Haemochromatosis

William JH Griffiths

Abstract

Hereditary haemochromatosis is now well recognized as an inherited disorder of iron loading that is entirely preventable if recognized early. Diagnosis is non-invasive – *HFE* genotyping principally for C282Y homozygosity – and treatment simple. Clinicians in primary and secondary care are more attuned to thinking about haemochromatosis, with much earlier presentations the norm; however, the diagnosis is still missed, and irreversible morbidity from joint disease affects quality of life. There is a greater opportunity now for non-invasive fibrosis testing to exclude those who might be at risk of hepatocellular carcinoma, but overlap with fatty liver disease is common; therefore just treating the iron overload is not always sufficient and lifestyle measures can also need addressing. With hospital services being stretched, ready access to therapeutic venesection can be challenging; clinicians should use the National Blood Service for their patients where, if they are eligible, donation can occur up to 6-weekly. Rarer forms of haemochromatosis can be excluded via next-generation sequencing of a panel of iron-related genes as a single test, readily accessible to clinicians via the National Genomic Testing Directory. Recently published UK guidelines on genetic haemochromatosis and management of raised serum ferritin will aid clinicians going forward.

Keywords Ferroportin; haemochromatosis; hepcidin; *HFE*; iron; liver; MRCP

Key points

- Hereditary haemochromatosis (HH) is common, with 0.5% of white individuals genetically predisposed
- *HFE* genotyping should be performed in the setting of raised ferritin and raised fasting transferrin saturation (>50% in male patients, >40% in females); C282Y homozygosity indicates HH
- If transaminases are raised and/or serum ferritin is >1000 micrograms/litre in HH, referral should be made to a hepatologist for further evaluation as there could be significant fibrosis or an additional disease such as non-alcoholic fatty liver disease
- Patients with HH and raised serum ferritin should undergo de-ironing and be directed to Haemochromatosis UK
- Blood donation should be considered for maintenance phlebotomy and in pre-symptomatic HH
- Magnetic resonance imaging is a useful non-invasive method to confirm iron overload and can, with specific software, be used to quantify iron
- Patients with unexplained hyperferritinaemia and/or iron overload but without typical *HFE* mutations can undergo more detailed genetic testing, which has now been simplified nationally in the UK
- The oral iron chelator deferasirox is an alternative to venesection in intolerant patients
- In 2018 the British Society for Haematology published new guidance documents on raised serum ferritin and genetic haemochromatosis

Definition

Hereditary haemochromatosis (HH; also known as type 1, genetic or HFE-related haemochromatosis) is an autosomal recessive disorder characterized by organ damage resulting from unregulated iron accumulation.

Incidence and presentation

Approximately 1 in 200 white individuals are homozygous for the C282Y mutation and therefore at risk of HH as a result of excess iron absorption from the gut. The gene is thought to be derived from 'Celtic' populations, in whom the frequency is higher. The compound heterozygous form (C282Y/H63D) causes mild iron loading but rarely the typical features of the disease.

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Female patients are largely protected before menopause because of natural iron losses but can still present early.

Typical symptoms include fatigue and joint pains, which affect quality of life and can be disabling for some. The arthralgia in haemochromatosis is specific, typically affecting the second and third metacarpophalangeal and first carpometacarpal joints, base of the thumbs, hips, knees, ankles and metatarsophalangeal joints. Penetrance was previously thought to be low but a recent study suggests quite significant morbidity related to the overall homozygote population. Historical 'bronze diabetes' is quite rare as patients are usually diagnosed earlier; however, it is important to recognize that, in severe cases, iron can be deposited in the pancreatic islet cells, heart, gonadotrophin-producing cells of the anterior pituitary and, rarely, adrenal and parathyroid glands, with consequent organ dysfunction.

It is important to exclude hepatic cirrhosis as it is associated with significantly reduced survival and a 100-fold increased risk of hepatocellular carcinoma (HCC), the most common cause of death in HH.

