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TOPIC HIGHLIGHT

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HFE gene in primary and secondary hepatic iron overload

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Abstract

Distinct from hereditary haemochromatosis, hepatic iron overload is a common finding in several chronic liver diseases. Many studies have investigated the prevalence, distribution and possible contributory role of excess hepatic iron in non-haemochromatotic chronic liver diseases. Indeed, some authors have proposed iron removal in liver diseases other than hereditary haemochromatosis. However, the pathogenesis of secondary iron overload remains unclear. The High Fe (*HFE*) gene has been implicated, but the reported data are controversial. In this article, we summarise current concepts regarding the cellular role of the HFE protein in iron homeostasis. We review the current status of the literature regarding the prevalence, hepatic distribution and possible therapeutic implications of iron overload in chronic hepatitis C, hepatitis B, alcoholic and non-alcoholic fatty liver diseases and porphyria cutanea tarda. We discuss the evidence regarding the role of *HFE* gene mutations in these liver diseases. Finally, we summarize the common and specific features of iron overload in liver diseases other than haemochromatosis.

Key words: Hereditary haemochromatosis; Chronic liver diseases; Chronic hepatitis C; Hepatic iron overload; *HFE* gene

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INTRODUCTION

Iron is an essential element for living cells because it is a cofactor for enzymes of the mitochondrial respiratory chain and it co-ordinates the binding of oxygen by myoglobin and haemoglobin. However, excess iron is toxic, causing increased oxidative stress; the production of reactive oxygen species is thought to be responsible for the observed oxidation of lipids, proteins and nucleic acids. Thus, iron overload can cause serious damage to organs including the liver, heart, joints and endocrine glands.

Hereditary haemochromatosis (HH) is the paradigm of heavy iron overload which can eventually lead to multiple organ failure. HH is an inherited disease of iron metabolism[1]. Absorption of dietary iron is inappropriately high in relation to body iron stores. This leads to increased deposition of iron, predominantly in parenchymal cells of the liver, heart, joints, pancreas and other endocrine organs. Undiagnosed and untreated, iron deposition can cause hepatocellular injury, activation of hepatic stellate cells (HSCs) and increased production of collagen and other components of the extracellular matrix[2]. The liver is the major site for storage of excess iron, which probably explains the increased risk of hepatocellular carcinoma (HCC) in HH[3].

In non-haemochromatotic liver diseases, hepatic iron may worsen liver injury or hepatic fibrosis. This has been investigated in common chronic liver diseases (CLDs) such as chronic hepatitis C (CHC) and alcoholic and non-alcoholic fatty liver diseases. In these CLDs, the progression towards end-stage liver disease is often unpredictable; many cofactors have been proposed to explain this variability. Iron overload has been proposed as such a cofactor, but its exact role remains unclear[4]. Iron has also been implicated in the progression of hepatitis B virus (HBV) infection[5], but this has not been widely studied. Other conditions that may cause CLD and that have been associated with iron overload include porphyria cutanea tarda (PCT) and insulin resistance-associated hepatic iron overload (IR-HIO) syndrome[6,7]. The IR-HIO syndrome encompasses iron overload with hyperferritinaemia and normal transferrin saturation, type 2 diabetes mellitus and non-alcoholic steatohepatitis (NASH). In these forms of secondary (acquired) iron overload, however, the hepatic iron concentration is generally lower than that seen in HH. Although major advances have been made in understanding the pathogenesis of primary iron overload in HH, the mechanism(s) whereby pre-existing CLD may lead to iron overload remain unclear. In HH, there is a strong relationship with missense mutations in the *HFE* (High Fe) gene[8]. In non-haemochromatotic diseases, several studies have investigated the possible role of *HFE* mutations in the pathogenesis of hepatic

iron overload, with somewhat discordant results. This review aims to describe the prevalence and role of *HFE* mutations in secondary iron overload. It will discuss the effects of moderate iron excess on the natural history and its possible relevance to therapeutic approaches in CLDs other than HH.

NOMENCLATURE OF IRON OVERLOAD

Hereditary, or primary, haemochromatosis is an inherited iron storage disease. Secondary iron overload is acquired as a result of another disease. The nomenclature and classification of iron overload states is shown in Table 1. Primary haemochromatosis may be due to mutations in the *HFE* gene, or to mutations in genes other than *HFE*. The main causes of acquired iron overload are haemolytic anaemias and CLDs. Untreated HH may develop severe iron overload, whereas in secondary iron overload due to CLDs, minimal to modest iron overload is usually seen[2].

Table 1 Nomenclature for iron overload states

Primary iron overload-hereditary haemochromatosis (HH)
HFE-associated HH
1 C282Y homozygosity
2 C282Y/H63D compound heterozygosity
3 Other mutations
Non *HFE*-associated HH
1 Juvenile haemochromatosis
2 TfR2-related haemochromatosis
3 Autosomal dominant haemochromatosis
Secondary iron overload-acquired
Iron-loading anaemias
1 Thalassaemia major
2 Sideroblastic anaemia
3 Chronic haemolytic anaemias
Chronic liver diseases
1 Hepatitis C
2 Alcoholic liver disease
3 Non-alcoholic steatohepatitis
4 Porphyria cutanea tarda
5 IR-HIO
6 Post-portacaval shunting
7 Transfusional and parenteral iron overload
8 Dietary iron overload
Miscellaneous
1 Iron overload in sub-Saharan Africa
2 Neonatal iron overload
3 Acaeruloplasminaemia
4 Congenital atransferritinaemia

GENETICS OF PRIMARY IRON OVERLOAD

There are two common mutations of the *HFE* gene. The first results in a change of cysteine at position 282 to tyrosine (C282Y); the second results in a change of histidine at position 63 to aspartate (H63D). Numerous studies have shown that homozygosity for C282Y is associated with typical phenotypic HH in Caucasians. C282Y homozygosity ranged from 64% of Italian haemochromatosis cases up to 100% of cases in Australia[9-11]. A recent study of almost 100 000 North American primary care patients analysed the distribution of *HFE* mutations in a racially diverse group[12]. It confirmed that homozygosity for C282Y is most common in whites, consistent with the hypothesis that this mutation originated in a Caucasian "founder" individual. Individuals who are compound heterozygous for C282Y and H63D may also have iron overload in the range diagnostic for haemochromatosis, although the penetrance of this genotype is lower than for C282Y homozygosity[13-15]. The H63D mutation is variably distributed worldwide. It is more prevalent than the C282Y mutation: approximately one in five of the European population are estimated to be H63D heterozygotes[9,16]. The C282Y/H63D compound heterozygous and H63D homozygous genotypes have mostly been associated with only biochemical evidence of mild iron overload. The clinical penetrance of these genotypes is low although there have been reports of varied phenotypic presentation[15,17].

A third point mutation (S65C) of the *HFE* gene was also identified. It was originally considered to be a neutral polymorphism, not associated with increased transferrin saturation[18]. However, other evidence implicated the mutation in mild iron overload[19,20]. Other, rare mutations have been described. Two missense mutations in exon 2 of the *HFE* gene (I105T and G93R) were detected in haemochromatosis patients with atypical *HFE* genotypes, such as heterozygosity for C282Y, H63D or S65C[21]. A splice-site *HFE* mutation (IVS3+1G/T) that prevented normal mRNA splicing was identified in a patient with classical HH who was heterozygous for the C282Y mutation. This highlighted the possibility that other rare *HFE* mutations could explain the classical HH phenotype, particularly in C282Y heterozygotes with iron overload[22]. In this review, we will consider only the two most frequent *HFE* mutations, C282Y and H63D. The other mutations are rare, thus they are unlikely to play a major role in CLDs other than HH.

