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Haemochromatosis

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Abstract

Haemochromatosis is defined as systemic iron overload of genetic origin, caused by a reduction in the concentration of the iron regulatory hormone hepcidin, or a reduction in hepcidin-ferroportin binding. Hepcidin regulates the activity of ferroportin, which is the only identified cellular iron exporter. The most common form of haemochromatosis is due to homozygous mutations (specifically, the C282Y mutation) in *HFE*, which encodes hereditary haemochromatosis protein. Non-*HFE* forms of haemochromatosis due to mutations in *HAMP*, *HJV* or *TFR2* are much rarer. Mutations in *SLC40A1* (also known as *FPN1*; encoding ferroportin) that prevent hepcidin-ferroportin binding also cause haemochromatosis. Cellular iron excess in *HFE* and non-*HFE* forms of haemochromatosis is caused by increased concentrations of plasma iron, which can lead to the accumulation of iron in parenchymal cells, particularly hepatocytes, pancreatic cells and cardiomyocytes. Diagnosis is noninvasive and includes clinical examination, assessment of plasma iron parameters, imaging and genetic testing. The mainstay therapy is phlebotomy, although iron chelation can be used in some patients. Hepcidin supplementation might be an innovative future approach.

In this Primer, haemochromatosis is defined as systemic iron overload of genetic origin caused by a deficiency of hepcidin, including decreased production or decreased activity of hepcidin-ferroportin binding. Normally, hepcidin restricts iron transport into plasma. The definition of haemochromatosis used herein excludes acquired iron overload, such as

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Competing interests

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overload caused by multiple blood transfusions, dyserythropoiesis or excessive parenteral iron supplementation. In addition, this definition excludes rare genetic disorders that lead to systemic iron excess by mechanisms other than primary hepcidin deficiency. These disorders include the loss-of-function ferroportin disease, atransferrinaemia, aceruloplasminaemia or divalent cation transporter 1 (DMT1; also known as NRAMP2)-related iron overload. Some of these disorders are occasionally considered as part of a wider haemochromatosis spectrum; indeed, ferroportin disease is officially referred to as type 4A haemochromatosis.

In most cases, haemochromatosis is caused by homozygous p.Cys282Tyr (C282Y) mutations in *HFE* (encoding hereditary haemochromatosis protein, or HFE, which has a role in hepcidin regulation). This mutation is found almost exclusively in white individuals and leads to *HFE*-associated haemochromatosis (also known as type 1 haemochromatosis). Haemochromatosis can also be caused by mutations in other genes (collectively referred to as non-*HFE* haemochromatosis), including *HAMP* (encoding hepcidin), *HJV* (also known as *HFE2*, encoding haemojuvelin) and *TFR2* (encoding transferrin receptor protein 2) in addition to gain-of-function mutations in *SLC40A1* (also known as *FPN1*, encoding ferroportin; BOX 1). Non-*HFE* haemochromatosis can affect both white and non-white individuals. Both *HFE*-associated and non-*HFE*-associated haemochromatosis lead to hepcidin deficiency, resulting in increased iron release from splenic macrophages and cells of the small intestine into plasma. Increased plasma iron levels lead to increased iron transport into parenchymal cells (particularly hepatocytes, pancreatic cells and cardiomyocytes), leading to predominantly hepatic, pancreatic and cardiac iron overload. The corresponding phenotype is characterized by increased plasma iron and transferrin saturation. Transferrin saturation is the ratio of the number of occupied iron binding sites to the total number of iron binding sites on plasma transferrin; increases in this parameter is critical for the diagnosis of haemochromatosis. In addition, strict interpretation of serum ferritin (the principal iron storage protein) concentration should be used for diagnosis. In general, patients with haemochromatosis are asymptomatic for many years and, in *HFE*-associated haemochromatosis, they develop symptoms only around 30–40 years of age. When symptoms manifest, they can be general and affect several organ systems (FIG. 1).

This Primer focuses on haemochromatosis caused by hepcidin deficiency and addresses the epidemiology, mechanistic and pathophysiological aspects of this disorder and the diagnosis, screening and management of patients. This Primer also discusses the quality of life of patients and summarizes some key remaining issues in the field. Other causes of iron overload aside from hepcidin deficiency are addressed, when relevant.

Epidemiology

The prevalence of the most common mutations in *HFE* (that is, C282Y and p.His63Asp (H63D)) that can cause haemochromatosis varies among ethnic groups¹. The HEIRS study evaluated the prevalence and genetic and environmental determinants, among other factors, of haemochromatosis in a multi-ethnic primary care-based sample of 100,000 adults over a 5-year period in the United States and Canada². Out of the 99,711 participants who did not have a family member participating in the study, 299 individuals were homozygous for C282Y. The estimated prevalence of C282Y homozygosity was 0.44% in non-Hispanic

white individuals, 0.11% in native and indigenous Americans, 0.027% in Hispanic individuals, 0.014% in black individuals, 0.012% in individuals of Pacific Island descent and 0.00039% in Asian individuals³. By contrast, the prevalence of C282Y homozygosity was 1.2% in Ireland⁴. Moreover, an average prevalence of 0.4% for C282Y homozygosity and 9.2% for C282Y heterozygosity was demonstrated in one review of 27 studies that included 6,302 samples from European countries⁵. The same study demonstrated a prevalence of 0.5% for C282Y homozygosity and 9% for C282Y heterozygosity in North America. In Asian, Indian subcontinent, African, Middle Eastern and indigenous Australasian populations (including Aboriginal and Vanuatuan Australians and Papuans), C282Y homozygosity was not detected in 3,752 samples, although the frequency of C282Y heterozygosity ranged from 0% to 0.5%. The prevalence of C282Y/H63D compound heterozygosity and H63D homozygosity was 2% (for both) in the European general population; in the Americas, the prevalence of compound heterozygosity was 2.5% and the prevalence of H63D homozygosity was 2.1%⁵. Other studies reported the highest frequency of C282Y in non-Finnish Europeans (an allele frequency of 5.14%)⁶. *TFR2*, *HJV* and *HAMP*-related haemochromatosis were rare, with allele frequencies ranging between 0.00007 and 0.0004.

SLC40A1 variants have an allele frequency of 0.0004 in several populations and were most prevalent in African individuals⁶. Iron overload in individuals in sub-Saharan Africa has been recognized for many years⁷ and is not uncommon among indigenous South Africans⁸. This syndrome is referred to as African iron overload, Bantu siderosis or dietary iron overload⁹. The prevalence of African iron overload is up to 20% in sub-Saharan Africa^{9,10}. In a survey of 505 rural Zimbabweans, iron overload was found almost exclusively in men who consumed traditional beer brewed in steel drums. In drinkers >45 years of age, 21% had high serum ferritin concentration and a transferrin saturation of >70%¹⁰.

Penetrance

Despite the high prevalence of C282Y homozygosity, only a minority of individuals will accumulate enough iron to cause organ damage. Given the autosomal recessive inheritance of C282Y, the frequency of C282Y homozygosity is similar in men and women, but the prevalence of clinical manifestations differs. Indeed, in one study, only 28.4% of men and 1.2% of women with C282Y homozygosity satisfied criteria for iron-related disease, consistent with a clinical diagnosis of haemochromatosis¹¹. However, at the start of the study, 81.8% of men and 55.4% of women had increased serum ferritin levels, suggesting that biochemical penetrance is higher than clinical penetrance. Other studies have estimated that the clinical manifestations of haemochromatosis develop in 25–60% of individuals homozygous for C282Y^{12,13}. Accordingly, other factors, such as genetic modifiers, environmental factors or lifestyle factors must modify susceptibility to haemochromatosis in individuals with C282Y homozygosity.

Modifier genes.—A single-nucleotide polymorphism (SNP) in *CYBRDI* (rs884409) is a modifier gene for haemochromatosis and has exclusively been found in individuals homozygous for C282Y¹⁴, although this polymorphism alone is not a major modifier of the haemochromatosis phenotype¹⁵. In addition, the p.D519G polymorphism in *GNPAT* is

associated with a high-iron phenotype in individuals with C282Y homozygosity¹⁶. Another study confirmed the association between the p.D519G polymorphism and increased iron stores in individuals homozygous for C282Y after correction for age, iron-related variables and alcohol consumption¹⁷. Mutations in other genes might also influence phenotypes in individuals homozygous for C282Y, including *HAMP*, *HJV*, *TFR2*, *SLC40A1* and *TMPRSS6* (REFS 18–25). In addition, mutations in *BMP6* (REFS 26,27) increase iron overload and a polymorphism in *PCSK7* is a strong genetic cofactor favouring the development of liver fibrosis in *HFE*-associated haemochromatosis²⁸. Nutritional factors that could have a major role in the development of fibrosis include excessive alcohol intake²⁹ and diabetes mellitus³⁰, which could increase the effects of oxidative stress.

