iron requirements to this regulatory system. However, it is widely believed that HFE and TFR2 are also involved in the iron-sensing process. How do they do this and what do they sense? There are several lines of evidence to suggest that circulating diferric transferrin is an important signal in regulating hepcidin expression (Refs 83, 93) (Fig. 4). Under normal physiological conditions, almost all the iron in the blood is bound to transferrin (Ref. 20). Transferrin contains two iron-binding sites and these may be unoccupied (apotransferrin), partly occupied (monoferric transferrin) or fully occupied (diferric transferrin). Diferric transferrin is the preferred substrate for TFR1, and thus is the major form in which iron is delivered to cells (Ref. 94). Diferric transferrin levels increase with iron loading and decrease with iron deficiency, and a positive correlation between the concentration of diferric transferrin and hepcidin expression has been demonstrated (Ref. 83). Mice that lack transferrin expression show reduced hepcidin and high iron absorption even in the presence of a high hepatic iron load (Refs 95, 96). This is consistent with a regulatory role for diferric transferrin, but the interpretation is complicated in this case because of the strongly enhanced erythropoiesis and hypoxia in these mice, which could independently regulate hepcidin.

A further link between diferric transferrin and hepcidin regulation comes from the analysis of TFR2 and HFE. TFR2 binds diferric transferrin, and this binding can stabilise the receptor (Ref. 97); hence it has the potential to act as a sensor of body iron levels. In cell culture studies it has been shown that the TFR2-mediated induction of *HAMP* requires diferric transferrin (Refs 98, 99). HFE itself does not bind diferric transferrin, but is able to bind to TFR1, with the binding site overlapping with one of the diferric-transferrin-binding sites on TFR1 (Ref. 100). Thus, the HFE–TFR1 complex could also act in an iron-sensing role, with diferric transferrin displacing TFR1-bound HFE and leaving it free to initiate a signal to increase hepcidin synthesis. Consistent with such a mechanism is the demonstration that mice engineered to prevent the HFE–TFR1 association showed enhanced hepcidin expression (Ref. 101). Despite considerable work in this area, much of it using proteins overexpressed in cell lines, we still do not have a clear picture of this iron-sensing mechanism, nor indeed whether diferric transferrin provides the key regulatory signal. For example, some studies have suggested that diferric transferrin will not stimulate *HAMP* expression in hepatocytes isolated from *Hfe*- or *Tfr2*-knockout mice (Ref. 98), whereas other studies have suggested that HFE is dispensible (Ref. 99).

Irrespective of the mechanisms by which iron status is assessed by hepatocytes, how do HFE and TFR2 direct changes in *HAMP* expression? The short answer is that we do not know. It has been proposed that HFE, TFR2, HJV and the BMP–receptor complex combine to form a 'super' complex that regulates hepcidin expression through the SMAD pathway (Ref. 102). However, the natural history of primary iron-loading disorders seems to argue against a mechanism where HFE and TFR2 are part of the same pathway (at least the same proximal pathway), and suggests that they perform independent, yet equivalent, roles. Mutations in the genes encoding HFE and TFR2 lead to adult-onset forms of iron overload – much milder disease than that due to mutations in HJV or HAMP (Ref. 3). In addition, when HFE is mutated (and possibly TFR2), iron absorption can still be regulated, albeit with a reduced sensitivity compared with unaffected individuals (Ref. 54). Furthermore, recent studies have shown that mice lacking both HFE and TFR2 have much lower hepcidin levels and develop more iron loading than animals lacking either gene alone (Ref. 37). These observations suggest that HFE and TFR2 do not act as part of the same pathway, but act independently to modulate hepcidin levels.

It is presumed that HFE and TFR2 activate signal transduction pathways to alter *HAMP* expression, and TFR2 accumulates in lipid rafts as many signalling proteins do (Ref. 103). Data from several sources now indicate that HFE and TFR2 can signal through the mitogen-activated protein kinase (MAPK) pathway to increase the phosphorylation of ERK1/2 [extracellular-signal-regulated kinase 1/2 (MAPK1/MAPK3)] (Fig. 4). Levels of phosphoERK are reduced in *Hfe*- and *Tfr2*-knockout mice (Ref. 37), and in primary mouse hepatocytes (Ref. 99) or K562 (erythroleukaemia) cells (Ref. 103) diferric transferrin can increase ERK1/2

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phosphorylation. Furthermore, inhibition of ERK can block *HAMP* stimulation by diferric transferrin (Ref. 99). There are some data that suggest HFE and TFR2 feed into the BMP–SMAD pathway, and reduced phosphorylation of SMAD1/5/8 has been reported in *Hfe*- and *Tfr2*-knockout mice (Ref. 37). Mice lacking either HJV or SMAD4 do not show an increase in hepcidin after iron administration (Refs 63, 104), indicating that an intact BMP–SMAD pathway is essential for hepcidin to respond to iron.