HH has been described as the most common monogenic disorder in Celtic populations. Certainly, homozygosity for the C282Y mutation occurs in about 1 in 300 people of Northern European origin, with an estimated carrier (heterozygote) frequency of 1 in 10[8]. However, the C282Y homozygous genotype has incomplete penetrance: not all patients with this genotype show an iron overload-related phenotype. The penetrance of HH is influenced by a variety of factors. Increasing age increases the penetrance, as the body has no means of active iron excretion, so iron accumulation progresses with time. Male gender also increases the penetrance, as women may be partially protected against iron overload by iron losses incurred in childbirth and menstruation. Dietary iron content may also influence penetrance. There has been considerable debate regarding the penetrance of HH. The "biochemical penetrance" of the C282Y homozygous state is generally agreed to be high, particularly in older males, but disease-related morbidity is less frequent. A study of almost a third (3011) of the residents of Busselton, Australia showed that four (all males) of the 16 C282Y homozygotes detected had fibrosis and/or cirrhosis. If attributable to iron overload, this equates to a disease-related morbidity of 25%[23]. In contrast, recent large studies have reported lower penetrance. Beutler and colleagues studied over

40 000 individuals attending a health appraisal clinic, where expression of haemochromatosis was investigated by questionnaire. The results for C282Y homozygotes were compared with the control group who were wild type for C282Y and H63D. The clinical penetrance of ill health or shortened lifespan in C282Y homozygotes was reported to be less than 1%[24]. Various factors, such as the exclusion of most patients who had a previous diagnosis of haemochromatosis from the assessment of ill health, would have tended to decrease this calculated clinical penetrance of C282Y homozygosity[25]. Despite this, a similar, low value was obtained in a survey of about 1 000 000 individuals in two health authority regions in South Wales. It was concluded that a diagnosis of haemochromatosis had been made for only 1.2% of adult C282Y homozygotes. When only male homozygotes older than 45 years were considered, the diagnosis rate rose to 2.8%[26]. Thus, the "severity" of the definition of penetrance (disease related morbidity or clinical expression as ill health/shortened life span) is an important factor influencing the observed value. It seems that the clinical penetrance of C282Y homozygosity is low.

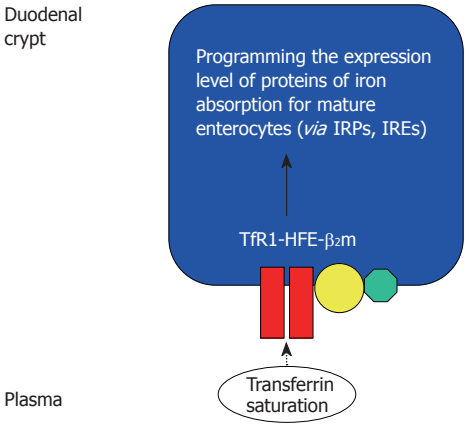

Figure 1 The duodenal "crypt cell hypothesis" of HFE function[36]. HFE at the basolateral membrane of duodenal crypt cells co-localizes with β2-microglobulin and TfR1. The saturation of circulating transferrin, which reflects body iron stores, was proposed to be "sensed" by the TfR1-HFE-β2-microglobulin complex, through transferrin-mediated endocytosis. Wild type HFE was proposed to facilitate transferrin-mediated iron uptake. In haemochromatosis, deficiency of functional HFE would therefore decrease the iron pool within the crypt cell, despite increased body iron stores. This would increase the activity of iron responsive proteins (IRPs), leading to increased expression of genes involved in iron absorption, such as DMT1 and ferroportin. This could contribute to the iron overload seen in HH[27,34,35]. However, recent evidence suggests that HFE may play more important roles in influencing iron metabolism in the liver.

HFE PROTEIN AND CELLULAR IRON HOMEOSTASIS

The predicted HFE protein has sequence homology to the major histocompatibility complex (MHC) class I molecules, a family of transmembrane glycoproteins of the immune system, which present peptide antigen to T cells[8]. Like the class I proteins, HFE is made up of three extracellular loops (α_1, α_2, α_3)[27], a transmembrane domain and a short cytoplasmic C-terminal region. Two disulphide bridges stabilize the tertiary structure of the HFE protein. One of these gives rise to the α_3 loop, that is crucial for interaction with β2-microglobulin and for translocation to the cell surface[28,29]. The C282Y mutation prevents formation of this disulphide bond, thereby inhibiting correct cell surface expression of HFE[8]. The H63D mutation causes a histidine to be replaced by aspartate in the α1 domain[8]. H63D is generally considered to have a milder effect on body iron stores than C282Y.

The role of HFE in cellular iron homeostasis remains only partially understood. Co-immunoprecipitation experiments showed that the HFE-β2-microglobulin complex interacted with transferrin receptor 1 (TfR1) in human placental membrane preparations[30] and in human embryonic kidney cells over-expressing wild type HFE[31]. Immunohistochemical studies of human duodenum showed that TfR1 staining overlapped that of HFE in the crypt cell enterocytes, consistent with the co-trafficking of these proteins[32] (Figure 1). These studies provided a link between HFE and the transferrin-mediated endocytosis pathway of iron uptake into cells. Under conditions of iron excess, circulating iron-saturated transferrin is understood to bind the TfR1-HFE-β2-microglobulin complex on the cell surface, which is internalised within an endosome. At the acidic endosome pH, iron dissociates from transferrin and enters an intracellular chelatable iron pool. The transferrin-TfR1 complex recycles to the cell surface, where transferrin dissociates at the pH of blood, around pH 7.4[27,33].

However, investigations of the HFE-TfR1 interaction and its potential effect on iron metabolism in transfected cell lines gave conflicting results. Several studies indicated that overexpression of HFE may ultimately reduce iron uptake into cells *via* transferrin-mediated endocytosis[34]. Biosensor- and radioactivity-based assays indicated that HFE competes with transferrin for binding to TfR1[35]. Conversely, in stably transfected Chinese hamster ovary cells, HFE increased the rate of TfR1-mediated iron uptake and cellular iron concentrations, but only when co-transfected with β2-microglobulin[36]. Also, in primary macrophages from haemochromatosis patients, transfection of the wild type *HFE* gene increased uptake of ^{55}Fe-transferrin and accumulation of ^{55}Fe in the ferritin iron pool[37]. These latter studies are consistent with the observation that in haemochromatosis, duodenal enterocytes are paradoxically iron-deficient[38].

Several hypotheses have been advanced to model the possible roles of HFE in the regulation of iron metabolism. It was originally proposed that the TfR1-HFE-β2-microglobulin complex on the basolateral surface of the duodenal crypt cell may sense the transferrin saturation (Figure 1). Where wild type HFE was present, the transferrin saturation was proposed to determine the crypt intracellular iron concentration, thereby setting the activity of iron responsive proteins 1 and 2 and ultimately the expression levels of key proteins of iron absorption in the mature enterocyte[36]. The pathway of iron absorption in the mature enterocyte is shown in Figure 2. Duodenal cytochrome b ferric reductase (Dcytb) is expressed on the luminal surface of the enterocyte and reduces dietary ionic iron from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state.

