

# Hereditary Hemochromatosis in the Post-*HFE* Era

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Following the discovery of the *HFE* gene in 1996 and its linkage to the iron overload disorder hereditary hemochromatosis (HH) there have been profound developments in our understanding of the pathogenesis of the biochemical and clinical manifestations of a number of iron overload disorders. This article provides an update of recent developments and key issues relating to iron homeostasis and inherited disorders of iron overload, with emphasis on *HFE*-related HH, and is based on the content of the American Association for the Study of Liver Diseases Single-Topic Conference entitled "Hemochromatosis: What has Happened After *HFE*?" which was held at the Emory Convention Center in Atlanta, September 7-9, 2007. (HEPATOLOGY 2008;48:991-1001.)

## Iron Homeostasis

**Overview of Iron Homeostasis** In healthy individuals, approximately 1-2 mg of iron are absorbed from the diet per day to maintain iron balance. Once absorbed, the iron is bound to plasma transferrin and is transported to the tissues where it is either utilized for the synthesis of heme or nonheme proteins, or stored as ferritin. Total body iron for a 70 kg person is approximately 4000 mg, comprising 2500 mg in erythrocyte hemoglobin, 1000 mg of storage iron (primarily in the liver), 300 mg in myoglobin and respiratory enzymes, and 4 mg bound to plasma transferrin. Approximately 0.8% of circulating erythrocytes are phagocytosed daily by macrophages and must be replaced. Macrophages degrade the erythrocyte-

derived hemoglobin and release the iron ( $\approx 20$  mg per day) into the plasma, so it can be reutilized for erythropoiesis in the bone marrow. Thus, iron is efficiently recycled within the body, with a daily plasma flux of  $\approx 20$  mg, and a daily loss of only 1-2 mg. If iron needs are increased, iron absorption is enhanced and storage iron is utilized; when iron demand declines, absorption and storage adjust accordingly.<sup>1</sup>

Within macrophages, heme derived from senescent erythrocytes is degraded by heme oxygenase and the liberated iron is either stored as ferritin or exits the cell via the iron export protein ferroportin (FPN). The released iron is then oxidized and binds to transferrin, and is available for utilization by the erythron or other tissues.<sup>2</sup> The amount of iron in the body is controlled at the level of dietary absorption, because iron excretion occurs by unregulated processes. Hereditary hemochromatosis (HH) is characterized by an increased rate of iron absorption, leading to iron overload.<sup>1-3</sup>

**Iron Absorption** Dietary iron is absorbed mainly in villus enterocytes of the duodenum. Iron is absorbed in two forms, nonheme iron and heme iron. Nonheme iron is reduced from ferric to ferrous ion by a ferrireductase at the apical surface of the brush border. Duodenal cytochrome B (DcytB) has ferrireductase activity and is highly expressed on the apical surface.<sup>4</sup> Recently, however, DcytB was shown to be not essential for iron absorption in mice.<sup>5</sup> Thus, another ferrireductase is likely to be involved and possible candidates are members of the six-transmembrane epithelial antigen of prostate protein (STEAP) family. Divalent metal transporter 1 (DMT1) is highly expressed in the apical surface of the duodenum and it transports ferrous ion across the brush border from the lumen into the enterocyte. DMT1 is a transmembrane glycoprotein that has the capacity to transport a number of divalent metals including zinc, cadmium, manganese,

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Abbreviations: BMP, bone morphogenetic protein; CIEBP $\alpha$ , CCAAT/enhancer-binding protein  $\alpha$ ; DcytB, duodenal cytochrome B; DMT1, divalent metal transporter 1; FPN, ferroportin; *HFE*-HH, *HFE*-related hereditary hemochromatosis; HH, hereditary hemochromatosis; HJV, hemojuvelin; HSC, hepatic stellate cell; JH, juvenile hemochromatosis; MRI, magnetic resonance imaging; SMAD4, Mothers against decapentaplegic homolog 4; STEAP, six-transmembrane epithelial antigen of prostate protein; TFR, transferrin receptor; ZIP14, Zrt-like and Irt-like protein 14.