Other factors.—Iron is absorbed from the diet as haem iron (present in meat and fish) or as non-haem iron (mainly present in vegetables, cereals and other foods, although this type of iron is also found in meat)^{31,32}. Haem iron is absorbed more readily than non-haem iron³³ and dietary haem iron content is a significant predictor of iron stores in individuals homozygous for C282Y^{34,35}. Indeed, the amount of dietary haem iron, but not non-haem iron, is positively associated with serum ferritin concentration^{36,37}. However, one study did not demonstrate a significant relationship between serum ferritin concentration and dietary haem iron content, dietary non-haem iron content or supplemental iron use in individuals homozygous for C282Y³⁸.

Heavy alcohol use by individuals with haemochromatosis increases the risk of cirrhosis³⁹. Indeed, 7% of individuals with haemochromatosis caused by C282Y homozygosity who consumed <60 g of alcohol per day had severe fibrosis and/or cirrhosis compared with 61% of patients who consumed excess alcohol (that is, individuals who consume >60 g alcohol per day). Patients with haemochromatosis who consume >60 g alcohol per day are approximately nine-times more likely to develop cirrhosis than those who drink <60 g per day⁴⁰.

Other environmental factors that could exert a protective effect against haemochromatosis in individuals homozygous for C282Y by decreasing iron absorption or increasing iron loss have been examined. For example, blood donations and physiological blood loss can influence iron stores and, accordingly, the phenotype of haemochromatosis. However, one study demonstrated that routine blood donation does not, on average, decrease the severity of iron overload in patients with haemochromatosis⁴¹ and one review concluded that these effects on disease penetrance are small⁴². The phenotypic expression of haemochromatosis is usually less pronounced in women than men, mainly due to menstruation, pregnancy or breastfeeding. In addition, hormonal factors — namely, testosterone — that can decrease hepcidin expression might contribute to the different phenotypic expression in men and women⁴³.

In an investigation of genetic modifiers of *HFE*-associated haemochromatosis caused by the H63D mutation, no correlation was found between potential genetic modifiers and phenotype⁴⁴. However, the H63D mutation together with alcohol, iron and calcium intake affects iron status⁴⁵. Recessively inherited haemochromatosis includes juvenile

haemochromatosis and *TFR2*-associated haemochromatosis, which have similar patterns of iron loading, albeit with differing severities and age at onset⁹.

Mechanisms/pathophysiology

Normal iron metabolism

Systemic iron regulation.—The maintenance of iron homeostasis includes the control of both iron concentration and biochemical forms of iron in plasma. Plasma iron is the main source of bioavailable iron for cells. Adequate body iron content and iron distribution require the maintenance of plasma iron concentration within normal limits (12–25 μM)⁴⁶ and the regulation of transferrin saturation. Normal transferrin saturation is between 20% and 45% and allows adequate iron delivery to cells, thereby avoiding diseases of iron metabolism⁴⁷. In mammals, iron is bound to transferrin in plasma, forming holotransferrin, which is the main biochemical form of iron in plasma. Transferrin is secreted by hepatocytes and receives up to two atoms of ferric iron (Fe(iii)) through an active process involving ferroxidase enzymes.

The main sources of plasma iron are enterocytes (which absorb iron from the gut lumen as non-haem or haem iron) and macrophages (which acquire iron from erythrophagocytosis; FIG. 2). The transport of iron from enterocytes and macrophages into the plasma occurs through ferroportin^{48–50}, which is highly expressed on the basolateral membrane of enterocytes and the plasma membrane of macrophages and is the only identified iron exporter in mammals. Hepcidin — an iron-regulated peptide that is secreted by hepatocytes — is the main regulator of ferroportin activity^{51–53}. Ferroportin-hepcidin binding induces the internalization, ubiquitination and degradation of ferroportin and, therefore, plasma hepcidin levels strongly affect plasma iron concentration⁵⁴. Indeed, low hepcidin levels increase the export of iron from enterocytes and macrophages, thereby increasing plasma iron concentration and transferrin saturation. When transferrin saturation increases, non-transferrin bound iron (NTBI) can appear in plasma, which can lead to cell toxicity (see Iron-induced toxicity, below). Accordingly, the control of hepcidin expression levels is a cardinal factor in the control of systemic iron homeostasis.

The transcription activity of *HAMP* is the main regulator of hepcidin expression. Several pathways have been implicated in the regulation of *HAMP* transcription, including cell signalling in response to alterations in iron stores or transferrin saturation (FIG. 3; reviewed in REF. 47). The signalling pathways controlling *HAMP* transcription from iron stores involve a complex of proteins localized on the hepatocyte membrane (including HJV and the bone morphogenetic protein receptor (BMPR) that is reactive to bone morphogenetic protein 6 (BMP6) and, to a lesser extent, BMP2 (REFS 55,56). Interestingly, transmembrane protease serine 6 (also known as matriptase-2; encoded by *TMPRSS6*) cleaves HJV and, therefore, strongly modulates this pathway; patients with *TMPRSS6* mutations have very high levels of hepcidin, leading to severe iron deficiency that is resistant to oral iron supplementation (iron-refractory iron deficiency anaemia)⁵⁷. Accordingly, modulating *TMPRSS6* expression by altering HJV expression could modify the phenotype of haemochromatosis in patients, as suggested from data from haemochromatosis mouse models⁵⁸. In addition, transferrin saturation is speculated to regulate hepcidin expression

through HFE^{59,60} and TFR2 (REFS 61,62), which are expressed on hepatocyte membranes (FIG. 3), although an alternative or complementary mechanism could be interactions between HFE and the BMPR-HJV complex⁶³. Hepcidin expression is downregulated by hypoxia through hypoxia-inducible factor (HIF)⁶⁴ and platelet-derived growth factor BB⁶⁵. Erythropoiesis might reduce hepcidin expression through erythropoietin that is excreted by erythroblasts⁶⁶ and transforming growth/differentiation factor 15 (REF. 67). Chronic inflammation induces hepcidin expression at least partly through IL-6 and signal transducers and activators of transcription 3 (STAT3) signalling^{68–71} (FIG. 3). Hormones can also modulate hepcidin expression; for example, oestrogens and testosterone increase and decrease hepcidin expression, respectively^{43,72}.

Intracellular regulation of iron metabolism.—Holo-transferrin binds to transferrin receptor 1 (TFR1) on the surface of every cell and is internalized by endocytosis⁷³. Thereafter, ferric iron is reduced to ferrous iron by metallo-reductase STEAP3 (REF. 74) and is transported into the cytosol by DMT1 (REFS 75,76). Cytosolic iron is used as a cofactor for many proteins and enzymes or is stored in a complex with ferritin. Ferritin is composed of 24 subunits of two different types (H and L) that can accumulate up to 4,500 iron atoms in a biochemically inactive form. The H subunit of ferritin has ferroxidase activity⁷⁷ that allows the iron ingress as ferric iron and in turn its subsequent deposition within the ferritin protein in a chemically inactive form after a nucleation process⁷⁸. This mechanism of iron sequestration avoids the oxidative stress that can occur through the Haber-Weiss and Fenton reaction when cytosolic iron concentration increases⁷⁹.

Both the cellular influx and storage of iron are controlled by the iron responsive element-iron regulatory protein (IRE-IRP) system⁸⁰. In cellular iron deficiency, the IRPs interact with the IRE nucleotide sequence located in the 3' untranslated region (UTR) of the *TFRC* mRNA (encoding TFR1) and the 5' UTR of ferritin mRNAs. The IRE-IRP interaction stabilizes *TFRC* mRNA, leading to increased TFR1 expression and increased holo-transferrin internalization⁸⁰. In addition, IRE-IRP interaction reduces ferritin translation and iron storage. Thus, IRP-IRE interaction increases cytoplasmic iron availability. Conversely, when iron accumulates in cells, IRE-IRP interaction is reduced, leading to decreased TFR1 expression and increased ferritin expression⁸⁰.

Iron-induced toxicity

Cellular iron toxicity due to iron excess leads to the formation of reactive oxygen species, which can cause organ damage. Increased transferrin saturation can lead to the formation of abnormal forms of iron in plasma, such as NTBI⁸¹. Thus, NTBI can form when transferrin saturation is >45% (such as in patients with *HFE*-associated haemochromatosis)⁸². NTBI is not fully characterized but seems bound to low molecular weight molecules, including citrate and acetate⁸³ or to albumin. In addition, small amounts of labile plasma iron (a highly reactive form of iron) can be detected in the plasma of patients with haemochromatosis when transferrin saturation is >75%^{84,85}. Labile plasma iron, which could be one type of NTBI but does not cover the whole NTBI, is involved in the production of reactive oxygen species through the Haber-Weiss and Fenton reactions⁸⁶. It is noteworthy that cellular iron excess alters mitochondrial function in the liver^{87,88}.