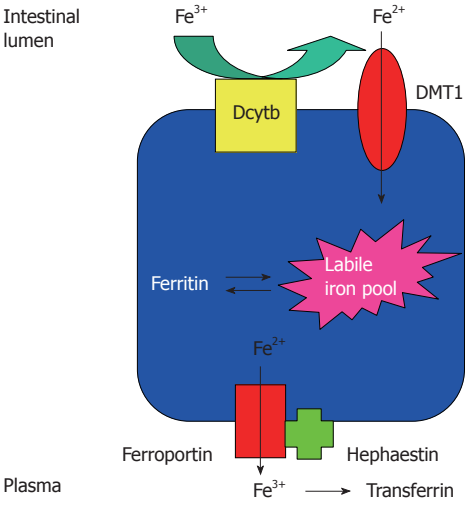

Figure 2 The pathway of iron absorption in the mature duodenal enterocyte. Dietary ferric iron is reduced to the ferrous state by ferric reductase(s), for example duodenal cytochrome b (Dcytb), which is expressed on the luminal surface of the enterocytes. Ferrous iron is taken up *via* DMT1 into the labile iron pool. Iron may be stored within the cell as ferritin or transferred across the basolateral membrane to the plasma by ferroportin. The exported iron is oxidised to the ferric state by hephaestin; ferric iron is then avidly bound by circulating transferrin[39].

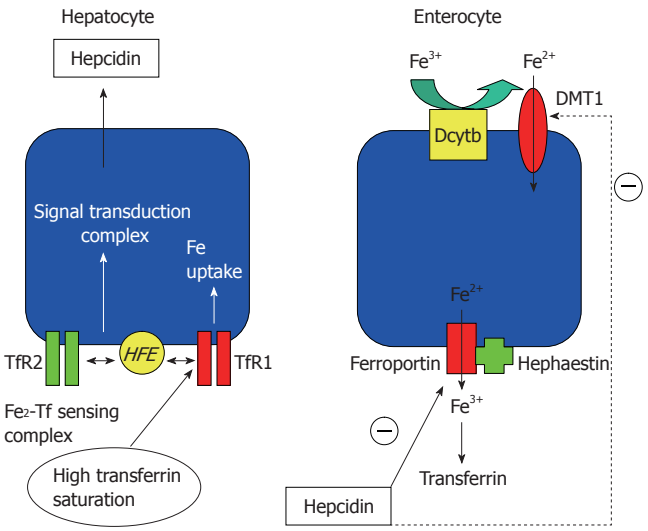

Figure 3 Current concepts regarding hepatic regulation of iron metabolism. Left panel, hepatocyte. At normal transferrin saturation, TfR1 may sequester HFE[191]. At increased transferrin saturation, diferric transferrin competes with HFE for binding to TfR1[55]. Freed HFE is proposed to bind TfR2; the complex conveys transferrin saturation status via a cytoplasmic signal transduction complex, leading to synthesis and secretion of hepcidin. In HH, mutations in the genes encoding HFE, TfR2, haemojuvelin or hepcidin may all disrupt this sensing system, leading to deficient hepcidin production and iron overload[53]. Right panel, enterocyte. Circulating hepcidin may reduce iron absorption by interacting with ferroportin, causing its internalization and degradation[49] and/or by reducing DMT1 expression[50].

The ferrous iron is then taken up by the apical transporter, divalent metal transporter 1 (DMT1). Iron may be stored within the cell as ferritin or exported across the basolateral membrane by ferroportin. The exported iron is re-oxidized to the ferric state by hephaestin. Upon exiting the villus enterocyte, ferric iron can be avidly bound by circulating transferrin[39].

The discovery of the peptide hormone hepcidin[40-42] revived an alternative hypothesis, that the liver may be central to the regulation of iron metabolism[43-46]. Hepatocytes act as a storage reservoir for iron, taking up dietary iron from the portal circulation and, in iron deficiency, releasing iron into the hepatic circulation. The liver is also the site of high expression of hepcidin, *TfR2* and haemojuvelin, three genes which when mutated result in HH.

Hepcidin is produced in the liver in response to dietary iron loading[42]. Mutation of the hepcidin gene results in juvenile haemochromatosis, characterised by early onset and a rapid rate of iron loading[47]. As in hepcidin-related haemochromatosis, inappropriately low hepcidin levels in relation to body iron stores are also seen in haemochromatosis resulting from mutation of the *HFE*, *TfR2* and haemojuvelin genes, suggesting that hepcidin may be the common pathogenic mechanism in haemochromatosis[43,48]. Therefore, HFE, TfR2 and haemojuvelin proteins may all be involved in the pathway sensing iron overload that leads to hepcidin synthesis. Experiments using cultured cells indicated that hepcidin may act by interaction with ferroportin, causing its degradation and reducing cellular iron export[49]. The overall effect would be to "trap" iron within enterocytes, hepatocytes and macrophages. However, other studies, using parenteral injection of hepcidin into mice and analysis of iron absorption in tied off lengths of duodenum, found that hepcidin inhibited the uptake step of iron absorption. This did not require HFE. In this study, hepcidin did not influence the proportion of iron transferred into the circulation[50]. Therefore it is not yet clear whether hepcidin acts at ferroportin in the enterocyte basolateral membrane, at DMT1 in the apical membrane, or both (Figure 3).

Because hepcidin is synthesised in hepatocytes, they have been proposed as the site of an iron sensing mechanism. TfR2 is expressed mainly in hepatocytes, haematopoietic cells and crypt cells of the duodenum, which are all also sites of HFE expression[51,52]. Recent expression studies of intact HFE and TfR2 proteins in cultured cells showed interaction between these two proteins[53]. It was proposed that when transferrin saturation is in the normal range, TfR1 may sequester HFE[54]. At increased serum transferrin saturation, diferric transferrin has been shown to compete with HFE for binding to TfR1[55]. Freed HFE is proposed to act as an iron sensor, binding TfR2 and conveying transferrin saturation status *via* TfR2 and a cytoplasmic signal transduction complex, leading to the synthesis of hepcidin (Figure 3). Haemojuvelin may form part of the signal transduction system. In HFE-, TfR2- or haemojuvelin-related haemochromatosis, mutations may disrupt this sensing system, leading to the observed deficiency of hepcidin; iron loading would therefore result[53]. Thus, latest ideas propose that HFE-TfR2 interaction in hepatocytes may sense transferrin saturation, as an index of body iron status, determining production of hepcidin, which governs iron absorption[53].