Other regulators of hepcidin expression

In addition to the predominantly iron-related regulatory mechanisms, hepcidin expression can also be modified by seemingly unrelated stimuli. The role of hepcidin in iron homeostasis was originally discovered using a mouse model that had upstream stimulatory factor 2 (USF2), a transcription factor involved in carbohydrate metabolism, knocked out. These animals were found to express very low levels of hepcidin and, consequently, develop severe iron loading (Ref. 105). It was subsequently shown that USF1 and USF2 form heterodimers with MYC/MAX and bind to E-boxes within the promoter region of hepcidin, regulating expression in both a cis and trans manner (Ref. 106). This provides tantalising evidence of a link between iron metabolism and the metabolism of carbohydrate and fat, where USF1 and USF2 are known to play an important role.

Alcohol consumption has been reported to increase body iron stores and can exacerbate the iron loading and morbidity associated with haemochromatosis. Ethanol has been shown to suppress hepcidin expression both in vivo and in vitro (Refs 107, 108, 109). It appears that ethanol-induced hypoxia leads to the formation of reactive oxygen species that downregulate hepcidin expression by reducing the DNA-binding activity of CCAAT/enhancer-binding protein alpha (C/EBPα) to the hepcidin promoter. This effect of ethanol on hepcidin expression can be prevented by antioxidants (Ref. 108) (Fig. 4).

Finally, polymorphic variations in the hepcidin promoter have been reported to regulate hepcidin expression and might have a role in determining the level of iron loading that occurs in primary haemochromatosis (Refs 110, 111).

Integrating the regulatory network

How these multiple signals integrate to regulate hepcidin expression in health and disease is incompletely understood, although an intact BMP–SMAD pathway seems essential for normal *HAMP* regulation, irrespective of the stimulus. Some of the components are likely to be involved in maintaining basal *HAMP* expression, whereas others may be involved in the adaptation to changes in body iron demand. Various arms of the system may respond differently to acute and chronic stimuli. For example, it has been demonstrated that in HFE-related haemochromatosis the acute response to iron administration is missing (Ref. 112) but subjects still respond to chronic iron loading (Ref. 113). The cellular site of this iron regulatory network also requires further analysis. Hepatic parenchymal cells express all the key components of the system and are probably the main regulatory site, but the finding that important players such as BMP6 and HJV are primarily expressed in other cell types (hepatic stellate cells and myocytes, respectively) (Refs 39, 114) suggests a complex cellular interplay. There is also a growing body of evidence that hepcidin can be synthesised locally in a range of tissues (Ref. 115), with macrophages being a potential source of the peptide in these situations. Finally, it should be pointed out that many of the studies to delineate pathways of hepcidin regulation have been carried out in cell culture models, often using exogenous protein overexpression. Hence these findings need to be validated in vivo.

Clinical implications and applications

Advances in our molecular understanding of the pathogenesis of iron-loading disorders have brought the promise of novel therapeutic approaches for these diseases. The recognition that hepcidin has a central role in regulating body iron intake has meant that most interest has centred on modulating hepcidin levels. However, as yet, none of these approaches has reached the clinic.

Modulating hepcidin levels

Because iron-loading diseases are characterised by reduced hepcidin expression, the administration of hepcidin or its analogues holds great promise in limiting iron loading. When mice overexpressing hepcidin are crossed with those deficient in HFE, iron loading in the *Hfe*-knockouts is prevented (Ref. 116), confirming in principle the utility of raising hepcidin levels.

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Several studies have also shown that administering hepcidin to mice will reduce iron levels (Refs 117, 118), and more recently it has been shown that short forms of hepcidin based on the N-terminal portion of the peptide can have similar effects (Ref. 119). These small-sized or 'minihepcidins' have potential as oral therapeutic agents. Hepcidin administration is equally applicable to both primary and secondary iron-loading disorders because hepcidin levels are reduced in each case. Increasing hepcidin levels will reduce or prevent the accumulation of additional iron, but will not necessarily lead to a reduction in existing body iron overload. Hence, it is likely that such therapies will be applied in conjunction with iron chelation to remove existing excess iron and prevent further iron accumulation. To remove accumulated iron, the newer-generation oral iron chelators such as deferasirox are likely to be the agents of choice (Ref. 120).