NTBI has specific kinetics that contribute to cell toxicity. Indeed, uptake of NTBI occurs independently of TFR1 (REF. 89) and can involve zinc transporter ZIP14 (REFS 90,91), which is expressed by hepatocytes, and ZIP8, which is expressed by cardiomyocytes. In addition, L-type calcium channels might have a role in NTBI uptake by cardiomyocytes⁹², and pharmacological blockade of these channels has been shown to reduce iron accumulation in animal models⁹³. NTBI uptake occurs despite iron overload⁹⁴, in contrast to transferrin iron uptake that is downregulated through the IRE-IRP system in such situations. Parenchymal cells are prone to take up NTBI, especially hepatocytes and cells of the pancreas and heart^{95,96}, which explains the main complications of haemochromatosis, such as liver fibrosis, cirrhosis, hepatocellular carcinoma, diabetes mellitus and heart failure.

Genetic haemochromatosis

Mutations causing plasma hypohepcidinaemia.—Plasma hypohepcidinaemia is caused by mutations in *HAMP* or in genes involved in the regulation of hepcidin levels. Mutations in *HFE* lead to *HFE*-associated haemochromatosis. HFE is a human leukocyte antigen (HLA) class I molecule that is expressed on cell membranes in association with β 2-microglobulin. As previously mentioned, most cases of haemochromatosis involve homozygosity for C282Y⁵⁹, which, owing to the disruption of a disulfide bond, affects the conformation of HFE and the interaction of this protein with β 2-microglobulin^{60,97} and, consequently, the interaction with other potential binding partners, including TFR2 and/or BMPR-HJV. The phenotypic expression of *HFE*-associated haemochromatosis is less severe than that of haemochromatosis caused by mutations in *HAMP*, as hepcidin levels, although not adapted to the iron status, remain detectable^{98,99}.

HAMP mutations cause haemochromatosis type 2B. This form of haemochromatosis is inherited in a recessive manner and is caused by homozygous or compound heterozygous mutations in the *HAMP* gene coding sequence that are extremely rare but lead to the juvenile form of haemochromatosis. Indeed, the absence of normal hepcidin in plasma leads to a biochemical phenotype that fully recapitulates haemochromatosis¹⁰⁰. Also, mutations in the *HAMP* promoter, although extremely rare, can affect hepcidin expression^{101,102}. The absence of hepcidin allows constant ferroportin expression independent of the iron status, which allows excessive iron transport into plasma. Increased plasma iron levels increase transferrin saturation and favour the production of plasma NTBI⁸⁹.

Homozygous mutations in *HJV* can cause haemochromatosis type 2A (also known as juvenile haemochromatosis), which is closely related to homozygous *HAMP* mutations²⁵. These mutations, by limiting the activity of the BMPR-HJV-SMAD pathway, impair the upregulation of *HAMP* transcription in response to cellular iron store increases, therefore underlying the major role of the BMPR-HJV-SMAD pathway in the iron-related regulation of hepcidin expression.

Mutations in *TFR2* can also lead to haemochromatosis^{103,104}, corresponding to haemochromatosis type 3. The phenotype is usually intermediate between *HFE*-associated, *HJV*-associated and *HAMP*-related haemochromatosis.

The combination of heterozygous mutations in two different genes involved in iron metabolism has been reported to cause iron overload^{105–108}. This finding suggests that mutations in different genes involved in the control of hepcidin transcription have a cumulative effect on hepcidin level and that additional rare mutations in *HFE* and in other genes that have a role in iron metabolism should be investigated¹⁰⁹. Moreover, every mechanism potentially modulating hepcidin expression in addition to iron gene mutations can modulate the phenotype of patients.

Mutations causing loss of activity of hepcidin.—A loss of hepcidin activity can mainly result from missense mutations in *HAMP* or *SLC40A1*. The biological activity of hepcidin requires an interaction with ferroportin on the cell membrane, which involves specific domains in both proteins.

In ferroportin, some amino acids that interact with hepcidin have been identified, and mutations that affect these amino acids cause a loss of ferroportin sensitivity to hepcidin^{110–114}. The identification of these amino acids explains why some mutations in *SLC40A1* can lead to an iron overload phenotype that is similar to hepcidin deficiency. This form of haemochromatosis corresponds to a rare form of iron overload caused by some rare mutations in *SLC40A1* and should be, in our view, named haemochromatosis type 4 (BOX 1). Other mutations in *SLC40A1* lead to ferroportin loss of function, and patients have a different phenotype to haemochromatosis (ferroportin disease).

A domain of hepcidin that mediates the biological effect on ferroportin has been reported¹¹⁵. It is noteworthy that missense mutations in *HAMP* could cause a decrease of hepcidin activity that could have a role in the development of iron overload owing to non-functionality of hepcidin. Similarly, mutations in the *HAMP* promoter that strongly decrease hepcidin expression might favour or aggravate iron overload phenotype^{101,102}.

Ferroportin disease.—Heterozygous mutations in *SLC40A1* that lead to ferroportin loss of function might lead to systemic iron overload, termed ferroportin disease. In our view, ferroportin disease does not correspond to haemochromatosis despite the current classification as haemochromatosis type 4A^{116,117}. Mutations in *SLC40A1* can be found all along the coding sequence of the gene¹¹⁸. The deficiency in iron export that occurs in ferroportin disease might result from a defect of intracellular ferroportin trafficking to the plasma membrane and/or loss of the iron-export function of ferroportin^{113,119}. In ferroportin disease, iron is sequestered in macrophages. Although macrophages deliver ~19–20 mg of iron per day to plasma, plasma iron concentration and transferrin saturation are rarely decreased in patients with ferroportin disease. Moreover, accumulation of iron in hepatocytes can occur, and increased transferrin saturation might be observed in the late stage of disease. Such findings suggest¹¹⁹ that macrophages are severely affected by ferroportin disease owing to the important volume of iron that they provide to plasma, whereas digestive iron absorption could be less affected, with the remaining normal gene being sufficient to ensure iron trafficking.

Diagnosis, screening and prevention

In general, diagnosis of haemochromatosis involves a sequential noninvasive strategy that combines clinical, imaging and biological data¹²⁰ (FIG. 4).

Clinical features

Haemochromatosis can be asymptomatic for several years. Indeed, the absence of symptoms can persist until adolescence or young adulthood in most forms of non-*HFE* haemochromatosis and up to 30–40 years of age in men and 40–50 years of age in women with *HFE*-associated disease. In general, symptoms are miscellaneous, variable and frequently of mundane nature (FIG. 1), which explains the frequent and damaging diagnostic delay^{121–123}. Clinically, multiple, diversely associated symptoms should suggest the possibility of iron overload.

Biological factors.—Several biological parameters, including increased serum ferritin levels and elevated transferrin saturation can indicate the presence of haemochromatosis.

Increased transferrin saturation (>45%) is the earliest biochemical sign observed in all haemochromatosis sub-types. However, mechanisms aside from iron overload can cause increased transferrin saturation, such as other causes of increased plasma iron (for example, haemolysis and cytolysis) or decreased plasma transferrin concentration (for example, by acquired hepatocellular failure, poor nutrition, proteinuria or genetic alterations)¹²⁴. Moreover, normal or even low transferrin saturation can be present despite overt body iron overload, such as in individuals with ferroportin disease¹¹⁷ or in hereditary aceruloplasminaemia¹²⁵ (BOX 2).

Whereas low ferritin levels always correspond to iron deficiency, increased ferritin levels (300 µg per litre in men and 200 µg per litre in women) need rigorous interpretation before they are assigned to iron overload. Thus, four main mechanisms that can account for increased ferritin levels independent of substantial iron overload should be ruled out: metabolic syndrome (which is the most frequent cause), alcoholism, inflammation and marked cytolysis. Despite these limitations, increased ferritin levels are critical for the diagnosis of haemochromatosis.

Several parameters can indicate organ-specific increased iron levels, for example, mild hypertransaminasaemia (less than three-times the upper normal limit) can indicate chronic hepatic iron excess, and hyperglycaemia can reflect pancreatic iron excess.

Imaging findings.—Radiological findings, such as chondrocalcinosis of the wrists or knees or subchondral arthropathy that can be detected using X-ray can suggest haemochromatosis. In addition, low T2-weighted signal on liver MRI can suggest haemochromatosis. Indeed, whenever available, iron excess should be confirmed by iron MRI. One of the most convenient techniques for iron MRI is based on the signal intensity ratio approach^{126–128}, which enables the visualization and quantification of hepatic, splenic and pancreatic iron excess by assessing T2-weighted hyposignal, without requiring specific 1.5 Tesla MRI equipment and using free online software (FIG. 5). When using 3 Tesla MRI,

which is becoming more widespread, the R2* method provides a more precise quantification of slight or moderate iron overload than signal intensity ratio, whereas signal intensity ratio is more accurate for high iron overload¹²⁹. The use of R2* measurements in both the liver and the pancreas have been proposed to predict the total amount of iron stores in patients with haemochromatosis¹³⁰. In addition, iron MRI can be used to differentiate haemochromatosis owing to hepcidin deficiency from ferroportin disease (FIG. 5). CT has a very poor sensitivity for visualizing iron excess, and ultrasonography cannot detect iron overload.