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copper, cobalt, and to a lesser extent nickel and lead.<sup>6</sup> Iron in the ferrous state is then transported across the basolateral membrane of the enterocyte by FPN.<sup>7</sup> Ferrous iron is oxidized to ferric iron by hephaestin, a multicopper oxidase located in the basolateral membrane, and ferric iron binds to transferrin in the blood.<sup>8</sup>

Heme iron is absorbed more efficiently than nonheme iron. It is likely transported across the brush border by a heme transporter, but the identity of this transporter remains uncertain. Once internalized, the heme is degraded and the liberated iron is thought to be handled by the enterocyte in the same manner as absorbed nonheme iron.<sup>1,2</sup>

**Cellular Iron Uptake** Transferrin-bound iron is taken up by almost all cells through a transferrin receptor 1 (TFR1)-mediated process. Diferric transferrin binds to TFR1 at the cell surface and is internalized into endosomes where the iron is released from transferrin by endosomal acidification. The ferric iron is reduced by STEAP3 and transported across the endosomal membrane by DMT1.<sup>9,10</sup> The iron is then utilized by the cell or stored as ferritin. The apotransferrin-TFR1 complex is recycled from the endosome to the cell surface where at pH 7.4, apotransferrin is released from TFR1 and additional holotransferrin binds to the receptor to deliver more iron to the cell.<sup>2</sup> Uptake of transferrin-bound iron is negatively regulated by cellular iron levels by an iron-responsive element-iron regulatory protein post-transcriptional mechanism that controls both TFR1 and ferritin expression.<sup>11</sup>

A second TFR (TFR2) is highly expressed by hepatocytes, but it has a lower binding affinity for holotransferrin than TFR1.<sup>12</sup> TFR2 is capable of mediating the internalization and recycling of transferrin and the delivery of iron to cells by a mechanism similar to that described for TFR1.<sup>12,13</sup> However, *in vivo* TFR2 accounts for only about 20% of total transferrin-bound iron uptake by the liver, suggesting that TFR2 plays a minor role in hepatic iron transport.<sup>14</sup>

Non-transferrin bound iron (NTBI) is present in the blood; it consists of iron bound with low-affinity to molecules other than transferrin, with the major component identified as ferric citrate.<sup>15</sup> The concentration of NTBI is normally low but increases when transferrin saturation is high; therefore, NTBI is of pathophysiological importance in iron overload disorders such as HH.<sup>16</sup> NTBI is toxic and is rapidly cleared by hepatocytes by a carrier-mediated process<sup>17</sup>: two candidate transporters are DMT1 and Zrt-like, Irt-like protein 14 (Zip14).<sup>17-19</sup>

**Hepcidin: An Iron-Regulatory Hormone** Hepcidin is an iron-regulatory hormone that plays a central role in iron homeostasis by coordinating iron absorption, mobi-

lization, and storage to meet the iron requirements of erythropoiesis and other iron-dependent processes.<sup>20</sup> Hepcidin is expressed predominantly in hepatocytes and is secreted into the circulation. It binds to FPN, which is highly expressed on macrophages and the basolateral surface of enterocytes, causing FPN to be internalized and degraded, and thereby inhibits iron export (Fig. 1A).<sup>21</sup> Hepcidin expression is regulated by iron status, erythropoiesis, inflammation, and hypoxia.<sup>22</sup> Excess iron and inflammation both induce hepcidin gene (*HAMP*) expression which, in turn, results in decreased iron absorption and diminished iron release from macrophages. In contrast, hepcidin expression is decreased by iron deficiency, erythropoiesis, and hypoxia, which results in increased iron absorption and enhanced iron release from macrophages.<sup>20,22</sup>

Stimulation of erythropoiesis by anemia causes a substantial increase in the demand for iron. It is proposed that an erythroid regulator molecule is released during erythropoiesis to maintain the supply of iron for erythropoiesis, regardless of iron storage levels.<sup>23</sup> During anemia, an increase in erythropoietic activity is required for the down-regulation of hepcidin.<sup>24</sup> This suggests that the erythroid regulator acts by decreasing hepcidin expression to supply the increased iron required for erythropoiesis. Recently, growth differentiation factor 15 has been identified as a candidate for the erythroid regulator, because its levels are elevated in the serum of thalassemia patients and it can down-regulate hepcidin expression.<sup>25</sup> In addition, hypoxia and erythropoietin, which both stimulate the production of red blood cells, can also down-regulate hepcidin expression.<sup>20</sup> Hypoxia stabilizes the hypoxia-inducible factor-1 that binds to the hypoxia responsive element in *HAMP* messenger RNA and inhibits transcription.<sup>26</sup> Erythropoietin, in addition to its effects on erythropoiesis, may also directly down-regulate hepcidin expression in hepatocytes by a mechanism involving the transcription factor CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ).<sup>27</sup>