It is also possible to increase hepcidin expression by enhancing signalling through the BMP–SMAD pathway. BMP6 is a clear candidate here and injection of Bmp6 has been shown to stimulate hepcidin expression in mice (Ref. 66). Along similar lines, antagonists of matriptase-2 could be used to enhance BMP signalling. In either case, however, the possibility of off-target effects will need to be thoroughly investigated. Gene therapy approaches for specific disorders might also be valuable in the future. For example, the virus-mediated delivery of *Hfe* and *Tfr2* transgenes into hepatocytes has been recently shown to partially correct the defects in *Hfe*- and *Tfr2*-knockout mice, respectively (Ref. 121). In these mice, serum hepcidin levels increased and transferrin saturation and hepatic iron levels decreased. A similar approach might also be suitable to correct HJV or hepcidin insufficiency. Although these possibilities are worth considering, the most practical solution to ameliorating iron loading in the foreseeable future is to directly administer hepcidin or hepcidin agonists.

Although increasing the hepcidin levels is appropriate for ameliorating iron overload, in other situations it may be desirable to reduce hepcidin levels or to interfere with its action. The anaemia of inflammation or chronic disease is associated with increased hepcidin and can ultimately lead to iron-deficient erythropoiesis, so reducing hepcidin to enhance iron supply could be potentially beneficial. In a murine model of the anaemia of inflammation (*Brucella abortus* infection), a neutralising monoclonal antibody against hepcidin was shown to reduce the anaemia when used in combination with erythropoietin treatment (Ref. 122). Serum iron levels were returned to near normal. Such approaches could also be valuable in treating patients with chronic kidney disease and those with refractory iron deficiency anaemia (which is frequently caused by matriptase-2 mutations). If the anaemia is secondary to an infection, increasing the serum iron levels by blocking hepcidin needs to be employed cautiously because iron is an important virulence factor for a range of pathogens.

Modulating iron transport

Therapeutics targeting the hepcidin–FPN axis are potentially valuable for limiting body iron intake by reducing the basolateral transport step of iron absorption. It may also be possible to block absorption using oral agents that interfere with iron transport across the brush-border membrane by DMT1, but this has not yet been achieved. Interestingly, however, L-type calcium-channel-blocking drugs such as nifedipine have been reported to modulate the activity of DMT1 systemically. They mobilise liver iron in primary and secondary iron-loading disorders and enhance the urinary excretion of iron (Ref. 123). Other L-type calcium-channel blockers such as amlodipine and verapamil have also been shown to reduce iron-overload cardiomyopathy (Refs 124, 125) and improve heart function and patient survival.

Hepcidin measurement as a diagnostic tool

Because hepcidin is a central controller of body iron trafficking, its measurement (in plasma or urine) has great potential as a clinical indicator of body iron status and it is almost certain that hepcidin evaluation will be part of a standard haematological/iron workup in the future. Unfortunately, the hepcidin peptide is a difficult antigen to work with and few robust antibodies to the peptide have been generated. Consequently, at the time of writing there is no reliable commercially available immunoassay for the peptide, although several groups have published hepcidin immunoassays as research tools (Refs 126, 127, 128). Despite this limitation, there has been considerable success in

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developing mass spectrometry (MS)-based methods for measuring hepcidin in biological fluids (Refs 129, 130, 131, 132, 133); several laboratories around the world offer the service of MS-based hepcidin measurement and a growing number of studies using these measurements are appearing (Refs 134, 135, 136). A commercially available assay for the hepcidin precursor prohepcidin is available, but it has been shown to have limited utility (Ref. 137). Prohepcidin levels do not correspond well with iron absorption, and there is a strong inverse correlation with the levels of the mature peptide.

Box 1. Outstanding research questions

- How does the hepatocyte sense body iron requirements? Diferric transferrin certainly makes sense as a candidate, but what are the roles of BMP6 and the iron-dependent regulation of furin?
- The concentration of diferric transferrin in the plasma is sufficiently high to seemingly saturate TFR1 and TFR2. Under these circumstances, how can it act to signal tissue iron requirements?
- How does iron regulate BMP6 expression? Does it respond to intracellular iron or extracellular cues?
- What role do cell types other than hepatocytes play in hepcidin regulation? For example, BMP6 appears to be most strongly expressed in hepatic stellate cells.
- How are all the signals that modulate hepcidin expression modulated at the whole-body level? Much of our detailed knowledge at present is derived from cell culture studies, so the in vivo relevance of these findings needs to be tested.
- What is the role of HJV in cardiac and skeletal muscle?
- What are the identities of the genes that modify disease penetrance during iron overload, and particularly in HFE-related haemochromatosis?
- How aggressively should patients with iron overload be treated by phlebotomy? The benefits of removing excess iron are clear, but too much iron depletion could reduce hepcidin and stimulate absorption.
- What is the role of BMP9 in inducing hepcidin? Does it respond to any systemic stimuli that regulate iron homeostasis?