Liver biopsy.—Liver biopsy is no longer necessary for asserting and quantifying iron excess and cellular iron distribution, and has been largely replaced by the combined evaluation of biological and imaging findings. However, liver biopsy can be important for assessing hepatic complications of haemochromatosis, such as fibrosis¹³¹.

Confirming genetic iron overload.—Confirming genetic iron overload requires excluding causes of acquired iron overload. Four main types of disorder should be excluded: haematological disorders, disorders due to excessive iron supplementation, metabolic syndrome and chronic alcoholism. In terms of haematological disorders, iron excess can be caused by transfusional iron overload¹³² and/or dyserythropoiesis^{133,134}, such as in congenital or acquired chronic anaemias (mostly thalassaemia and myelodysplastic syndromes), as well as hereditary spherocytosis, which might be related to dyserythropoiesis¹³⁵. In addition, excessive parenteral iron supplementation can lead to iron overload, especially in the setting of chronic renal failure with haemodialysis¹³⁶. Dysmetabolic iron overload syndrome likely has a multifactorial pathogenesis and can lead to mild iron excess, which is sometimes observed in association with one or several metabolic alterations (increased body mass index, increased blood pressure, hyperlipidaemia, hyperglycaemia, hyperuricaemia or hepatic steatosis). Dysmetabolic iron overload syndrome is associated with increased ferritin levels and mild hepatic iron overload; the key differential parameter from haemochromatosis is that plasma transferrin saturation is not increased¹³⁷. Finally, chronic alcoholism can cause moderate hepatic iron excess, possibly owing to alcohol-related hepcidin deficiency¹³⁸.

Identifying the genetic cause of the disease is easy for *HFE*-associated haemochromatosis¹³⁹. For example, this disorder is almost always found in white individuals by identifying C282Y homozygosity, which can be carried out by numerous laboratories. As mentioned previously, simple C282Y heterozygosity cannot cause iron excess and C282Y/H63D compound heterozygosity can cause some plasma transferrin saturation increase but not substantially increased ferritin levels or considerable organ iron overload. At most, compound heterozygosity might lead to mild iron overload in situations such as chronic alcoholism or metabolic syndrome. Notably, rare *HFE* variants are more prevalent than variants in other genes involved in iron metabolism in individuals with suggestive hepcidin deficiency-related haemochromatosis but without C282Y homozygosity^{109,140}. Thus, the detection of C282Y heterozygosity in a patient with considerable systemic iron overload should lead to the search for other genetic and/or acquired causes of iron excess.

The genetic confirmation of non-*HFE* haemochromatosis requires testing carried out by specialized laboratories after the appropriate selection of required genetic tests by an expert reference centre. High-throughput techniques (especially next-generation sequencing) offer powerful diagnostic tools, although the interpretation of these tests is more demanding than rigorous clinical characterization and selection.

Haemochromatosis severity

After the diagnosis is confirmed, the severity of haemochromatosis should be assessed by determining the amount of body iron excess and the extent of organ damage. A five-grade classification can be used for haemochromatosis related to hepcidin deficiency, which is similar to the classification initially proposed for *HFE*-associated haemochromatosis¹⁴¹ (FIG. 6). This classification is based on the presence or absence of increased plasma transferrin saturation, increased plasma ferritin, affected quality of life and signs jeopardizing prognosis. For example, plasma ferritin levels >1,000 µg per litre at diagnosis have been reported to correspond to an increased risk of death in *HFE*-associated haemochromatosis¹⁴². This classification does not apply for the loss-of-function ferroportin disease¹⁴³.

Screening and prevention

HFE-associated haemochromatosis is theoretically the archetype of genetic diseases, justifying active screening and prevention for several reasons. For example, this disorder is one of the most prevalent genetic diseases, diagnosis is minimally invasive and the disease can be treated with phlebotomy, which is efficient, simple and inexpensive. As soon as an individual has been diagnosed with haemochromatosis, they must be considered as a proband, and cascade family screening should be initiated.

The methodology of screening for haemochromatosis is based on genetic testing. For all forms of haemochromatosis, the absence of the proband's mutation in their relative means that the relative is free of any risk of that form of haemochromatosis. For individuals with recessive subtypes of haemochromatosis (that is, types 1, 2 and 3), the presence of simple heterozygosity means they do not have a risk of developing the disease but risk transmission to their offspring, whereas for individuals with dominant subtypes of haemochromatosis (such as haemochromatosis type 4), heterozygosity means risk of developing the disease. Siblings of the proband are the preferred target for screening after a family member has been diagnosed with recessive forms of haemochromatosis. However, in individuals with *HFE*-associated haemochromatosis, the high prevalence of the C282Y mutation among white individuals justifies the screening of offspring (although this screening is usually deferred until the offspring reach adulthood). Despite the efficiency of cascade screening and the fact that this method can determine the pathogenicity of a given genetic variant, this screening strategy is too rarely adopted, likely owing to the practical difficulties in reaching scattered relatives and in collating family data. Specific centres devoted to family screening are the solution but remain quite rare.

Growing evidence highlights the importance of conducting population screening for *HFE*-associated haemochromatosis^{144,145}. The HEIRS study did not recommend conducting *HFE*

genotyping before phenotyping¹⁴⁶; thus, initially phenotyping individuals might be the preferred option. In practice, plasma transferrin saturation could be checked once in young adults and once again, for example, 5 years after menopause, which could form the basis of population screening and allow every individual with increased transferrin saturation to undergo testing for C282Y and, if warranted, treatment for haemochromatosis.

Management

Iron removal

Phlebotomy.—Phlebotomy remains the mainstay of treatment for *HFE*-associated haemochromatosis. For non-*HFE*-associated haemochromatosis that is related to hepcidin deficiency, phlebotomy is the preferred treatment¹⁴⁷, but adjunctive oral chelation can be used in the most severe cases (for example, individuals with juvenile haemochromatosis). Phlebotomies are also efficient for treatment of patients with loss-of-function ferroportin disease but should be carried out on a less intensive schedule given the risk of anaemia owing to poor iron recycling in these patients. Many physicians and patients insist that high serum ferritin levels equal iron overload and should be treated by phlebotomy. However, as previously mentioned, patients without C282Y homozygosity might not always have iron overload.

The goal of phlebotomy therapy is to remove excess iron to prevent any further tissue damage. For ethical reasons, phlebotomy therapy has not been investigated in randomized clinical trials, which has hindered our understanding of the natural history of untreated disease^{148,149}. Although some individuals, albeit rarely, have suggested phlebotomy therapy has no supporting evidence¹⁵⁰, most experts believe that this form of iron depletion can improve chronic fatigue and cardiac function, stabilize liver disease, reverse hepatic fibrosis and reduce skin pigmentation in patients with haemochromatosis¹⁴⁷. However, joint symptoms respond poorly to phlebotomy therapy and can worsen¹⁵¹. The effectiveness of phlebotomy is good provided it has been started before the development of cirrhosis. Adverse effects of phlebotomy occur in 37–50% of patients¹⁵² and include phlebitis, malaise and fatigue. If adverse effects occur, hot compress to the skin, oral fluid replacement and increasing the interval between treatments can be considered.

For phlebotomy induction therapy, patients usually attend an ambulatory care facility where the treatment is performed by a nurse. Typically, a volume of 400–500 ml of blood is removed over 15–30 minutes with the patient in the reclining position. This process is carried out on a weekly basis until the serum ferritin level is ~50 µg per litre. Haemoglobin levels are also assessed, and the phlebotomy schedule is modified if haemoglobin levels are <11 g per decilitre (for example, to 400–500 ml of blood removal every other week)¹⁴⁷. The concomitant oral administration of liquid (such as a salty sport beverage) of an equivalent volume to the volume of blood removed by phlebotomy is important for maintaining plasma volume during the procedure. The duration of induction therapy depends on the severity of iron overload and can range from months to years.

After the induction phase, maintenance phlebotomies are carried out to maintain serum ferritin levels at ~50 µg per litre. If phlebotomy is continued until serum ferritin levels are

<20 µg per litre, iron deficiency or iron reaccumulation can occur¹⁵³. Serum hepcidin levels can decrease with phlebotomy therapy¹⁵⁴. Maintenance phlebotomies are carried out on average two to four times per year, and this depends on the rate of iron reaccumulation, which is variable between patients^{155,156}. Checking serum ferritin levels 3–6 months after induction therapy has ended can be useful to estimate the rate of iron reaccumulation. Assessing serum ferritin levels can be carried out monthly during induction phlebotomy and weekly when the serum ferritin has decreased to 100 µg per litre. Once serum ferritin is 50 µg per litre, assessment of serum ferritin levels annually or at the time of each maintenance phlebotomy can be considered. Although the evidence supporting the use of maintenance therapy is lacking, many patients appreciate this therapy, particularly if they can be voluntary blood donors^{157,158}.