Other regulators of hepcidin include bone morphogenetic protein (BMP), inflammation, and oxidative stress. BMPs stimulate hepcidin expression via a hemojuvelin (HJV)-facilitated Mothers against decapentaplegic homolog 4 (SMAD4) signaling pathway.<sup>28,29</sup> The inflammatory cytokine interleukin-6 up-regulates hepcidin via signal transducer and activator of transcription 3 signaling, causing iron retention in macrophages and decreased iron absorption.<sup>30</sup> The resultant hypoferrremia plays a major causal role in the anemia of chronic disease. Reactive oxygen species inhibit hepcidin expression via a C/EBP $\alpha$ -mediated mechanism which is likely to account for the

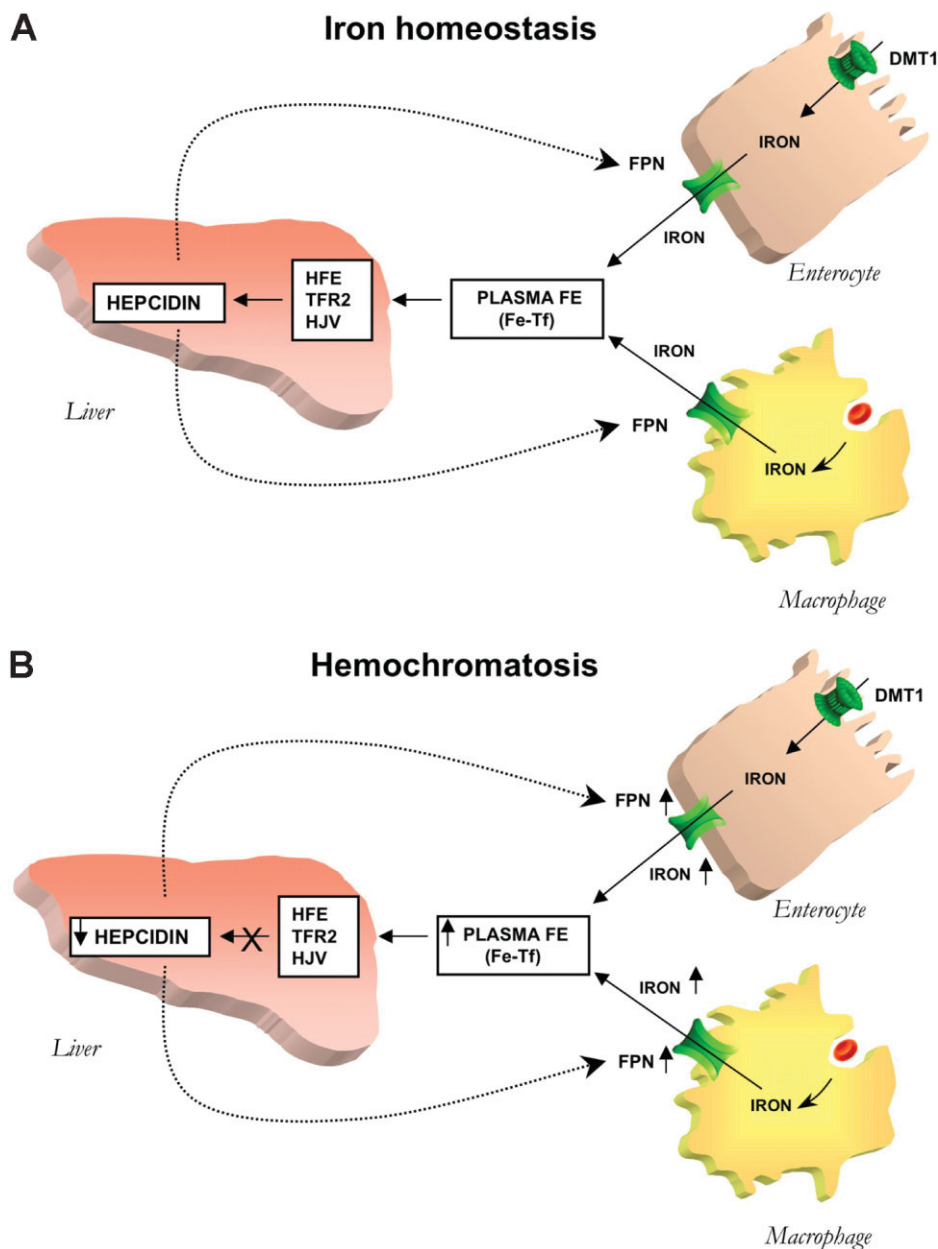


Fig. 1. (A) Under normal conditions, plasma transferrin saturation regulates the expression of liver hepcidin via a HFE, TFR2, and BMP-HJV signaling pathway is secreted into the blood, binding to FPN in the intestine and macrophages inducing FPN internalization and degradation, limiting intestinal Fe absorption and Fe recycling by macrophages to maintain plasma transferrin saturation. (B) In HH, mutations in *HFE*, *HJV*, and *TFR2* impair hepcidin synthesis, increasing FPN levels and Fe release from intestinal cells and macrophages, elevating plasma transferrin saturation and causing deposition of iron in the liver and other tissues.

hepatic iron loading associated with alcoholic liver disease and chronic hepatitis C.<sup>31,32</sup>

## Definition and Classification of Hemochromatosis

Hemochromatosis can be broadly defined as any disorder characterized by iron deposition and tissue injury in multiple organs.<sup>33</sup> The most common of the inherited forms of hemochromatosis, and the principal focus of this review, is type 1 or *HFE*-related HH (hereafter termed *HFE*-HH). It is an autosomal recessive disorder resulting in iron overload and variable multiorgan dysfunction. The *HFE* gene was identified on the short arm of chromosome 6 by Feder et al. in 1996.<sup>34</sup> Approximately 90%