Abbreviations: BMP, bone morphogenic protein; HJV, haemojuvelin; TF, transferrin; TFR, transferrin receptor.

Research in progress and outstanding research questions

Defining the mechanisms of body iron homeostasis and their relationship to disorders of iron metabolism, particularly iron-loading disorders, is an extremely active field at present and new discoveries are regularly being published. Despite these recent advances, much more remains to be learned in this important field. Perhaps the most significant unresolved question in our understanding of the biology of hepcidin is how HFE and TFR2 modulate expression of the protein. Do they act independently or together? Do they feed into the BMP–SMAD signalling pathway? Do they respond to the same systemic signals? Some of the other significant issues requiring attention are summarised in Box 1. Studies over the past decade have clearly shown the potential of hepcidin-related diagnosis and therapies for iron-overload disorders. Translating this into clinical practice will be the focus of the next decade, and this in turn will be guided by elucidating the unresolved research questions listed above.

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References

1 Zimmermann, M.B. and Hurrell, R.F. (2007) Nutritional iron deficiency. Lancet 370, 511-520
2 O'Neil, J. and Powell, L. (2005) Clinical aspects of hemochromatosis. Seminars in Liver Disease 25, 381-391
3 Lee, P.L. and Beutler, E. (2009) Regulation of hepcidin and iron-overload disease. Annual Review of Pathology 4, 489-515
4 Gattermann, N. (2009) The treatment of secondary hemochromatosis. Deutsches Arzteblatt International 106, 499-504
5 Rechavi, G. and Rivella, S. (2008) Regulation of iron absorption in hemoglobinopathies. Current Molecular Medicine 8, 646-662
6 Forth, W. and Rummel, W. (1973) Iron absorption. Physiological Reviews 53, 724-792
7 Brittenham, G.M. (1994) The red cell cycle. In Iron Metabolism in Health & Disease (Brock J.H.,

Accession information: doi:10.1017/S1462399410001687; Vol. 12; e36; November 2010
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https://doi.org/10.1017/S1462399410001687 Published online by Cambridge University Press

Halliday J.W. and Pippard M.J., eds), pp. 31-62, W.B. Saunders Company Ltd, London, UK
8 Powell, L.W., Jazwinska, E. and Halliday, J.W. (1994) Primary iron overload. In Iron Metabolism in Health and Disease (Brock J.H., Halliday J.W. and Pippard M.J., eds), pp. 227-270, W.B. Saunders Company Ltd, London, UK
9 McKie, A.T. et al. (2001) An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291, 1755-1759
10 Fleming, M.D. et al. (1998) Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proceedings of National Academy of Sciences USA 95, 1148-1153
11 Gunshin, H. et al. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482-488
12 Abboud, S. and Haile, D.J. (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. Journal of Biological Chemistry 275, 19906-19912
13 Donovan, A. et al. (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403, 776-781
14 McKie, A.T. et al. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Molecular Cell 5, 299-309
15 Vulpe, C.D. et al. (1999) Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nature Genetics 21, 195-199
16 Conrad, M.E., Jr and Crosby, W.H. (1963) Intestinal mucosal mechanisms controlling iron absorption. Blood 22, 406-415
17 Conrad, M.E., Weintraub, L.R. and Crosby, W.H. (1964) The role of the intestine in iron kinetics. Journal of Clinical Investigation 43, 963-974
18 Frazer, D.M. et al. (2003) A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut 52, 340-346
19 Chen, H. et al. (2003) Systemic regulation of hephaestin and Ireg1 revealed in studies of genetic and nutritional iron deficiency. Blood 102, 1893-1899
20 Huebers, H.A. and Finch, C.A. (1987) The physiology of transferrin and transferrin receptors. Physiological Reviews 67, 520-582
21 Anderson, G.J. et al. (2007) Regulation of systemic iron homeostasis: how the body responds to changes in iron demand. Biometals 20, 665-674
22 Donovan, A. et al. (2005) The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metabolism 1, 191-200
23 Nemeth, E. and Ganz, T. (2009) The role of hepcidin in iron metabolism. Acta Haematologica 122, 78-86
24 Nemeth, E. et al. (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. Journal of Clinical Investigation 113, 1271-1276
25 Allen, K.J. et al. (2008) Iron-overload-related disease in HFE hereditary hemochromatosis. New England Journal of Medicine 358, 221-230
26 Neves, J.V. et al. Hemochromatosis and pregnancy: iron stores in the Hfe-/- mouse are not reduced by multiple pregnancies. American Journal of Physiology Gastrointestinal and Liver Physiology 298, G525-G529
27 Benyamin, B. et al. (2009) Variants in TF and HFE explain approximately 40% of genetic variation in serum-transferrin levels. American Journal of Human Genetics 84, 60-65
28 Galaris, D. and Pantopoulos, K. (2008) Oxidative stress and iron homeostasis: mechanistic and health aspects. Critical Reviews in Clinical Laboratory Sciences 45, 1-23
29 Ramm, G.A. and Ruddell, R.G. (2005) Hepatotoxicity of iron overload: mechanisms of iron-induced hepatic fibrogenesis. Seminars in Liver Disease 25, 433-449
30 Camaschella, C. et al. (2000) The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nature Genetics 25, 14-15
31 Chloupkova, M., Zhang, A.S. and Enns, C.A. (2010) Stoichiometries of transferrin receptors 1 and 2 in human liver. Blood Cells Molecules and Diseases 44, 28-33
32 Kawabata, H. et al. (2000) Transferrin receptor 2-alpha supports cell growth both in iron-chelated cultured cells and in vivo. Journal of Biological Chemistry 275, 16618-16625
33 Fleming, R.E. et al. (2002) Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proceedings of the National Academy of Sciences of the United States of America 99, 10653-10658
34 Kawabata, H. et al. (2005) Expression of hepcidin is down-regulated in TfR2 mutant mice manifesting a phenotype of hereditary hemochromatosis. Blood 105, 376-381
35 Nemeth, E. et al. (2005) Hepcidin is decreased in TFR2 hemochromatosis. Blood 105, 1803-1806