Despite successful iron depletion, transferrin saturation will remain increased in several patients. One report suggested that maintaining normal transferrin saturation might improve symptoms to a greater degree than lowering ferritin but not transferrin saturation¹⁵⁹. However, transferrin saturation might not decrease until the patient is almost iron deficient, so maintaining appropriate ferritin levels and transferrin saturation might be difficult.

Some patients have been treated by erythrocytapheresis¹⁶⁰, but this technique is more expensive and less available than phlebotomy. One study in Australia demonstrated an improvement in a fatigue scale in patients (with ferritin levels of 300–1,000 µg per litre) receiving erythrocytapheresis and who had iron reduction compared with patients receiving sham pheresis, suggesting the potential benefit of blood removal even in mild iron overload^{161,162}.

Chelation therapy.—Chelation therapy with deferoxamine is not recommended for any subtype of haemochromatosis. Deferoxamine is expensive, inefficient and cumbersome and can lead to adverse effects, such as blurred vision, dizziness, tinnitus, flushing, skin rash and diarrhoea¹⁶³.

Other therapies and dietary factors

Patients with haemochromatosis are advised to avoid oral iron therapy and alcohol abuse, but there are no proven dietary restrictions³⁸. Patient support groups have been discouraged by the iron fortification of foods, although most of this iron is an inexpensive form with poor bio-availability. Iron fortification of foods has been stopped in Sweden, and a reduction in the mean serum ferritin levels has been demonstrated in the general population¹⁶⁴. Tea consumption¹⁶⁵ and the use of proton pump inhibitors have been shown to decrease intestinal iron absorption¹⁶⁶. Indeed, one trial demonstrated a reduction in annual phlebotomy requirements by 1.33 units per year in individuals with C282Y homozygosity who receive daily proton pump inhibitor therapy¹⁶⁶. Patients with haemochromatosis should avoid consuming raw shellfish, especially in subtropical areas, as patients have an increased risk of *Vibrio* spp. infections¹⁶⁷.

Liver transplantation

Despite the high prevalence of haemochromatosis, it remains an uncommon indication for liver transplantation. For example, in Brisbane, Australia, 0.6% of liver transplants were carried out for haemochromatosis¹⁶⁸. The main indication for liver transplantation in haemochromatosis is hepatocellular carcinoma¹⁶⁸. Many individuals diagnosed with haemochromatosis and who received liver transplantation owing to decompensated cirrhosis are more likely to have had iron overload secondary to cirrhosis from other causes¹⁶⁹. Pretransplant phlebotomy might improve cardiac function and is recommended if tolerated. The lack of recurrence of iron overload together with normalization of plasma hepcidin levels after liver transplantation have provided the clinical proof that hepcidin is central to the pathogenesis of haemochromatosis¹⁷⁰.

Hepcidin therapies

As low hepcidin levels or hepcidin activity have been implicated in the pathogenesis of haemochromatosis, hepcidin analogues have been proposed as a therapy¹⁷¹. Early reports suggest that this approach is better suited for maintenance therapy as these drugs do not decrease iron storage in the liver¹⁷². These treatments will likely be parenteral and expensive, which will be difficult to compete with phlebotomy therapy.

Quality of life

Several studies have demonstrated a negative effect of haemochromatosis on health-related quality of life (HRQOL). Elevated iron stores, particularly transferrin saturation and serum ferritin levels and the presence of comorbidities, contribute to reduced HRQOL.

The Short Form 36 (SF-36) is the most commonly used instrument to assess HRQOL for patients with haemochromatosis¹⁷³. The SF-36 consists of eight domains that provide physical component and mental component scores that range between 0 and 100 (higher scores indicate a higher HRQOL). In the United States, the population normative score is 50 (with a s.d. of 10)¹⁷⁴. Individuals with increased transferrin saturation (45% for females and 50% for males) have a worse HRQOL assessed using the SF-36 than participants with normal transferrin saturation¹⁷⁵. Both the physical and mental component scores were significantly lower in individuals with increased transferrin saturation¹⁷⁵. Although not examined in this study, the association between higher iron stores and lower HRQOL might be due to increased fatigue and lethargy^{176,177}. The effect of haemochromatosis-related comorbidities on QOL has also been evaluated using the SF-36 (REF. 178). Interestingly, arthritis was associated with a lower overall HRQOL than cirrhosis or diabetes mellitus in patients with completed phlebotomy treatment (with serum ferritin levels <50 µg per litre)¹⁷⁸. In addition, one study reported a physical component score of 30.5 (which is 2 s.d. lower than the mean values in the United States) and a mental component score of 77.7 (which is almost 3 s.d. above the normal values in the United States) in patients with painful osteo-arthritis of the hind foot^{174,179}. Patients with a recent diagnosis of haemochromatosis who were deemed clinically affected by the disorder had a significantly lower mean physical component score and a trend towards a lower mental component score¹⁸⁰.

Some studies have used HRQOL utility instruments, such as the EuroQol-5D (EQ-5D) and the Assessment of Quality of Life-4D (AQOL-4D), to assess HRQOL in patients with haemochromatosis. These instruments estimate utility values, which enable the estimation of how both quantity and quality of life are affected by a disease, health state and/or intervention¹⁸¹. Utility is measured on a scale of 0 to 1, of which 0 represents death and 1 represents optimal health. Based on self-reported symptoms, a cross-sectional study demonstrated significantly lower mean utility values in patients with either elevated iron levels and early symptoms (0.60) or elevated iron levels and organ damage (0.50), compared with asymptomatic individuals (0.81) and the general population (0.81)¹⁷⁷. Further, a negative correlation between the number of haemochromatosis-related symptoms and/or comorbidities and utility was reported.

Only one randomized clinical trial has assessed HRQOL and/or utility in patients receiving different treatments. However, no significant differences were observed in HRQOL in individuals receiving erythrocytapheresis or phlebotomy when assessed using either the SF-36 or the EQ-5D¹⁸².

Outlook

Although iron deficiency remains a major health problem worldwide, the clinical effect of hereditary conditions associated with iron overload is now increasingly recognized. Haemochromatosis is the paradigmatic clinical condition associated with systemic iron overload. Whereas haemochromatosis was originally considered an unusual autopsy finding probably owing to excessive alcohol intake, the condition is now recognized as a unique syndromic entity owing to the ground-breaking discovery that a lack of hepcidin causes this disorder.

Evolutionary perspectives

Iron is a vital micronutrient for humans and pathogens, and fighting pathogens (and avoiding starvation) has most likely represented the main challenge in human evolution^{183,184}. Accordingly, it is interesting that hepcidin, an antimicrobial peptide that evolved to withhold iron from blood during pathogen invasion, and a protein used by hepcidin to 'sense' blood iron (that is, HFE) are centrally involved in the regulation of body iron homeostasis and in the pathogenesis of haemochromatosis. Yet we still do not understand its profound meaning from an evolutionary perspective. What do the spreading and strong evolutionary pressure of the C282Y polymorphism in white populations have to do with innate immunity and the battle against pathogens (for example, does HFE also have an immunological role? Does the macrophage-deprived environment of haemochromatosis have a role in the response to intracellular pathogens?) and, more generally, does this potential selective advantage still have some relevance in terms of physical performance? These questions remain to be clarified^{184,185}. In addition, an 'iron-thrifty' phenotype might have evolved in humans to store energy and accumulate iron reserves¹⁸⁴. Although this might have been evolutionarily advantageous to survive long periods of famine, this might be detrimental for contemporary lifestyles and dietary habits that favour iron accumulation. In individuals with chronic and degenerative diseases, a genetic predisposition to a slight excess of pro-oxidant iron in blood

owing to variants of *HFE* or other iron-loading genes could contribute to and amplify the underlying pathophysiological event, which could impact the course and outcome of chronic diseases. This process has been suggested in studies in diabetes¹⁸⁶.

Mechanisms of disease

Although the hepcidin-centred pathogenetic basis of haemochromatosis has been largely elucidated, some details are still under investigation, such as the interaction and putative cooperation of different haemochromatosis-associated proteins (such as HFE and TFR2) in hepcidin signalling and the role of BMPs in haemochromatosis^{187–191}. In addition, one concern is that available animal models have been useful to explain iron overload in haemochromatosis, but they do not explain the resulting multiorgan disease. Several general questions still await answers, such as if a role exists for HFE outside the hepatocyte-hepcidin-centred model, for example, in crypt enterocytes (where the HFE-TFR1 complex was originally identified in humans¹⁹²) or in macrophages (where hepcidin-independent HFE activity has been hypothesized)^{193–196}.