Variability of expression

As stated above, the principal influence on iron absorption is gender (Figure 1). Environmental factors that increase iron loading and can modify disease expression include excess alcohol, an iron-rich diet and iron supplements — these factors should be sought from the history. Conversely, proton pump use has been shown to reduce iron absorption and venesection requirements so could explain the relatively low iron loading in some patients; a history of previous blood donation could also be relevant here. Factors that affect hepatic fibrosis, independent of iron loading, include obesity and alcohol excess. Iron loading on its own tends not to cause fibrosis until at least grade 3 (unpublished data).

Genetic modifiers are important determinants of disease expression in C282Y homozygotes. Genome-wide association studies in *HFE*-related haemochromatosis have identified the transferrin gene as a significant modifier of iron status, and proprotein convertase subtilisin/kexin type 7 (PCSK7) as a host risk factor for liver cirrhosis. The A736V polymorphism in the iron regulatory transmembrane serine protease 6 (TMPRSS6) gene and the D519G variant of glyceronephosphate *O*-acyltransferase (GNPAT) gene have also been separately implicated in influencing disease expression in C282Y homozygotes.

Pathophysiology

Iron absorption from the small intestine is regulated homeostatically according to body iron status in healthy individuals. Importantly, there is no physiological mechanism to excrete excess iron when overload occurs, hence the gradual build up in HH from adulthood because the gene defect results in an inability to reduce iron absorption appropriately (Figure 2). The initial biochemical finding is a raised plasma transferrin saturation as excess iron loads onto transferrin after entering the circulation via the enterocyte brush border. Serum ferritin subsequently rises in accordance with tissue iron loading. Total body iron, 4 g in a normal adult, typically rises to >10 g in a patient with haemochromatosis.

Study of iron-regulatory genes in addition to *HFE* has advanced our understanding of the molecular control of iron homeostasis. Shortly after cloning of the *HFE* gene, the C282Y mutation was noted to abrogate the binding of β_2-microglobulin to the homeostatic iron regulator (HFE) protein, thereby preventing cell surface expression and interaction with transferrin receptors. Although this provided a ready explanation for cellular iron entry modulation, it was not clear how HFE influenced the 'crypt programming' of iron absorption in the small intestine. Evidence now points to a central hepatic role for HFE along with a number of other mediators that influence iron loading.

Hepcidin, an antimicrobial peptide synthesized in hepatocytes, is a key negative regulator of iron absorption. In iron-deficient states, hepcidin synthesis is reduced in order to stimulate gastrointestinal uptake; conversely, hepcidin synthesis is increased in secondary iron overload. The receptor for hepcidin is the protein ferroportin, which, when bound, is inhibited from releasing iron into the circulation from reticuloendothelial cells and enterocytes. In HH, however, hepcidin synthesis is reduced by a direct effect of mutant HFE at the hepatocyte membrane and, consequently, iron release into the circulation because of unregulated ferroportin activity.