Accession information: doi:10.1017/S1462399410001687; Vol. 12; e36; November 2010
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https://doi.org/10.1017/S1462399410001687 Published online by Cambridge University Press

36 Pietrangelo, A. (2005) Non-HFE hemochromatosis. Seminars in Liver Disease 25, 450-460
37 Wallace, D.F. et al. (2009) Combined deletion of Hfe and transferrin receptor 2 in mice leads to marked dysregulation of hepcidin and iron overload. Hepatology 50, 1992-2000
38 De Gobbi, M. et al. (2002) Natural history of juvenile haemochromatosis. British Journal of Haematology 117, 973-979
39 Papanikolaou, G. et al. (2004) Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nature Genetics 36, 77-82
40 Roetto, A. et al. (2003) Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nature Genetics 33, 21-22
41 De Domenico, I. et al. (2006) Iron overload due to mutations in ferroportin. Haematologica 91, 92-95
42 McCance, R.A. and Widdowson, E.M. (1937) Absorption and excretion of iron. Lancet ii, 5
43 Yang, L.P., Keam, S.J. and Keating, G.M. (2007) Deferasirox : a review of its use in the management of transfusional chronic iron overload. Drugs 67, 2211-2230
44 Vichinsky, E. (2008) Clinical application of deferasirox: practical patient management. American Journal of Hematology 83, 398-402
45 Li, H. et al. (2010) Transferrin therapy ameliorates disease in beta-thalassemic mice. Nature Medicine 16, 177-182
46 Wheby, M.S. (1978) Medicinal iron-induced hepatic cirrhosis: reversal by phlebotomy: studies on pathogenesis. Transactions of the American Clinical and Climatological Association 89, 100-108
47 Gordeuk, V.R. (2002) African iron overload. Seminars in Hematology 39, 263-269
48 Hamill, R.L., Woods, J.C. and Cook, B.A. (1991) Congenital atransferrinemia. A case report and review of the literature. American Journal of Clinical Pathology 96, 215-218
49 Morita, H. et al. (1995) Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopathological study of a Japanese family. Annals of Neurology 37, 646-656
50 Whitington, P.F. (2007) Neonatal hemochromatosis: a congenital alloimmune hepatitis. Seminars in Liver Disease 27, 243-250
51 Deugnier, Y. and Turlin, B. (2007) Pathology of hepatic iron overload. World Journal of Gastroenterology 13, 4755-4760
52 Sarkany, R.P. (2001) The management of porphyria cutanea tarda. Clinical Experimental Dermatology 26, 225-232
53 Hutchinson, C. et al. (2008) Post-prandial iron absorption in humans: comparison between HFE genotypes and iron deficiency anaemia. Clinical Nutrition 27, 258-263
54 McLaren, G.D. et al. (1991) Regulation of intestinal iron absorption and mucosal iron kinetics in hereditary hemochromatosis. Journal of Laboratory Clinical Medicine 117, 390-401
55 Cazzola, M. et al. (1983) Juvenile idiopathic haemochromatosis: a life-threatening disorder presenting as hypogonadotropic hypogonadism. Human Genetics 65, 149-154
56 Bridle, K.R. et al. (2003) Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet 361, 669-673
57 Spasic, M.V. et al. (2008) Hfe acts in hepatocytes to prevent hemochromatosis. Cell Metabolism 7, 173-178
58 Vujic Spasic, M. et al. (2007) Physiologic systemic iron metabolism in mice deficient for duodenal Hfe. Blood 109, 4511-4517
59 Babitt, J.L. et al. (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nature Genetics 38, 531-539
60 Chen, D., Zhao, M. and Mundy, G.R. (2004) Bone morphogenetic proteins. Growth Factors 22, 233-241
61 Xia, Y. et al. (2008) Hemojuvelin regulates hepcidin expression via a selective subset of BMP ligands and receptors independently of neogenin. Blood 111, 5195-5204
62 Truksa, J. et al. (2009) Suppression of the hepcidin-encoding gene Hamp permits iron overload in mice lacking both hemojuvelin and matriptase-2/TMPRSS6. British Journal of Haematology 147, 571-581
63 Wang, R.H. et al. (2005) A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. Cell Metabolism 2, 399-409
64 Mleczko-Sanecka, K. et al. (2010) SMAD7 controls iron metabolism as a potent inhibitor of hepcidin expression. Blood 115, 2657-2665
65 Babitt, J.L. et al. (2007) Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. Journal of Clinical Investigation 117, 1933-1939
66 Andriopoulos, B. Jr. et al. (2009) BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nature Genetics 41, 482-487