Genetics

In principle, the lack of any gene that limits iron transfer into the blood could cause haemochromatosis. In this context, mutations causing the loss of the iron-retention capability of enterocytes could cause unrestricted iron transfer into blood, leading to haemochromatosis. Indeed, deletion of *Fth* (encoding H ferritin) causes a haemochromatosis phenotype in mice¹⁹⁷, and an unconfirmed report identifying haemochromatosis owing to mutations in *SRD5A2* (encoding H ferritin) in humans suggests this is a possibility¹⁹⁸.

Other open questions in haemochromatosis relate to the penetrance of *HFE*-associated haemochromatosis. As previously mentioned, the clinical expression of haemochromatosis varies substantially between patients and follows a gradient ranging from the least penetrant form (*HFE* and C282Y homozygosity) to the fully penetrant forms (owing to *HJV* or *HAMP* mutations)¹⁹⁹. Although environmental and physiological factors are important for the clinical expressions of *HFE*-associated haemochromatosis, genetic factors might also have a role. For example, polymorphic variants in a number of predictable and apparently iron-unrelated genes that potentially affect the phenotype of haemochromatosis patients have been reported^{16,26,200–202}. However, to reach definite conclusions about these variants, the major haemochromatosis referral centres should collaborate to design prospective next-generation sequencing studies in large cohorts of patients and controls.

Diagnosis and management

Although a substantial advancement in the diagnosis and optimal management of the main clinical manifestations of haemochromatosis has been achieved over the past few decades, several areas require further study. For example, the pathophysiology of haemochromatosis arthropathy is still elusive, and this symptom has an unpredictable clinical onset and complex management and is often frustrating for patients and doctors; further study of the pathogenetic basis of haemochromatosis arthropathy is required.

As in the case of diabetes, we can now envisage new and more-effective approaches for the diagnosis and treatment of haemochromatosis¹⁹⁹. For example, the measurement of serum hepcidin levels is considered a useful add-on in the diagnostic workup of individuals with suspected haemochromatosis, and, in the near future, hepcidin-sensitivity tests might be available to aid diagnosis of haemochromatosis owing to hepcidin resistance, such as ferroportin-related haemochromatosis. As to the treatment, there is no doubt that phlebotomy is a safe and effective treatment for haemochromatosis. However, severe cases are still cumbersome and difficult to treat, and a hormonal hepcidin-based replacement or phlebotomy-adjuvant therapy might be invaluable in these patients.

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Box 1 |**Haemochromatosis subtypes**

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- Type 1: caused by mutations in *HFE*
 - Type 2A: caused by mutations in *HJV* (also known as *HFE2*)^a
 - Type 2B: caused by mutations in *HAMP*^a
 - Type 3: caused by mutations in *TFR2*^b
 - Type 4: caused by mutations in *SLC40A1* that lead to ferroportin gain of activity (this disorder was previously classified as type 4B and is different from ferroportin disease, which is caused by ferroportin loss of activity and is classified as type 4A)
-

^aDenotes juvenile haemochromatosis.

^bSometimes corresponds to juvenile haemochromatosis.

Box 2 |**Other genetic disorders leading to systemic iron load**

Hereditary aceruloplasminaemia is caused by homozygous mutations in *CP* (encoding ceruloplasmin). This disorder is characterized by very low levels of plasma iron and transferrin saturation, which causes anaemia, but patients have systemic iron excess. One explanation is the loss of the ferroxidase activity of ceruloplasmin²⁰³, limiting iron release from macrophages into plasma. However, in patients, the spleen is not overloaded with iron, and the liver has iron deposition in hepatocytes, suggesting that the precise mechanisms governing organ iron distribution are not yet understood¹²⁵. The potential effect of anaemia in favouring iron excess through the downregulation of hepcidin expression has been proposed. Whether compensatory ferroxidase activity by hephaestin has a role in ensuring iron export from macrophages despite low ceruloplasmin-related ferroxidase activity should be considered^{204,205}.

Mutations in *TF* (encoding transferrin) lead to the development of anaemia owing to the absence of transferrin-bound iron available for erythroid cells. These mutations lead to very low plasma transferrin levels, which favour the appearance of non-transferrin bound iron (NTBI). Indeed, NTBI is the predominant form of plasma iron in these individuals. In addition, hepcidin expression is decreased owing to anaemia, leading to subsequent improved iron absorption, which, together with plasma NTBI, contributes to the development of iron overload in the liver, pancreas and heart²⁰⁶.

Mutations in *DMT1* leading to DMT1-related iron overload affect both iron absorption (owing to the role of DMT1 in non-haem iron uptake by enterocytes) and intracellular iron trafficking (owing to the role of DMT1 in iron transfer from endocytic vesicles towards the cytosol, particularly in erythroblasts). Thus, anaemia is one of the major consequences of *DMT1* mutations and presents early in life. It is noteworthy that visceral iron overload is found in parallel, suggesting that other mechanisms are involved in the disease, including the potential role of increased digestive iron absorption by an adaptive mechanism and the presence of NTBI^{207,208}.

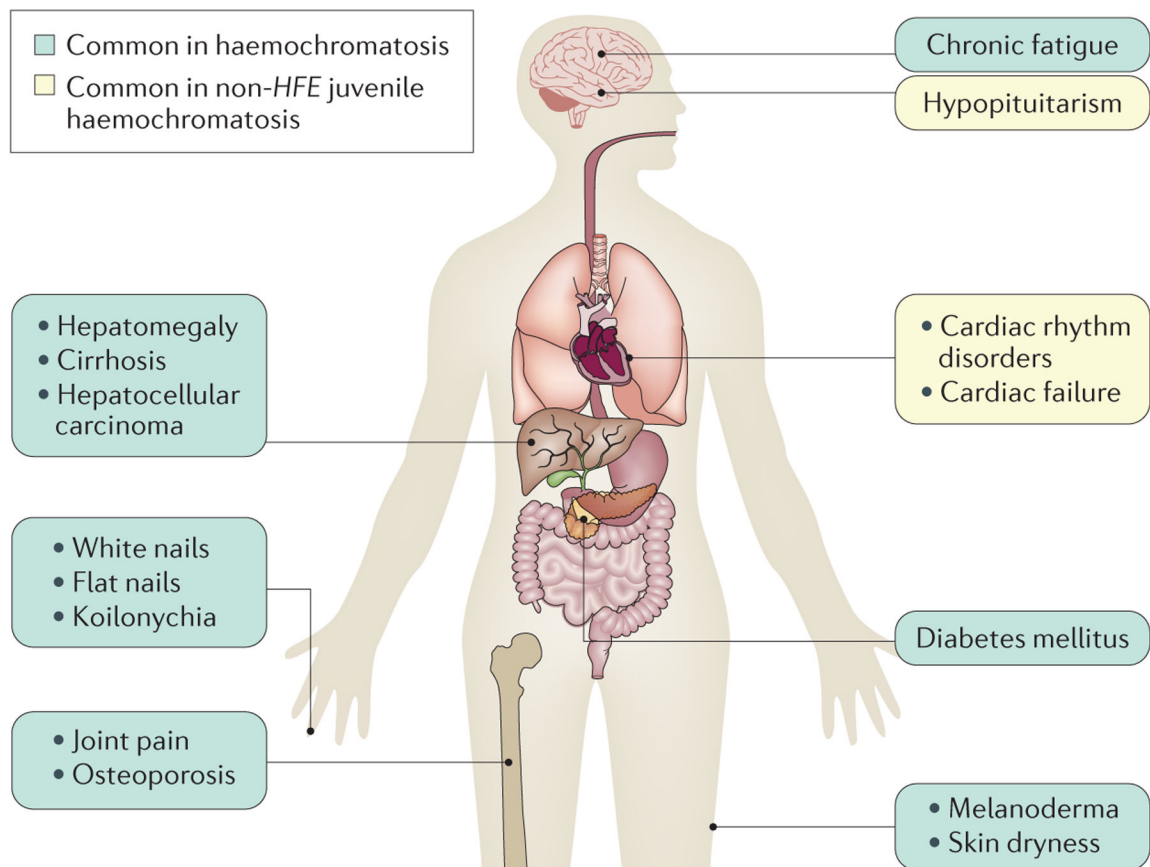


Figure 1 |. Symptoms of haemochromatosis.