IRON AND *HFE* MUTATIONS IN CHRONIC HEPATITIS C

CHC remains a major health problem with around 200 million individuals affected worldwide[56]. The natural course of CHC is characterised by progressive fibrosis in the inflamed liver with cirrhosis and haemodynamic changes which may be followed by end-stage complications[57]. The progression of fibrosis in CHC is highly variable. Several factors may favour progression, including alcohol, young age at the time of infection and male gender. The role of iron in the pathogenesis of CHC has been debated. An association between iron and viral hepatitis was first described by Blumberg and colleagues[5]. Following those observations, several studies noted elevated serum iron indices in CHC[58-60]. Iron has been proposed as a cofactor that may both promote the progression of liver disease and reduce the response to antiviral therapy[56,61,62]. Mechanisms proposed include production of reactive oxygen species, increased fibrogenesis through activation of HSCs and impairment of the host immune response[63]. Several studies of CHC reported hepatic iron deposits in HSCs, which may contribute to liver damage[64,65]. Many studies have also tried to investigate the pathogenesis of iron overload in CHC. Necroinflammation due to ongoing viral infection is considered the most important cause of iron overload. Additionally, the viral infection *per se* or the associated activation of cytokines have been proposed to modify iron metabolism in liver cells[60,66]. A recent study showed that TfR1 expression was increased in CHC hepatic tissue irrespective of the degree of hepatic iron overload. This might, therefore, contribute to the accumulation of hepatic iron in CHC[67]. Viral genotype may be another factor. In a large study of 242 patients, one of us analyzed the relationship between hepatitis C virus (HCV) genotypes and liver iron deposits in CHC[68]. We found a higher prevalence of hepatic iron deposits in HCV-3 infected cases, concluding that hepatic iron deposition is HCV-genotype dependent. Indeed, the distribution of hepatic iron was mostly parenchymal in HCV-3 cases, while in non-HCV-3 patients, hepatic iron deposits were more frequently detected in the reticuloendothelial cells. These findings support the hypothesis that the expression of HCV-3 proteins in infected hepatocytes might cause specific metabolic changes resulting in enhanced oxidative stress. The non-HCV-3 cases had more severe fibrosis. Therefore their lesser hepatic iron deposits might reflect a more advanced stage of liver inflammation. A further relationship between viral genotype and iron was found in another study: Izumi and colleagues reported higher hepatic iron concentrations in HCV-1b compared with HCV-2 infected cases[69].

A role of proteins that are involved in iron homeostasis has also been hypothesized. In a detailed histopathological study, Corengia and colleagues investigated *HFE* genotypes and hepatic iron score in hepatitis C[70]. They found a significant relationship between *HFE* genotypes and iron deposition in hepatocytes. This suggested that some iron accumulation in CHC derives from increased iron absorption due to mutated HFE protein. Hepcidin has also been implicated. A study by Aoki and colleagues evaluated the possible role of hepcidin in determining iron overload in hepatitis C[71]. In patients with HCV, they demonstrated that hepatic hepcidin mRNA correlated with hepatic iron concentration (HIC). This suggested that iron stores regulate hepcidin expression normally; iron loading in CHC is not due to inappropriate hepcidin expression.

Serum iron indices are frequently abnormal in patients with CHC. Elevation of the serum ferritin concentration has been reported in 20%-60% of patients with chronic hepatitis C[59,68,72,73]; stainable hepatic iron deposits were detected in 3%-38%. Furthermore, many cases of CHC, including those with elevated serum ferritin, elevated transferrin saturation, or both, showed no significant increase in HIC[59,61,68,73-74]. In most of these studies, hepatic iron overload was generally mild to moderate and often not sufficient to be hepatotoxic *per se*[61,75,76]. Elevated serum ferritin concentration in CHC may be explained by several associated conditions, in the absence of iron overload. Serum ferritin could be elevated as an acute phase protein because of the cytolitic necroinflammation that is common in hepatitis C. Moreover, hepatic steatosis is frequent in patients with hepatitis C[77,78] and has been associated with raised levels of serum ferritin[79,80]. A recent study suggested that increased serum ferritin in CHC may be mainly due to diabetes mellitus, which is commonly associated with CHC[81]. In contrast, other observations from one of us suggested that elevated serum ferritin in CHC may be mostly multifactorial[82].

The distribution of iron has been examined in several studies, with some discrepancies. Some studies reported iron deposits mostly in hepatocytes[70,83,84], others in reticuloendothelial cells[85-87] and others reported a mixed distribution[68,88,89]. This is a notable difference with HH where, until iron loading is severe, hepatic iron deposition is almost exclusively parenchymal.

The role of *HFE* mutations as a risk factor for iron overload in CHC has been studied in different populations, with somewhat discordant results (Table 2). Piperno and colleagues studied 110 Italian patients with chronic B or C viral hepatitis; they found that all male heterozygotes for the C282Y mutation had iron overload. The H63D mutation was significantly more frequent in patients with marked hepatic siderosis than in those with mild or no siderosis and in controls[73]. A study of 137 CHC patients from the North of England reported that patients who carried the C282Y mutation had higher serum iron indices and more frequently had stainable hepatic iron, together with more advanced fibrosis or cirrhosis, than patients without the C282Y mutation[90]. Similar results were obtained in other studies which correlated the presence of *HFE* mutations with increased serum iron indices, hepatic iron deposits and severe hepatic fibrosis[70,91-94]. In an Austrian study of 184 patients with CHC versus 487 controls, Kazemi-Shirazi and colleagues found that serum iron indices were increased in patients carrying *HFE* mutations. In contrast with other studies, however, there was no evidence for more hepatic siderosis or advanced fibrosis in patients with *HFE* mutations[95]. Other studies reported no relationship of *HFE* mutations with hepatic iron deposits and severe hepatic fibrosis[83,89]. In a Scottish study, Thorburn and colleagues prospectively investigated

Table 2 Studies of the relationship between chronic hepatitis C, iron and *HFE* mutations

Reference	Cases *n*	Ethnicity	*HFE* relationship with serum iron indices	*HFE* relationship with HI	*HFE* relationship with fibrosis
[70]	206	Italian	Yes	Yes	Yes
[83]	120	Mostly Swiss and Italian	No	No	No
[88]	242	Mostly Caucasian	No	No	No
[89]	164	Mostly Caucasian	No	No	No
[90]	137	Caucasian	Yes	Yes	Yes
[91]	135	Brazilian	Yes	Yes	Yes
[92]	119	Mostly Caucasian, non-Hispanic	Yes	Yes	Yes
[93]	316	Mostly White	Yes	Yes	Yes
[94]	401	Mostly German	Yes	Yes	Yes
[95]	184	White, non-Hispanic	Yes	No	No
[96]	273	NA	Yes	Yes (only H63D)	No
[97]	1051	Mostly White, non-Hispanic	Yes	Yes	No

NA: not available; HI: hepatic iron (histological and/or biochemical evidence).

164 consecutive patients with HCV infection. They did not find a role for *HFE* mutations in the accumulation of iron or the progression of liver disease[89]. Overall, only a few studies have suggested an increased prevalence of *HFE* mutations in CHC patients, with respect to the general population[90,92,95]; this observation was not confirmed in other studies[73,91,94].

A role for the H63D mutation in the iron overload of CHC was proposed in a few of the studies. Lebray and colleagues found that the histological hepatic iron score was higher in patients who were homozygous or heterozygous for H63D; this was surprisingly associated with an increased response rate to antiviral therapy[96]. Another study reported increased hepatic iron deposits in male patients carrying the H63D mutation[97]. Thus, the role of the H63D mutation is unclear and, as observed in HH, is minor with respect to the C282Y mutation.