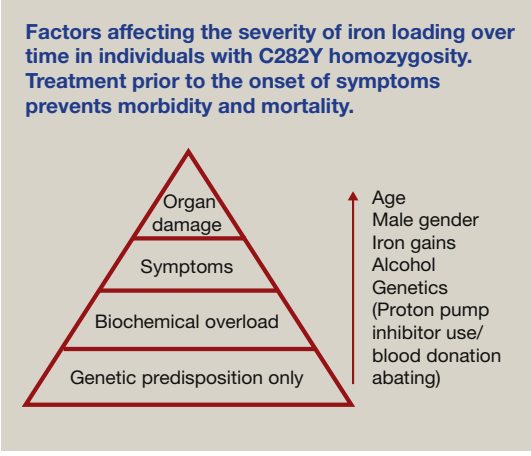


Figure 1

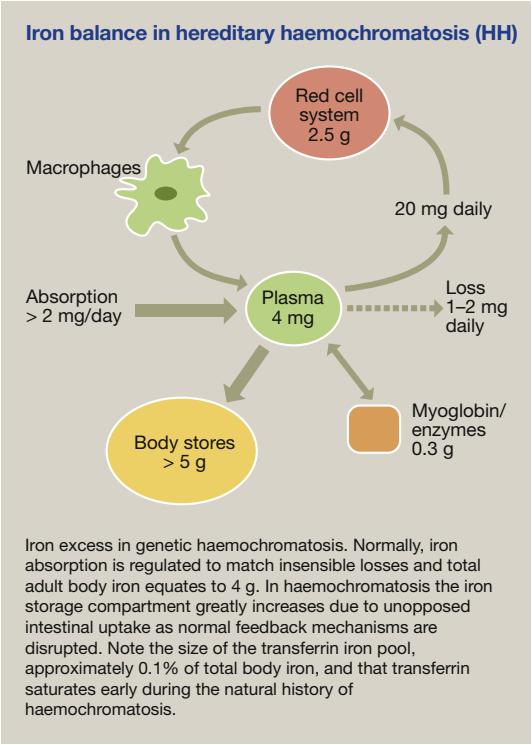


Figure 2

Diagnosis and work-up

The *HFE* gene on chromosome 6p was discovered in 1996 and completely altered the diagnostic pathway for HH, providing a specific tool for non-invasive diagnosis, screening and estimation of prevalence.[1] Diagnosis of HH rests principally on identification of C282Y homozygosity, which accounts for 90% of cases, in conjunction with demonstration of iron loading, typically from biochemical indices with or without compatible symptoms. The combination of elevated serum ferritin and raised fasting transferrin saturation (>50% in male individuals, 40% in females) is highly suggestive of the condition. Simple genotyping of the *HFE*

gene is straightforward and accessible from primary care. The compound heterozygous form (C282Y/H63D) accounts for approximately 4% of cases, and the iron burden is usually mild.

Hyperferritinaemia with normal transferrin saturation often occurs in the setting of excess alcohol and/or non-alcoholic fatty liver disease (NAFLD).[2] Fatty liver disease resulting from alcohol or NAFLD can coexist with HH and overestimate serum ferritin level; this is particularly the case in compound heterozygotes. It is therefore important to undertake a liver-related history and examination as well as baseline hepatic ultrasonography in HH with raised transaminases or these risk factors. Hepatic iron deposition can be demonstrated using T2-weighted magnetic resonance imaging (MRI) sequences and quantified with reasonable accuracy using specific software. The latter can be informative when *HFE* analysis is negative and there is concern regarding significant iron overload.

Liver biopsy is reserved for patients who do not have a recognizable genotype or who have a risk of significant liver fibrosis. It is important to identify the latter as 6-monthly surveillance for HCC using α-fetoprotein and ultrasonography is indicated in those with incipient or established cirrhosis. In C282Y homozygotes without additional liver disease risk factors, serum ferritin concentrations >1000 micrograms/litre are associated with the development of liver fibrosis. Iron depletion does not necessarily reduce the risk of HCC in cirrhosis. In homozygotes with normal serum aminotransferase values, no hepatomegaly and serum ferritin <1000 micrograms/litre, the risk of significant fibrosis is negligible; these patients do not require further liver assessment.

Transient elastography is now relatively commonplace within secondary care hepatology units and accurately stages 60% of homozygotes with ferritin >1000 micrograms/litre and/or raised transaminases. This method reduces the requirement for biopsy in at-risk patients to match those with intermediate values (6.4 −13.9 kPa).[3]

Joint assessment is important and may require referral to a rheumatologist and/or orthopaedic surgeon. Cardiac assessment is only required if iron overload is very severe or there are clinical findings to suggest involvement; in this instance an echocardiogram should be performed with or without MRI with T2-weighted sequencing to assess for excess iron. Any suggestion of low libido in men should be investigated via testosterone/luteinizing hormone/follicle-stimulating hormone measurement. Diabetes mellitus is more likely to relate to NAFLD/metabolic syndrome, i.e. insulin resistance, but needs to be excluded in severe cases where iron deposition in the pancreas can be evident on MRI.

Family screening is important for case-finding, and first-degree relatives of homozygotes should be tested for HH. Practically speaking, iron indices and *HFE* gene testing should be requested. Where children are concerned, the other parent can be checked for carrier status to determine whether or not the offspring are at risk (C282Y heterozygous state approximately 1:10 in white individuals).

Treatment

Weekly removal of a unit of blood is an effective method for clearing excess iron, and all HH patients with serum ferritin concentrations above normal should be considered for this. The terms 'venesection' and 'phlebotomy' are used interchangeably to describe this process. This method works by stimulating the erythron to use iron for further red cell production and in doing so physiologically remove it from the tissues, particularly the liver. Each time a unit of blood is removed, the serum ferritin falls by approximately 50 micrograms/litre, although ferritin levels can initially behave erratically.