When symptoms develop, chronic fatigue is prominent. In addition, joint pain is frequent and is caused by acute or chronic monoarthritis, oligoarthritis or polyarthritis; arthritis in the second and third metacarpophalangeal joints and the ankles is particularly suggestive of haemochromatosis. Spontaneous fractures (particularly of the vertebrae) can occur owing to early-onset osteoporosis²⁰⁹. Dermatological signs are primarily melanoderma (darkening of the skin) but can also include skin dryness and nail changes, such as white nails, flat nails and koilonychia (that is, abnormally thin nails that curve inwards, also called ‘spoon’ nails). The main hepatic symptom is hepatomegaly. In contrast to most other liver diseases (notably due to alcohol use, viral infection or nonalcoholic fatty liver), cirrhotic livers in patients with haemochromatosis are well functioning, such that neither hepatocellular insufficiency or portal hypertension are usually observed in the absence of hepatotoxic cofactors. This finding explains why patients with haemochromatosis rarely develop complications of liver failure but develop hepatocellular carcinoma. In addition, diabetes mellitus and, more rarely, adrenal insufficiency or hypopituitarism can occur. Cardiac symptoms consist of cardiac rhythm disorders and cardiac failure. Anaemic syndrome is not observed in haemochromatosis; the presence of this symptom together with signs of iron excess suggests congenital atransferrinaemia²¹⁰, hereditary aceruloplasminaemia¹²⁵ and divalent cation transporter 1 (DMT1)-related iron overload²⁰⁸. Arthropathies are a frequent complication of haemochromatosis that frequently persists in patients despite otherwise successful iron depletive treatments, suggesting that this manifestation is not directly related to iron

excess²¹¹. Whether persistent high plasma transferrin saturation levels¹⁵⁹ with the presence of labile plasma iron are involved in arthropathies needs further investigation.

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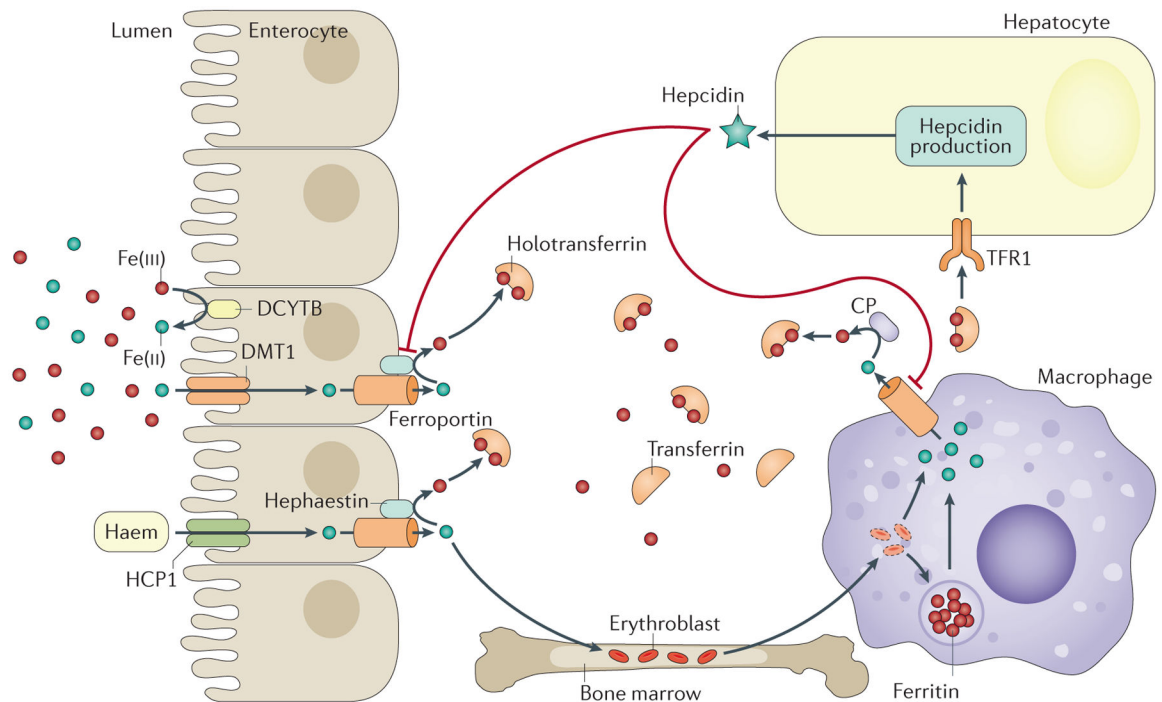


Figure 2 | Iron uptake, cycling and distribution within the body.

The main sources of plasma iron are enterocytes and macrophages. Non-haem iron is absorbed through divalent cation transporter 1 (DMT1), which is found on the apical surface of enterocytes²¹² after conversion of iron from its ferric (Fe(iii)) to its ferrous (Fe(ii)) form by a reduction process that can involve the duodenal cytochrome *b* reductase 1 (CYBDR1; also known as DCYTB) or other enzymes²¹³. The mechanism of haem iron uptake by enterocytes is not fully elucidated, although it likely involves proton-coupled folate transporter (SLC46A1; also known as HCP1). By contrast, macrophages acquire iron from erythrophagocytosis (whereby erythrocytes are degraded and the contained iron is recycled). Iron is released from enterocytes and macrophages into plasma through ferroportin. Iron is then oxidized by ceruloplasmin (CP, which circulates in plasma) and hephaestin (which is anchored to enterocytes) and binds to transferrin (to form holotransferrin)^{203,214}. CP has a role in the control of iron export from reticuloendothelial cells²¹⁵, and both hephaestin and CP might have a role in cell iron metabolism in other tissues^{204,205}. Holotransferrin delivers iron to every cell type, although erythroblasts (immature erythrocytes) are the main consumers. Holotransferrin levels are also sensed by hepatocytes that take up iron through transferrin receptor 1 (TFR1). In response to high levels of holotransferrin, hepcidin — which induces the degradation of ferroportin — is secreted into plasma to control the iron export. Figure adapted from REF. 216, Macmillan Publishers Limited.

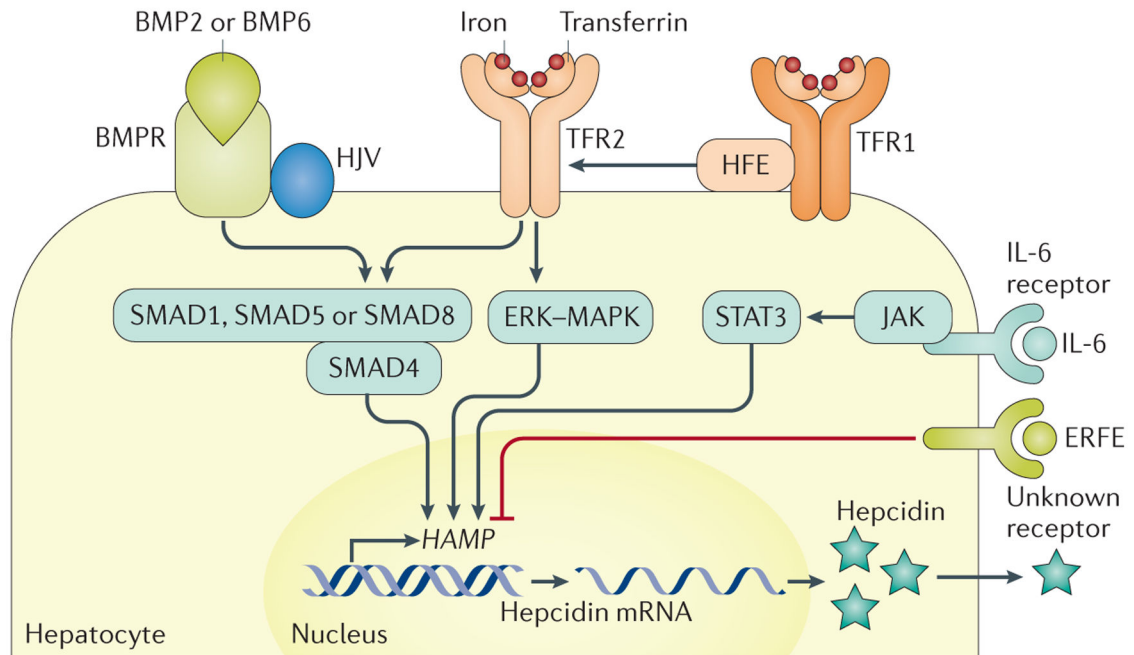


Figure 3 | Hepcidin regulation.

Increased cell iron stores lead to increased bone morphogenetic protein 6 (BMP6) expression in liver cells (sinusoidal cells are probably the major producers^{217,218}, but hepatocytes and stellate cells might also be involved). BMP6 binds to the heterodimeric BMP receptor (BMPR) type 1 and type 2, which are bound to haemojuvelin (HJV)^{219,220}. BMP-BMPR binding leads to BMPR phosphorylation, which leads to the phosphorylation of mothers against decapentaplegic homologue 1 (SMAD1), SMAD5 or SMAD8 (REF. 221). These proteins form a complex with SMAD4, which is transported into the nucleus and interacts with BMP-responsive elements on the *HAMP* promoter, leading to *HAMP* transcription and hepcidin expression^{102,222–224}. Transferrin saturation is also involved in hepcidin regulation. This process could involve a shift of the interactions between hereditary haemochromatosis protein (HFE), transferrin receptor 2 (TFR2) and transferrin receptor 1 (TFR1) with increased transferrin saturation¹⁹⁰, leading to signalling — potentially through mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK)²²⁵ — to increase *HAMP* transcription, although this process is not fully understood. The HFE-TFR1-TFR2 complex could also interact with the BMPR-HJV complex⁶³. Other regulators of hepcidin expression include chronic inflammation, which is mediated by IL-6 produced by inflammatory cells and induces Janus kinase (JAK) and signal transducers and activators of transcription 3 (STAT3) activation^{68–71}, in addition to erythroferrone (ERFE), which is produced by erythroblasts and interacts with unknown partners to decrease *HAMP* transcription. Figure adapted from REF. 216, Macmillan Publishers Limited.