Several studies suggested that higher levels of serum ferritin or HIC were associated with a diminished likelihood of response to antiviral therapy[84,85,97]. Data from a recent Italian multicenter study indicated that iron removal by phlebotomy improved the rate of response to interferon[98]. The influence of *HFE* mutations on the outcome of antiviral treatment has been investigated in a few studies. Chapman and colleagues suggested that a single mutation in the *HFE* gene had no impact on the outcome of interferon treatment; this was confirmed by subsequent reports[99-101]. However, a study of 242 patients found that presence of the C282Y mutation was positively correlated with sustained response in a multivariate analysis[88]. The reports by Lebray *et al* and Distante *et al* suggest that *HFE* may be part of a pattern of host genes which together influence response to antiviral therapy[88,96]. Indeed, clearance of HCV is believed to be associated with different HLA alleles; the C282Y mutation of the *HFE* gene is part of an extended founder haplotype which includes the HLA region on the short arm of chromosome 6[101]. The positive effect of the C282Y mutation may reflect linkage disequilibrium between the *HFE* mutated allele and alleles at other loci implicated in the virological response, close to the *HFE* gene on chromosome 6. Several MHC class I and II loci have been shown to be associated with a sustained virological response to interferon treatment[102-105]. Similarly, an American study investigated the role of iron overload and *HFE* mutations in the response to antiviral therapy in over 1000 patients with advanced CHC[97]. The authors found that *HFE* mutations correlated with histological hepatic iron score in CHC. Subjects harbouring *HFE* mutations, particularly H63D, had significantly higher likelihood of both on-treatment virological responses (at 24 and 48 wk) and sustained virological responses (24 wk after the end of lead-in therapy) to re-treatment with pegylated interferon alpha-2α plus ribavirin. Again, both the *HFE* mutation and/or associated genetic variants were considered as possible causes of the improved response to therapy.

In conclusion, elevation of serum iron indices and hepatic iron deposits are a common feature in CHC. Hepatic iron overload is generally mild to moderate and it rarely reaches the severity seen in HH. In contrast to HH, the intrahepatic iron accumulation is generally mixed, with both parenchymal and reticuloendothelial distribution. The exact mechanism of hepatic iron accumulation in hepatitis C is still not clear. The pathogenesis is likely to be multifactorial and viral and host factors have been evaluated. The viral factors suggested by several studies include necroinflammation due to viral infection, direct influence on iron homeostasis mediated by cytokines, or HCV genotypes. Among the host factors, a role for *HFE* mutations has been proposed and extensively evaluated. On the basis of current knowledge, we conclude that *HFE* mutations may have a role in the elevation of serum iron indices and hepatic iron deposition observed in CHC, but they do not fully explain the observed abnormalities of iron homeostasis. Iron may also contribute to the rate of response to antiviral therapy.

IRON AND *HFE* MUTATIONS IN CHRONIC HEPATITIS B

Chronic hepatitis B (CHB) remains a serious global health concern. Appro-ximately 350 million people are chronically infected, and 500 000 to 1.2 million deaths per year are attributed to HBV-associated complications[106]. Among patients with active viral replication, cirrhosis will develop in 15%-20% within five years[107]. For patients with cirrhosis, acute exacerbation can occur and the disease may progress to end stage complications[107]. The histopathological pathway of progressive liver disease is characterised by fibrosis leading to increasing distortion of the hepatic architecture, that is the hallmark of evolution to cirrhosis. Liver fibrosis is the result of chronic injury and plays a direct role in the pathogenesis of hepatocellular dysfunction and portal hypertension. The progression of liver fibrosis is due to many viral and host

factors. In CHC it has been proposed that iron may be a cofactor, but data for patients with CHB are more scarce. An association between iron and hepatitis B was first described by Blumberg and colleagues[5]. They found that serum iron indices were higher in patients who developed chronic hepatitis than in those who eliminated the virus. The same team also observed that haemodialysed patients with higher serum iron indices were less likely to achieve spontaneous recovery after acute hepatitis B[108]. Other authors, assessing the presence of hepatic iron in Kupffer cells of patients with CHB, deduced that it is derived from hepatocytes destroyed by the virus[109,110]. Zhou and colleagues studied 40 patients with hepatocellular carcinoma, 80% of whom were hepatitis B surface antigen (HBsAg) positive. They found a significant correlation between the presence of hepatitis B core antigen (HBcAg) and iron in hepatic tissue, suggesting that iron may accumulate predominantly in the hepatocytes in which HBV replication takes place[111]. More recently, Martinelli and colleagues evaluated the prevalence of serum iron biochemical abnormalities and iron deposits in the liver of CHB patients[112]. They found elevated transferrin saturation in 27.1% and liver iron deposits in 48.7% of cases. Patients with liver iron deposits presented with higher scores of necroinflammatory activity and fibrosis. The authors found no relationship between *HFE* mutations and elevation of serum iron indices or liver iron.

The effect of iron on the outcome of interferon alpha therapy in patients with CHB has been investigated by very few studies. In chronic viral hepatitis, Van Thiel and colleagues reported that low hepatic iron content may predict response to interferon therapy[61]. More recently, a Polish group investigated iron metabolism and prognostic factors in interferon therapy in children with CHB. They showed that seroconversion for hepatitis Be antigen (HBeAg) was more frequently observed in children with lower iron and ferritin values[113].

In conclusion, only a few studies have evaluated the prevalence and the physiopathological significance of iron overload in CHB. It has been suggested that iron deposits may occur mostly in hepatocytes. It seems that patients with higher levels of serum iron indices are less likely to achieve spontaneous recovery after acute hepatitis B. No role for the *HFE* gene mutations in iron overload has been detected. Iron could influence the response to antiviral therapy, but there is not sufficient evidence to permit a definitive conclusion.

IRON AND *HFE* MUTATIONS IN ALCOHOLIC LIVER DISEASE

Alcoholic liver disease (ALD) is one of the leading causes of end-stage CLD. It is well established that only a minority of heavy drinkers, estimated at between 10 and 30%, will ever develop advanced ALD; the risk increases with cumulative alcohol intake[114,115]. Hence, in addition to alcohol, other factors are deduced to act synergistically to enhance its hepatotoxic effects. Patients with ALD commonly have elevation of transferrin saturation and serum ferritin concentration; significant hepatic iron deposition is not infrequent[116-118]. However, most patients with ALD have normal or slightly elevated HIC, with a mixed parenchymal and reticuloendothelial pattern of distribution[119,120]. There is growing evidence that a mild degree of iron overload is sufficient to enhance alcohol-induced liver injury. The paradigm of synergy between iron and alcohol is HH. Patients with HH and significant alcohol consumption have a higher incidence of cirrhosis and hepatocellular carcinoma than those without heavy alcohol consumption or a history of alcohol abuse[121,122]. In ALD, stainable hepatic iron was positively correlated with fibrosis in a multivariate analysis of risk factors in 268 French alcoholic patients[123].

Table 3 Studies of the relationship between alcoholic liver disease, iron and *HFE* mutations

Reference	Cases *n*	Ethnicity	HFE relationship with serum iron indices	HFE relationship with HI	HFE relationship with ALD
[120]	257	Caucasian	NA	No	No
[121]	254	Caucasian	No	Yes	No
[129]	179	White Hispanic	Yes (C282Y)	NA	No
[130]	61	White, non-Hispanic	NA	NA	NA

NA: not available; HI: hepatic iron; ALD: alcoholic liver disease (histological and/or biochemical evidence).

There are several potential causes for hepatic iron overload in alcoholic liver disease, including increased ingestion of iron, increased intestinal iron absorption, up-regulation of hepatic TfR1, secondary anaemia due to haemolysis, hypersplenism, ineffective erythropoiesis, hypoxaemia due to intrapulmonary shunts and portosystemic shunts[4,124,125]. Increased iron absorption could arise through three main mechanisms: an increase in the reduction of luminal iron to the ferrous state; up-regulation of DMT1 in duodenal enterocytes; upregulation of ferroportin in duodenal enterocytes. The latter mechanism may be influenced by the down-regulation by ethanol of the hepatic production of hepcidin[126].