Accession information: doi:10.1017/S1462399410001687; Vol. 12; e36; November 2010
© Cambridge University Press 2010

https://doi.org/10.1017/S1462399410001687 Published online by Cambridge University Press

67 Meynard, D. et al. (2009) Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nature Genetics 41, 478-481
68 Kautz, L. et al. (2008) Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. Blood 112, 1503-1509
69 Lee, D.H. et al. (2010) Neogenin inhibits HJV secretion and regulates BMP-induced hepcidin expression and iron homeostasis. Blood 115, 3136-3145
70 Zhang, A.S. et al. (2009) Hemojuvelin-neogenin interaction is required for bone morphogenic protein-4-induced hepcidin expression. Journal of Biological Chemistry 284, 22580-22589
71 Du, X. et al. (2008) The serine protease TMPRSS6 is required to sense iron deficiency. Science 320, 1088-1092
72 Lin, L., Goldberg, Y.P. and Ganz, T. (2005) Competitive regulation of hepcidin mRNA by soluble and cell-associated hemojuvelin. Blood 106, 2884-2889
73 Silvestri, L., Pagani, A. and Camaschella, C. (2008) Furin-mediated release of soluble hemojuvelin: a new link between hypoxia and iron homeostasis. Blood 111, 924-931
74 Zhang, A.S. et al. (2007) Evidence that inhibition of hemojuvelin shedding in response to iron is mediated through neogenin. Journal of Biological Chemistry 282, 12547-12556
75 Folgueras, A.R. et al. (2008) Membrane-bound serine protease matriptase-2 (Tmprss6) is an essential regulator of iron homeostasis. Blood 112, 2539-2545
76 Finberg, K.E. et al. (2008) Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nature Genetics 40, 569-571
77 Guillem, F. et al. (2008) Two nonsense mutations in the TMPRSS6 gene in a patient with microcytic anemia and iron deficiency. Blood 112, 2089-2091
78 Chambers, J.C. et al. (2009) Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nature Genetics 41, 1170-1172
79 Ganesh, S.K. et al. (2009) Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nature Genetics 41, 1191-1198
80 Tanaka, T. et al. (2010) A genome-wide association analysis of serum iron concentrations. Blood 115, 94-96
81 Silvestri, L. et al. (2008) The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metabolism 8, 502-511
82 Finberg, K.E. et al. (2010) Down-regulation of Bmp/Smad signaling by Tmprss6 is required for maintenance of systemic iron homeostasis. Blood 115, 3817-3826
83 Frazer, D.M. et al. (2004) Delayed hepcidin response explains the lag period in iron absorption following a stimulus to increase erythropoiesis. Gut 53, 1509-1515
84 Peyssonnaux, C. et al. (2007) Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). Journal of Clinical Investigation 117, 1926-1932
85 Tanno, T. et al. (2009) Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells. Blood 114, 181-186
86 Tanno, T. et al. (2007) High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nature Medicine 13, 1096-1101
87 Kanda, J. et al. (2008) Serum hepcidin level and erythropoietic activity after hematopoietic stem cell transplantation. Haematologica 93, 1550-1554
88 Chang, C. et al. (2001) Twisted gastrulation can function as a BMP antagonist. Nature 410, 483-487
89 Ross, J.J. et al. (2001) Twisted gastrulation is a conserved extracellular BMP antagonist. Nature 410, 479-483
90 Nicolas, G. et al. (2002) The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. Journal of Clinical Investigation 110, 1037-1044
91 Agarwal, N. and Prchal, J.T. (2009) Anemia of chronic disease (anemia of inflammation). Acta Haematologica 122, 103-108
92 Wrighting, D.M. and Andrews, N.C. (2006) Interleukin-6 induces hepcidin expression through STAT3. Blood 108, 3204-3209
93 Wilkins, S.J. et al. (2006) Iron metabolism in the hemoglobin-deficit mouse: correlation of diferric transferrin with hepcidin expression. Blood 107, 1659-1664
94 Huebers, H. et al. (1985) Molecular advantage of diferric transferrin in delivering iron to reticulocytes: a comparative study. Proceedings Proceedings of the Society for Experimental Biology and Medicine 179, 222-226
95 Buys, S.S. et al. (1991) Iron absorption in hypotransferrinemic mice. Blood 78, 3288-3290