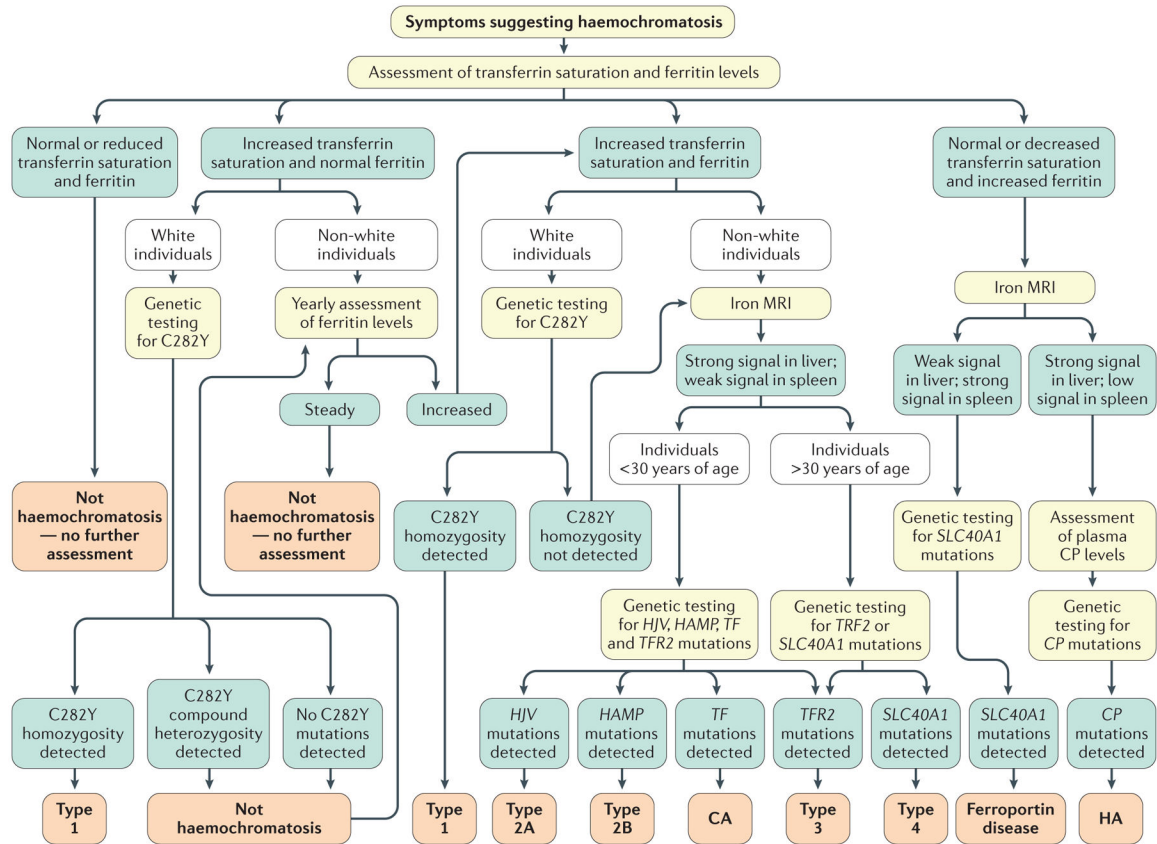


Figure 4 | Diagnostic chart for systemic iron overload of genetic origin.

This algorithm excludes the diagnosis of acquired iron overload owing to blood transfusions, dyserythropoiesis or parenteral iron supplementation. Several factors should be considered as part of the initial diagnostic workup for haemochromatosis, such as patient ethnicity (as *HFE*-associated haemochromatosis is observed almost exclusively in white individuals) and sex (as the phenotypic expression of haemochromatosis is usually less pronounced in women). In addition, patient age should be taken into consideration, as *HFE*-associated (type 1) or *TFR2*-associated (type 3) haemochromatosis is generally observed in individuals >30 years of age, whereas clinical expression in younger individuals is typical of *HJV*-related (type 2A) or *HAMP*-related (type 2B) haemochromatosis. The diagnostic approach should also consider that non-*HFE* haemochromatosis diseases are rare, in contrast to *HFE*-associated haemochromatosis⁶. Type 4 refers to haemochromatosis caused by gain-of-function mutations in *SLC40A1*, previously named haemochromatosis type 4B. CA, congenital atransferrinaemia; CP, ceruloplasmin; HA, hereditary aceruloplasminaemia.

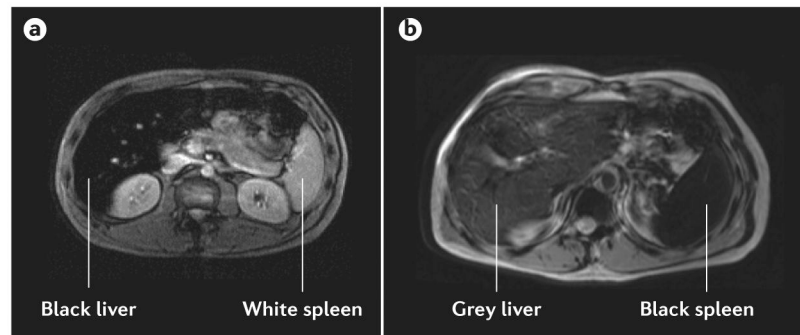


Figure 5 | MRI findings in haemochromatosis owing to hepcidin deficiency and ferroportin disease.

The signal intensity ratio technique with T2-weighted MRI can be used to differentiate patients with haemochromatosis and those with ferroportin disease. **a** | In patients with hepcidin-deficient haemochromatosis, ‘black’ liver corresponds to a highly iron-overloaded liver, and ‘white’ spleen corresponds to the absence of iron overload. The appearance of the liver and spleen is similar in patients with types 1, 2A, 2B, 3 and 4 haemochromatosis. **b** | In ferroportin disease, the spleen appears black (highly iron-overloaded) and the liver appears grey (moderately iron-overloaded) or black (highly iron-overloaded) on T2-weighted MRI⁴⁷.

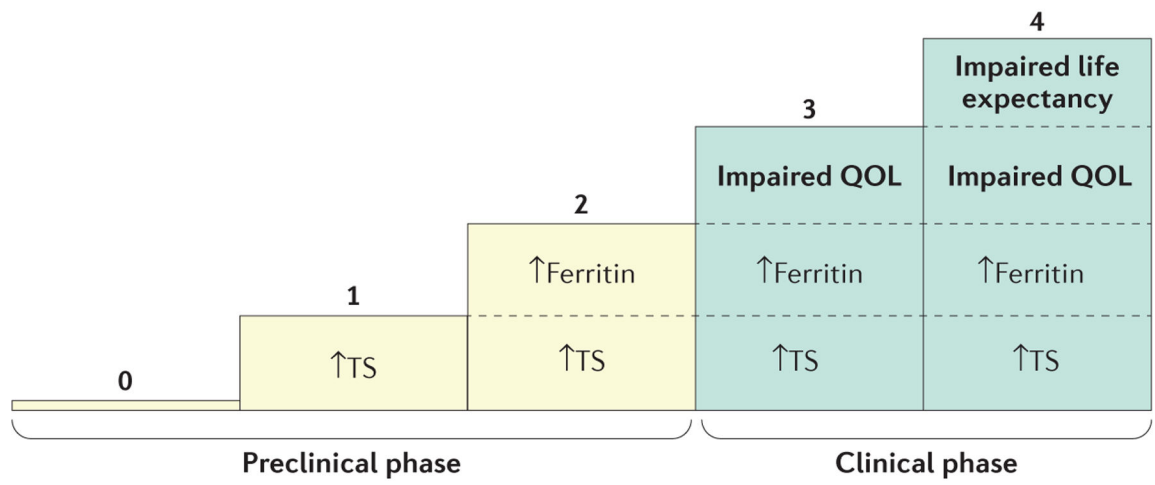


Figure 6 |. Proposed phenotypic classification of haemochromatosis related to hepcidin deficiency.

Increased ferritin: 300 µg per litre for men and 200 µg per litre for women; increased transferrin saturation (TS): >45% (often 60–100%). QOL, quality of life. Adapted with permission from REF. 141, Elsevier.