Some patients only tolerate loss of half a unit but the aim is as much as can be removed in one sitting. Patients are advised to eat and drink plenty prior to each visit and should have a booklet to record their venesection activity. It is important not to remove the blood too quickly. A model venesection service has protocols in place to ensure safety and ready access to the phlebotomists and the overseeing clinician.

Dietary restriction of red meat, liver and iron-enhanced food is advised; a number of other theoretical dietary alterations can be made with information from the Haemochromatosis UK (www.haemochromatosis.org.uk). Other typical treatments are lifestyle measures and testosterone replacement in male patients. Joint symptoms can prove difficult to treat, tend not to improve with de-ironing and indeed worsen over time. No drugs have yet been shown to modify the arthropathy in haemochromatosis, although trials are being considered.

Recent UK guidance suggests that venesection should continue until the serum ferritin falls to 20−30 micrograms/litre and transferrin saturation to <50%.[4] After completing the therapeutic phase, patients should be on a regular maintenance schedule, which is preferable to the 'watch-and-wait' approach sometimes historically practised. During maintenance, the serum ferritin and transferrin saturation should ideally remain <50 micrograms/litre and <50%, respectively. Of note, however, there is some debate regarding the need to maintain a low transferrin saturation, although a recent French study suggested that this might reduce general and joint-related symptoms.[5] Blood obtained via hospital services is discarded as not safe for donation, but the National Blood Service readily accept patients with haemochromatosis who are otherwise eligible. Patients can donate up to 6-weekly if required but their iron indices should be monitored by their clinician at least annually.

Treatment before the onset of cirrhosis or diabetes mellitus had previously been shown to ensure normal survival. However, a more recent study suggested that homozygotes with a serum ferritin >1000 micrograms/litre at diagnosis have a 5-fold relative risk of death even with treatment, which reinforces the need, where possible, to intervene before patients reach this threshold. Interestingly, longitudinal studies have shown that rates of iron accumulation are variable and progressive iron loading does not always occur, particularly in female patients. Pre-symptomatic individuals with modest ferritin elevation could be considered for blood donation from the outset, with monitoring, and in older patients monitoring alone may suffice where the clinical benefit of de-ironing may be small. If in doubt, therapeutic venesection is advised along with lifestyle measures for any coexisting fatty liver process. Of note, venesection has been shown to cause regression of liver fibrosis.

An alternative for patients who are intolerant of venesection is the oral iron chelator deferasirox. This has been used in Phase II trials in haemochromatosis, and a dose of 10 mg/kg reduces

serum ferritin more slowly than weekly venesection but nonetheless effectively. The drug requires monitoring and is not licensed for this use. HH is a relatively uncommon indication for liver transplantation, usually in the context of HCC, and according to recent data outcomes are no worse than for comparable forms of chronic liver disease.

HFE and liver disease

HFE mutations have historically been associated with fibrosis in hepatitis C, and phlebotomy is a useful adjunct, but this is no longer relevant given the effectiveness of current antiviral regimens. *HFE* mutations have not been associated with the siderosis observed in alcohol-related liver disease; excess of iron here may be mediated via a direct suppression of circulating hepcidin. In NAFLD, an association between hepatic iron and *HFE* mutations has been observed, although effects on fibrogenesis have not been confirmed. Phlebotomy has been shown in separate studies to improve insulin sensitivity and histology in patients with a combination of NAFLD and hyperferritinaemia, although a recent Australian study has cast doubt on these findings. Venesection might play a role in established chronic liver disease resulting from to alcohol or NAFLD where there is significant non-HFE related siderosis: studies have shown a correlation between iron grade and outcome in terms of fibrosis, HCC and overall survival.

Finally, a link between *HFE* mutations and iron is seen in the context of porphyria cutanea tarda, where iron overload is associated with a high prevalence of *HFE* mutations and where de-ironing typically rectifies sun-sensitive skin lesions.