Several studies suggested a genetic component to disease susceptibility. Significant associations have been reported between ALD risk and polymorphisms of the genes encoding cytochrome P450 and tumour necrosis factor (TNF) α[126,127]. Few studies assessed the possible role of *HFE* mutations as genetic cofactors in the development of ALD (Table 3). Lauret and colleagues found a significant association between carriage of the C282Y mutation and elevation of serum iron indices, but another study did not replicate this finding[120,128]. One study found a relationship between *HFE* mutations and hepatic iron deposits[120] but this observation was not confirmed by others[119]. Moreover, none of these studies found that *HFE* mutations influenced the severity of ALD[119,120,128,129].

In conclusion, patients with ALD tend to show mild increases in hepatic and serum measures of iron status. Iron is thought to play a role in worsening the course of ALD, although the mechanisms responsible are not resolved. To date, evidence from the literature does not

suggest a role for *HFE* mutations in determining iron overload or in influencing the course of ALD.

IRON AND *HFE* MUTATIONS IN NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as the most prevalent liver disease, at least in the West. In the adult population of the USA, 31% of men and 16% of women were found to have NAFLD[130]. The disease has a spectrum ranging from fatty liver alone to steatohepatitis, and progressive steatofibrosis. Although fatty liver alone is considered non progressive, up to 20% of patients with non-alcoholic steatohepatitis (NASH) may develop cirrhosis, liver failure and HCC[131]. Many cases of cryptogenic cirrhosis may be end-stage forms of NASH[132]. The pathogenesis of NAFLD and the reasons why some patients with fatty liver develop NASH and have progressive liver disease are not entirely understood. A "two-hit" hypothesis has been proposed, involving the accumulation of fat in the liver ("first hit"), together with a "second hit" that gives rise to increased oxidative stress. Hepatic steatosis has been recognised as the first of two hits in the pathogenesis of NASH, since the presence of oxidisable fat within the liver is enough to trigger lipid peroxidation[133]. However, many patients with fatty liver do not progress to steatohepatitis. Potential second hits for the development of NASH include all mechanisms contributing to the development of inflammation and fibrosis. Increased expression of ethanol-inducible cytochrome P450 2E1 (CYP2E1) and an increase in the intrahepatic concentration of free fatty acids could result in oxidative stress via peroxisomal oxidation[134]. TNFα has also been implicated, since administration of anti-TNFα antibody ameliorated liver damage in animal models[135].

There is controversial evidence that hepatic iron may play a role in the pathogenesis of NASH. It has been proposed that iron, in relatively low concentrations, could synergize with lipid overload and induction of CYP2E1 to increase oxidative stress in hepatocytes[136]. Elevation of serum iron indices has been found in several studies[126,127]. In most cases hepatic siderosis was mild and HIC was only rarely elevated[137-140]. The distribution of hepatic iron was mixed parenchymal and sinusoidal[79]. Strong support for an important role of iron in NAFLD and NASH was recently provided by studies on iron-depletion therapy, which improved both serum aminotransferases and insulin sensitivity[141,142].

The role of iron has been re-evaluated since the discovery of the strict pathogenetic link between NASH and insulin resistance[143]. Iron has been proposed to contribute to the development or exacerbation of insulin resistance, which is the most important risk factor for development of NAFLD and NASH[79,80]. Mendler and colleagues reported a new syndrome characterized by mild to moderate hepatic iron overload and features of insulin resistance; the new syndrome was named insulin resistance-hepatic iron overload (IR-HIO). In their study, liver steatosis and NASH were present in 25% and 27% of IR-HIO cases, respectively[7,144]. A subsequent study confirmed the high prevalence of NAFLD (59.7%) in patients with insulin resistance-associated iron overload[145]. Moreover, as in NASH, phlebotomy allows for normalization of body iron stores in IR-HIO[146]. Indeed, IR-HIO shares some features with NASH, such as hyperferritinemia with mostly normal transferrin saturation. However, not all patients with IR-HIO have hepatic steatosis. It is likely that NASH and IR-HIO share some pathogenetic mechanisms. It has been proposed that IR-HIO may represent one end of the spectrum of NASH in which HIC is increased. Another point of view is that IR-HIO may represent a coincidental convergence of hepatic iron overload with a very common liver disorder in the general population[128].

Table 4 Studies of the relationship between NAFLD-NASH, iron and *HFE* mutations

Reference	Cases *n*	Ethnicity	HFE relationship with serum iron indices	HFE relationship with HI	HFE relationship with fibrosis
[139]	51	Australian	Yes with transferrin saturation, no with ferritin	Yes	Yes
[142]	31	Italian	No	Yes	No
[146]	263	Italian	Yes (C282Y)	No	No
[151]	57	Caucasian	Yes	Yes	Yes
[152]	32	Mostly Caucasian	No	No	No
[153]	38	Asian	No	No	No
[154]	31	Asian Indian	No	No	No
[155]	93	Mostly Caucasian	No	Yes	No

NA: not available; HI: hepatic iron (histological and/or biochemical evidence).

Genetic factors have also been proposed to play a role in the pathogenesis of NASH. Investigations of the possible contribution of *HFE* mutations in the pathogenesis of NASH have given sometimes discordant results (Table 4). George and colleagues reported increased intrahepatic iron (Perls' grade > 1) in 41% of cases; 23% of patients had HIC above the upper limit of normal[138]. The increased HIC was attributed to the higher prevalence of homozygotes and heterozygotes for C282Y in these Australian patients with NASH versus control subjects (31% *vs* 13%, respectively). No significant difference in the prevalence of the H63D mutation was reported. A subsequent study by Bonkovsky and colleagues reported a significantly increased prevalence of H63D heterozygosity in patients versus controls (44.4% *vs* 26.4%)[147]. Heterozygosity for C282Y was not statistically different between the two groups. Fargion and coworkers also reported an increased prevalence of *HFE* mutations in patients with respect to controls (65% *vs* 26%). Patients carrying *HFE* mutations did not present with increased serum iron indices, but showed increased HIC[79]. In contrast to these results, other investigators have failed to observe significant associations between hepatic iron accumulation, *HFE* mutations and the severity of liver disease in patients with NASH[139,140,80,148-151]. Angulo and colleagues found that HIC was normal in patients with NASH and abnormal serum iron indices[139]. Another study by Chitturi and collegues did not find any correlation

between HIC and any features of NASH, including the severity of fibrosis[151]. The authors did not find any increase in stainable hepatic iron in the majority (90%) of liver biopsies from patients with NASH. Although they, like others, found a higher prevalence of C282Y heterozygosity in patients with NASH, no relationship with fibrotic severity was identified. Other studies did not find either significant abnormalities of serum and liver iron or an increased frequency of *HFE* mutations[80,148,149].

Explanations for discrepant results include ascertainment bias, varied power of the studies and possible ethnic differences in the study populations. It should be underlined that elevation of serum iron indices in NASH, especially serum ferritin, may be due to insulin resistance, steatosis and inflammation, rather than to iron overload. Indeed, the discrepancies could be partially attributed to the differences between databases: the patients could be selected among subjects with hepatic iron overload or among population routinely attended a gastroenterology outpatient clinic. The association between NASH and *HFE* mutations has been described mainly in Australian and North American Caucasians[138,147]. This hypothesis is supported by a recent study by Chitturi and colleagues that found an increased frequency of C282Y heterozygosity in NASH only in Anglo-Celtic patients[151]. However, studies showing a positive correlation may have come from tertiary centres with an interest in iron storage disorders.