Accession information: doi:10.1017/S1462399410001687; Vol. 12; e36; November 2010
© Cambridge University Press 2010

https://doi.org/10.1017/S1462399410001687 Published online by Cambridge University Press

96 Weinstein, D.A. et al. (2002) Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood 100, 3776-3781
97 Johnson, M.B. and Enns, C.A. (2004) Diferric transferrin regulates transferrin receptor 2 protein stability. Blood 104, 4287-4293
98 Gao, J. et al. (2009) Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metabolism 9, 217-227
99 Ramey, G., Deschemin, J.C. and Vaulont, S. (2009) Cross-talk between the mitogen activated protein kinase and bone morphogenetic protein/hemojuvelin pathways is required for the induction of hepcidin by holotransferrin in primary mouse hepatocytes. Haematologica 94, 765-772
100 West, A.P., Jr et al. (2001) Mutational analysis of the transferrin receptor reveals overlapping HFE and transferrin binding sites. Journal of Molecular Biology 313, 385-397
101 Schmidt, P.J. et al. (2008) The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metabolism 7, 205-214
102 Andrews, N.C. (2008) Forging a field: the golden age of iron biology. Blood 112, 219-230
103 Calzolari, A. et al. (2006) TfR2 localizes in lipid raft domains and is released in exosomes to activate signal transduction along the MAPK pathway. Journal of Cell Science 119, 4486-4498
104 Niederkofler, V., Salie, R. and Arber, S. (2005) Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. Journal of Clinical Investigation 115, 2180-2186
105 Nicolas, G. et al. (2001) Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proceedings of the National Academy of Sciences of theUnited States of America 98, 8780–8785
106 Bayele, H.K., McArdle, H. and Srai, S.K. (2006) Cis and trans regulation of hepcidin expression by upstream stimulatory factor. Blood 108, 4237-4245
107 Bridle, K. et al. (2006) Hepcidin is down-regulated in alcoholic liver injury: implications for the pathogenesis of alcoholic liver disease. Alcoholism: Clinical and Experimental Research 30, 106-112
108 Harrison-Findik, D.D. et al. (2006) Alcohol metabolism-mediated oxidative stress down-regulates hepcidin transcription and leads to increased duodenal iron transporter expression. Journal of Biological Chemistry 281, 22974-22982
109 Flanagan, J.M., Peng, H. and Beutler, E. (2007) Effects of alcohol consumption on iron metabolism in mice with hemochromatosis mutations. Alcoholism: Clinical and Experimental Research 31, 138-143
110 Bayele, H.K. and Srai, S.K. (2009) Regulatory variation in hepcidin expression as a heritable quantitative trait. Biochemical and Biophysical Research Communications 384, 22-27
111 Andreani, M. et al. (2009) Association of hepcidin promoter c.-582 A > G variant and iron overload in thalassemia major. Haematologica 94, 1293-1296
112 Piperno, A. et al. (2007) Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. Blood 110, 4096-4100
113 van Dijk, B.A. et al. (2008) Serum hepcidin levels are innately low in HFE-related haemochromatosis but differ between C282Y-homozygotes with elevated and normal ferritin levels. British Journal of Haematology 142, 979-985
114 Knittel, T. et al. (1997) Bone morphogenetic protein-6 is expressed in nonparenchymal liver cells and upregulated by transforming growth factor-beta 1. Experimental Cell Research 232, 263-269
115 Theurl, I. et al. (2008) Autocrine formation of hepcidin induces iron retention in human monocytes. Blood 111, 2392-2399
116 Nicolas, G. et al. (2003) Constitutive hepcidin expression prevents iron overload in a mouse model of hemochromatosis. Nature Genetics 34, 97-101
117 Laftah, A.H. et al. (2004) Effect of hepcidin on intestinal iron absorption in mice. Blood 103, 3940-3944
118 Chung, B. et al. (2009) Hepcidin decreases iron transporter expression in vivo in mouse duodenum and spleen and in vitro in THP-1 macrophages and intestinal Caco-2 cells. Journal of Nutrition 139, 1457-1462
119 Nemeth, E. (2010) Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. Advances in Hematology 2010, 750643, doi: 10.1155/2010/750643
120 Paradkar, P.N. et al. (2008) Iron depletion limits intracellular bacterial growth in macrophages. Blood 112, 866-874
121 Gao, J. et al. (2010) Hepatocyte-targeted HFE and TFR2 control hepcidin expression in mice. Blood 115, 3374-3381
122 Sasu, B.J. et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. Blood 115, 3616-3624