Uncommon inherited iron overload syndromes

A number of uncommon pathogenic variants in *HFE* have since been reported such that full sequencing of the gene could reveal, for example, a second rare allele not picked up via initial genotyping. Importantly, after the initial discovery of *HFE*, the ensuing decade saw the characterization of several novel haemochromatosis syndromes (Table 1).

One such condition is juvenile haemochromatosis (JH), which is associated with very severe iron overload; it typically presents before the age of 30 years and affects both sexes equally. Inheritance is recessive, and hypogonadism and cardiomyopathy are usually evident. Heart failure can be life-threatening but is often responsive to aggressive iron chelation therapy. Mutations in the *HJV* gene on chromosome 1 account for most JH (type 2A), with homozygosity for G320V accounting for half of cases. JH is also associated with mutations in the *HAMP* gene on chromosome 19, which encodes hepcidin (type 2B). A less severe form of haemochromatosis is seen with homozygosity for transferrin receptor 2 *(TfR2)* mutations (type 3). JH and TfR2 haemochromatosis resemble HFE-related haemochromatosis in terms of specific organ susceptibility to iron loading, but involve more faster iron loading from the gut and are more severe.

'Ferroportin disease' deserves a specific mention and is caused by heterozygous mutations in the *SLC40A1* gene. Ferroportin protein controls iron release from a number of cell types, including hepatocytes, but its principal action in terms of iron homeostasis is on the surface of enterocytes and macrophages, where it acts in conjunction with circulating hepcidin. Mutations in *SLC40A1* are not restricted to white individuals and are associated with autosomal dominantly inherited type 4 haemochromatosis.

The classical form of this disorder is characterized by a raised serum ferritin and normal or low transferrin saturation, with a tendency towards anaemia after venesection. Iron loading occurs predominantly within the reticulo-endothelial system, and splenic uptake can be visible on MRI (Figure 3). The distribution of iron in the liver is different from that in HFE-related haemochromatosis, with Kupffer cell iron deposition occurring early. The clinical significance of iron loading and the role of venesection in ferroportin disease have not been defined. The differential diagnosis includes hereditary hyperferritinaemia with or without cataracts; patients with this diagnosis have mutations in

Haemochromatosis syndromes

Disorder	Type	Gene	OMIM	Protein
HH	1	*HFE*	235200	HFE
JH	2A	*HJV*	608374	Hemojuvelin
	2B	*HAMP*	606464	Hepcidin
TfR2	3	*TFR2*	604250	TfR2
Ferroportin	4	*SLC40A1*	606069	Ferroportin
H-ferritin	5	*FTH1*	615517	H-ferritin

OMIM, Online Mendelian Inheritance in Man.

Table 1

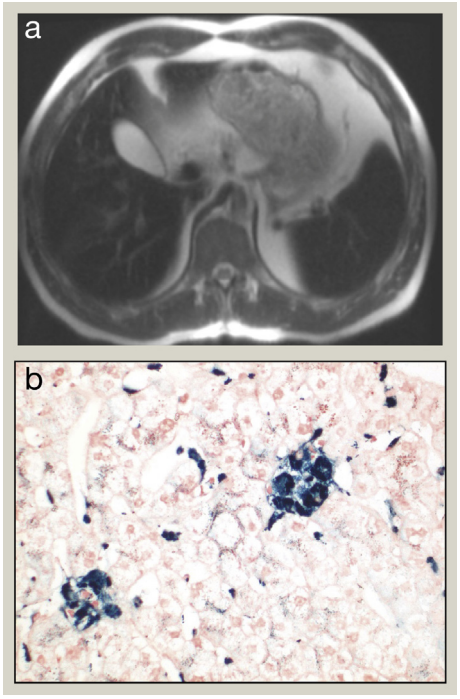
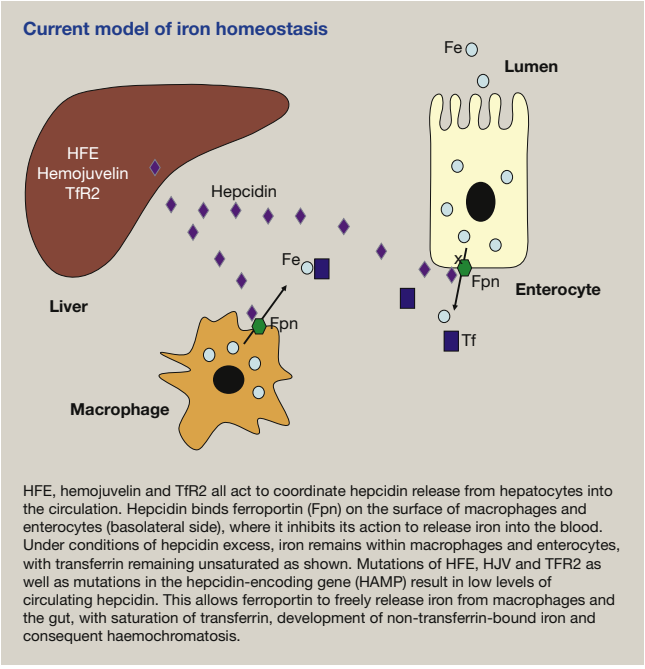