An increased prevalence of C282Y and H63D mutations has also been reported in patients with IR-HIO, but this finding was not connected with increased iron burden[7]. A recent study of ours described four cases of H63D homozygosity associated with hyperferritinemia, macrovesicular steatosis, mild parenchymal and sinusoidal hepatic siderosis, with a granular pattern that could be related to NASH. Furthermore, three of the cases had one or more metabolic disorders which are part of the insulin resistance syndrome. The study could be consistent with proposals of a possible biological effect of the H63D mutation in IR-HIO and/or fatty liver[152].

In conclusion, NAFLD has a heterogeneous spectrum of disease. It can progress to NASH and to end-stage liver disease, but the exact mechanism of fibrosis progression is not completely clear. A "two hits" hypothesis has been proposed and iron has been evaluated as a potential "second hit" that can cause progression of simple fatty liver to NASH. The available data are discordant about prevalence and effect of iron overload in NAFLD and NASH. Patients with coexisting NASH and hepatic iron stores undoubtedly exist, but the discordant data of the literature suggest that iron overload in NASH may be an epiphenomenon rather than have a main causative role. Insulin resistance syndrome has emerged as a key player in NAFLD and in the development of NASH. Iron may exacerbate insulin resistance; although iron overload is fairly common in NAFLD, its amount is rarely clinically significant when considered in isolation. A possible role of *HFE* mutations in NASH has been described in two studies from countries with predominantly Caucasian populations. However, this was not confirmed in subsequent studies of more heterogeneous populations. *HFE* mutations may be part of a genetic pattern contributing to the progression of NASH in populations of Celtic origin.

Table 5 Studies of the relationship between porphyria cutanea tarda, iron and *HFE* mutations

Reference	Cases *n*	Ethnicity	HFE relationship with serum iron indices	HFE relationship with HI	HFE relationship with fibrosis
[163]	41	Caucasian	NA	NA	NA
[164]	108	Australian	Yes	Yes	No
[165]	70	North American	NA	NA	NA
[167]	23	Brazilian	Yes	No	Yes
[168]	36	Southern France	No	NA	NA
[170]	190	German	No	No	No
[172]	68	Italian	No	NA	NA
[174]	62	German	Yes	NA	NA

NA: not available; HI: hepatic iron (histological and/or biochemical evidence).

IRON AND *HFE* MUTATIONS IN PORPHYRIA CUTANEA TARDA

Porphyria cutanea tarda (PCT) is the most common of the human porphyrias. It is caused by deficient activity of hepatic uroporphyrinogen decarboxylase. Most cases of PCT are acquired; the major risk factors are CHC, alcohol abuse, iron overload and oestrogen use. The familial form of the disease is observed in 20%-25% of patients[4]. Both sporadic and familial PCT are iron-dependent disorders. The association of PCT with iron overload has been recognized for decades. Independent of the cause of liver disease, the majority of patients with sporadic PCT have biochemical evidence of iron overload, liver siderosis and increased body iron stores. However, hepatic siderosis is generally mild or moderate, reaching the lower end of the haemochromatosis range in less than 10% of cases[153-154]. The causes of iron overload in patients with PCT appear to be heterogeneous. Indeed, altered iron status may be secondary to cofactors such as alcohol and chronic infection with HCV, that are frequently associated with sporadic PCT[4]. Clinical and experimental data suggest that an iron-dependent process reversibly inactivates uroporphyrinogen decarboxylase[155]. Indeed, as initially observed by Lundvall, venesection therapy may induce remission of cutaneous lesions and an improvement of liver function tests, whereas replenishment of iron stores leads to relapse[156,157]. Phlebotomy may also be beneficial in patients without biochemical or histological evidence of iron overload[157]. Altered iron homeostasis, even in the absence of systemic iron overload, could reduce uroporphyrinogen decarboxylase activity[158].

Several studies have investigated the possible association between PCT and mutations of the *HFE* gene (Table 5). Investigators from UK reported that 44% of patients with PCT *vs* 11% of the control group carried the C282Y mutation, whereas no significant difference was found for the H63D mutation[159]. Similar results were

reported by Stuart and colleagues in Australian patients and by Bonkovsky and colleagues in patients from North America[160,161]. A detailed study comprising 108 US patients with PCT reported a C282Y frequency of 30% compared to 6% in controls[162]. HIC, transferrin saturation and serum ferritin were highest in PCT patients who were homozygous for the C282Y mutation. The authors also reported a high frequency of comorbidity due to factors such alcohol and hepatitis C. They concluded that homozygosity for the C282Y mutation and HCV infection, especially with heavy alcohol consumption, are the strongest risk factors for PCT. Martinelli and colleagues reported an association between C282Y and PCT with respect to controls (17.4% *vs* 4%) in a population of Southern European ancestry[143]. Similar results were obtained in two studies of French patients[164,165]. In a large study of 190 sporadic PCT cases from Germany the C282Y and H63D mutations were found in 39% and 45%, respectively[166]. C282Y was significantly more frequent in patients than controls. Serum iron, transferrin saturation, ferritin concentration, HIC and liver enzymes did not differ significantly between patients with or without *HFE* mutations. Investigators from South Africa determined the frequency of *HFE* mutations in a racially-mixed group of patients with PCT in Cape Town[167]. They found that both the C282Y and H63D mutations were highly prevalent in South Africans of European origin. In cases of mixed or Asian origin, the H63D mutation was common but the C282Y mutation was very rare. Neither mutation was found in any African subject. They concluded that both mutations were associated with PCT, but the association was dependent on the ethnic origin of the patient. Interestingly, one study from Italy reported a strong association of PCT with the H63D mutation, which was present in half of the patients. The presence of the H63D mutation was not related to the iron status of patients. However, a subtle abnormality of iron metabolism induced by this mutation could escape detection by the standard parameters of iron status[168]. Some major factors may account for some differences between these studies. Firstly, the C282Y mutation is more frequent in cases of Celtic ancestry, although the prevalence of this mutation appears to be lower in Southern European countries[9,11]. Secondly, the distribution of factors predisposing for PCT also shows relevant geographical differences. In Italy and other Mediterranean countries, hepatitis C is present in 70%-90% of PCT patients while it is rare in Northern European countries, where alcohol is the prevalent aetiological agent for CLD associated with PCT[169]. Stolzel and colleagues investigated the relationship between *HFE* gene mutations and response to chloroquine in PCT patients[170]. Chloroquine therapy was accompanied by clinical remission and reduced urinary porphyrin excretion in 39% of patients without *HFE* mutations versus 56% of *HFE* heterozygous patients. Interestingly, all patients homozygous for the C282Y mutation had high serum iron, transferrin saturation and serum ferritin concentration, and failed to respond to chloroquine treatment.