Accession information: doi:10.1017/S1462399410001687; Vol. 12; e36; November 2010
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https://doi.org/10.1017/S1462399410001687 Published online by Cambridge University Press

123 Ludwiczek, S. et al. (2007) Ca2+ channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1. Nature Medicine 13, 448-454
124 Oudit, G.Y. et al. (2003) L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nature Medicine 9, 1187-1194
125 Sugishita, K. et al. (2009) A case of iron overload cardiomyopathy: beneficial effects of iron chelating agent and calcium channel blocker on left ventricular dysfunction. International Heart Journal 50, 829-838
126 Ganz, T. et al. (2008) Immunoassay for human serum hepcidin. Blood 112, 4292-4297
127 Grebenchtchikov, N. et al. (2009) High-sensitive radioimmunoassay for human serum hepcidin. British Journal of Haematology 146, 317-325
128 Busbridge, M. et al. (2009) Development of a novel immunoassay for the iron regulatory peptide hepcidin. British Journal of Biomedical Science 66, 150-157
129 Ward, D.G. et al. (2008) SELDI-TOF-MS determination of hepcidin in clinical samples using stable isotope labelled hepcidin as an internal standard. Proteome Science 6, 28
130 Kemna, E. et al. (2005) Novel urine hepcidin assay by mass spectrometry. Blood 106, 3268-3270
131 Murphy, A.T. et al. (2007) Quantitation of hepcidin from human and mouse serum using liquid chromatography tandem mass spectrometry. Blood 110, 1048-1054
132 Li, H. et al. (2009) Development of a method for the sensitive and quantitative determination of hepcidin in human serum using LC-MS/MS. Journal of Pharmacological and Toxicological Methods 59, 171-180
133 Kroot, J.J. et al. (2009) Results of the first international round robin for the quantification of urinary and plasma hepcidin assays: need for standardization. Haematologica 94, 1748-1752
134 Iqbal, T. et al. (2009) Is iron overload in alcohol-related cirrhosis mediated by hepcidin? World Journal of Gastroenterology 15, 5864-5866
135 Peters, H.P. et al. (2010) Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrology, Dialysis, Transplantation 25, 848-853
136 van Deuren, M., Kroot, J.J. and Swinkels, D.W. (2009) Time-course analysis of serum hepcidin, iron and cytokines in a C282Y homozygous patient with Schnitzler's syndrome treated with IL-1 receptor antagonist. Haematologica 94, 1297-1300
137 Young, M.F. et al. (2009) Serum hepcidin is significantly associated with iron absorption from food and supplemental sources in healthy young women. American Journal of Clinical Nutrition 89, 533-538

Further reading, resources and contacts

Weiss, G. (2010) Genetic mechanisms and modifying factors in hereditary hemochromatosis. Nature Reviews Gastroenterology and Hepatology 7, 50-58
This recent review describes the genetic and environmental factors that modify the severity and clinical penetrance of hereditary haemochromatosis.

Adams, P.C. and Barton, J.C. (2010) How I treat hemochromatosis. Blood 22, 317-325
Pietrangelo, A. (2010) Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology 139, 393-408
These two recent reviews cover the clinical aspects of haemochromatosis.

Features associated with this article

Figures
Figure 1. Systemic regulation of iron homeostasis.
Figure 2. Regulation of iron homeostasis in primary iron-overload disorders.
Figure 3. Regulation of iron homeostasis in secondary iron-overload disorders.
Figure 4. Molecular regulation of hepcidin expression.

(continued on next page)

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Features associated with this article *(continued)*

Table
Table 1. Classification of some iron-loading disorders.

Box
Box 1. Outstanding research questions.

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