Figure 3 MRI and histology in a patient with classical ferroportin disease. On T2-weighted imaging, the spleen and liver can show reduced signal in ferroportin iron overload, indicating iron in both organs as a result of reticulo-endothelial cell loading (**a**). This is also illustrated using the Perls' stain on liver biopsy sections, with iron seen predominantly within Kupffer cells (**b**, ×40 magnification).



HFE, hemojuvelin and TfR2 all act to coordinate hepcidin release from hepatocytes into the circulation. Hepcidin binds ferroportin (Fpn) on the surface of macrophages and enterocytes (basolateral side), where it inhibits its action to release iron into the blood. Under conditions of hepcidin excess, iron remains within macrophages and enterocytes, with transferrin remaining unsaturated as shown. Mutations of HFE, HJV and TFR2 as well as mutations in the hepcidin-encoding gene (HAMP) result in low levels of circulating hepcidin. This allows ferroportin to freely release iron from macrophages and the gut, with saturation of transferrin, development of non-transferrin-bound iron and consequent haemochromatosis.

Figure 4

the light chain ferritin (*FTL*) gene promoter or exon 1, respectively. Venesection is not required but the diagnosis can be overlooked if a history of cataracts is not obtained and/or specific genetic testing not pursued.

Identification of non-HFE haemochromatosis syndromes have contributed to our understanding of iron homeostasis. The hemojuvelin and TfR2 proteins act in a similar way to HFE, stimulating hepcidin production within the liver with consequent downstream actions via ferroportin (Figure 4). The gene products sit on the hepatocyte membrane and coordinate hepcidin synthesis in response to iron; mutations disrupt molecular pathways that maintain a suppressive effect of hepcidin on iron absorption, and consequently iron loading ensues. These pathways are being targeted for potential novel therapies that can modify iron homeostasis. ◆

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TEST YOURSELF

To test your knowledge with the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 35-year-old woman, with a family history of the disease, was screened for haemochromatosis. She was asymptomatic.

Investigations

- Serum ferritin 130 micrograms/litre (15–300)
- Transferrin saturation 98%
- HFE genotyping confirmed C282Y homozygosity

What is the ideal management approach here?

A Observe and monitor
B Venesection
C Advise blood donation with monitoring
D Low-iron diet
E Liver ultrasound

Question 2

A 55-year-old man presented with a recent diagnosis of hereditary haemochromatosis (HH) with a serum ferritin of 730 micrograms/litre (15–300), normal alanine aminotransferase and a degree of fatigue. He had previously been an intravenous drug user but negative for viruses. He reported that venesection had previously failed because of the state of his veins.

Which of the following management actions would be most appropriate?

A Observe and monitor
B Refer back for venesection
C Refer for blood donation instead
D Consider deferasirox
E Discharge back to primary care

Question 3

A 45-year-old man was being investigated for haemochromatosis.

On clinical examination, there was central obesity, with a body mass index 35 kg/m^2.

Investigations

- Serum ferritin 560 microgram/litre (15–300)
- Transferrin saturation 66%
- Alanine aminotransferase 87 U/litre (5–35)
- HFE genotyping showed C282Y/H63D

What is the most appropriate next step?

A Venesection
B Liver ultrasound
C Liver biopsy
D Weight loss
E Fibroscan