In conclusion, PCT is frequently associated with elevated serum iron indices and sometimes with hepatic iron overload. The pathogenesis of altered iron homeostasis in PCT is not completely understood, although iron is thought to reduce the activity of uroporphyrinogen decarboxylase. Venesection therapy may be beneficial in PCT patients. Several cofactors have been proposed to play a role in the impairment of iron homeostasis observed in PCT, such as alcohol and CHC. A role of *HFE* mutations has also been reported. The available data support a role for the C282Y mutation in many cases of PCT, especially the C282Y homozygous genotype. This is particularly true in Celtic ancestry cases, thus confirming that the importance of *HFE* mutations as modifiers of disease varies according to the ethnic group. Most studies did not detect a relationship between *HFE* mutations and serum and hepatic iron overload. This suggests that *HFE* mutations, particularly C282Y, may contribute to the pathogenesis of PCT either through immunological mechanisms that could be iron-independent, or via subtle changes in hepatic iron metabolism that may act in concert with other co-factors to inhibit the activity of uroporphyrinogen decarboxylase.

IRON AND *HFE* MUTATIONS IN HEPATOCELLULAR CARCINOMA OCCURRING IN CLDS OTHER THAN HH

HCC is a common cause of death in patients with compensated cirrhosis[171]. European studies have reported HCC as the cause of liver-related deaths in 54%-70% of cases of compensated cirrhosis of varied aetiology and in 50% of cases with cirrhosis due to HCV[172]. The annual incidence of HCC in patients with liver cirrhosis has been estimated at 3%-5%[173,174]. Thus, it is important to identify patients at high risk of HCC, to increase the rate of early detection. Several risk factors for the development of HCC have been identified in Western patients with cirrhosis, including male sex, age, persistently raised serum α-fetoprotein levels, severity of cirrhosis and genetic background[173]. A possible carcinogenic role for iron has been suggested by *in vitro* studies. Iron may promote cellular oxidative stress through the production of reactive oxygen species. These have the potential to cause lipid peroxidation as well as damage to other cellular components, including proteins and nucleic acids[151]. *In vitro* studies showed that iron can reduce the levels of two systems that normally protect against reactive oxygen species, vitamin E and superoxide dismutases[175-176]. Furthermore, *in vivo* studies in mammals showed that tumor growth is enhanced by iron supplementation and inhibited by iron deficiency[175,177]. Finally, neoplastic cells highly express TfR1 and can synthesize their own transferrin[177].

Apart from the experimental evidence, the role of iron in the step by step process that leads to HCC in CLDs other than HH is controversial. A report of 133 cases by Boige and colleagues did not find any difference in the grade of hepatic iron staining between cirrhotic patients with and without HCC[178]. This finding was also in keeping with a previous study where no significant relationship was observed between the hepatic iron score and the occurrence of HCC in alcoholic and HCV-related

cirrhosis[179]. A role of *HFE* mutations as part of a genetic pattern promoting carcinogenesis has been evaluated in some studies, with somewhat discordant results. In a German study, C282Y heterozygosity was significantly more common in 137 HCC cases with no history of HH versus 107 cirrhotic patients without HCC and 126 healthy controls. C282Y heterozygote HCC patients had significantly increased hepatic iron score in both HCC and non-tumourous tissue[180]. Other studies reported that the prevalence of C282Y heterozygosity was increased above control levels in patients with HCC and alcoholic and virus-related liver disease[129,182]. An excess of the C282Y mutation, mostly in the heterozygous genotype, has also been reported in patients with HCC developed in non cirrhotic liver. In that study, Blanc and colleagues showed that mild iron overload is frequent (54%) and that in patients with HCC in non cirrhotic liver and iron overload, C282Y mutations are frequent (36.8% of cases) and significantly increased compared to HCC in non cirrhotic liver without iron overload[183]. In contrast with the above results, some studies did not find a relationship between HCC and mutations in the *HFE* gene. Cauza and colleagues evaluated the prevalence of *HFE* mutations in patients with HCC developed on cirrhosis of viral and alcoholic aetiology. The authors found that, except for C282Y homozygotes, *HFE* mutations did not increase the risk of HCC in patients with cirrhosis[184]. A prospective study of 133 consecutive cirrhotic patients without HH did not find an increased prevalence of *HFE* mutations in cirrhotic patients who developed HCC compared to cases without HCC[178]. Similarly, a study from Italy found that in patients with HCC in the absence of HH, the frequency of *HFE* mutations was not increased, compared to the controls. The authors concluded that mutations of the *HFE* gene do not play a significant role in the pathogenesis of HCC[185].

In conclusion, iron has a clear pathogenetic role in the development of HCC in HH. The carcinogenic role of iron is deduced to be mediated through production of reactive oxygen species leading to lipid peroxidation and damage of proteins, nucleic acids and other cellular components in hepatocytes. However, similar results have not been obtained for other CLDs. The available studies about the role of *HFE* mutations in HCC in liver diseases other than HH indicate that C282Y heterozygosity may play a role in liver iron deposition and could contribute to hepatocarcinogenesis, possibly by playing a part in the immunogenetic pattern of the patient or through subtle changes in iron metabolism acting together with other cofactors.

HIGHLIGHTS

Apart from HH, a number of CLDs cause hepatic iron overload. These include hepatitis C and B, alcoholic and nonalcoholic steatohepatitis and PCT. Secondary iron overload due to CLD presents with some clinical, histological and genetic differences with respect to HH. Since the occurrence of HH is clearly related to the presence of specific genetic patterns, the role of mutations of the gene most commonly responsible for HH has been investigated in liver diseases other than HH. Liver diseases that cause secondary iron overload share some common features, but they also show differences (Table 6). The evidence in the literature regarding secondary iron overload in comparison to HH are consistent in showing that: (1) in HH iron overload is widespread in many organs while in secondary iron overload due to CLDs, iron is confined to the liver; (2) in HH the histological distribution of iron is mostly parenchymal while in secondary iron overload due to CLDs, iron shows mostly a mixed distribution, with both reticuloendothelial and parenchymal localization; (3) point mutations in the *HFE* gene are the most common genetic factor underlying HH; their prevalence varies according to geographic area and ethnic group. In secondary iron overload, data are controversial. When present, the penetrance of *HFE* mutations may be more influenced by cofactors, since the aetiology of CLDs is multifactorial; (4) in HH, genetics is the "primum movens" of the disease while in secondary haemochromatosis it has been proposed as a cofactor that may increase the severity of disease expression. Evidence from the literature suggests that the C282Y mutation may play a role in NASH, PCT and possibly CHC in cases of Celtic ancestry. The importance of *HFE* mutations as modifiers of disease, therefore, varies between different ethnic groups. H63D has been reported by few authors as having a role in some cases of mild iron overload in NAFLD and PCT, although any effect is generally minor, in agreement with the findings in HH. *HFE* mutations do not seem to determine iron overload or to influence the course of ALD and CHB. Iron may contribute to the rate of response to antiviral therapy in CHC and to chloroquine in PCT and a positive influence of *HFE* mutations has been suggested in CHC.

Table 6 Secondary iron overload: common features and differences

Common features
1 Mixed distribution of hepatic iron (parenchymal and reticuloendothelial)
2 Mild to moderate iron overload in most cases, rarely severe iron overload
3 Phlebotomy possibly improves the course of disease and response to therapy
Differences
1 C282Y: accepted role in PCT, possible cofactor in CHC and NAFLD (especially in populations of Northern European descent)
2 H63D: unclear role in NAFLD and PCT
3 No role for HFE mutations in ALD or CHB

PCT: porphyria cutanea tarda; CHC: chronic hepatitis C; NAFLD: non-alcoholic fatty liver disease; ALD: alcoholic liver disease; CHB: chronic hepatitis B; CLDs: chronic liver diseases.

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