of subjects with HH who are of northern European descent are homozygous for the missense mutation that results in the substitution of tyrosine for cysteine at amino acid 282 (C282Y).<sup>35</sup> A more common mutation is the substitution of aspartate for histidine at amino acid 63 (H63D); this mutation may contribute to minor increases in iron levels but rarely causes iron overload in the absence of C282Y. Approximately 1%-5% of subjects with HH may be compound heterozygous for both the C282Y and the H63D mutations.<sup>36,37</sup> Other rare *HFE* mutations have also been described.<sup>38</sup>

Other well-defined but rare genetic disorders of iron metabolism result from mutations in additional recently discovered genes (Table 1). Type 2 or juvenile hemochro-

**Table 1. Classification of Iron Overload Syndromes**

<b>Hereditary Hemochromatosis</b>
Type 1 ( <i>HFE</i> -related)
C282Y homozygous
C282Y/H63D compound heterozygous
Other <i>HFE</i> mutations
Type 2 (juvenile hemochromatosis)
A. Hemojuvelin ( <i>HJV</i> ) mutations
B. Hepcidin ( <i>HAMP</i> ) mutations
Type 3 ( <i>TFR2</i> mutations)
Type 4 (ferroportin mutations)
A. Loss-of-function
B. Gain-of-function
<b>Secondary Iron Overload</b>
Iron-loading anemia
Parenteral iron overload
Long-term hemodialysis
Chronic liver disease
Alcoholic liver disease
Hepatitis B or C
Porphyria cutanea tarda
Nonalcoholic steatohepatitis
<b>Miscellaneous</b>
Congenital alloimmune hepatitis (neonatal iron overload)
African iron overload
Aceruloplasminemia
Atransferrinemia

Adapted from Galhenage et al.<sup>143</sup>

matoxis (JH) differs from *HFE*-HH in that it has an earlier age of onset of manifestations of iron overload, usually by the second or third decade of life.<sup>39,40</sup> JH is an autosomal recessive disorder affecting both genders equally and is relatively rare.<sup>40</sup> Clinical manifestations are severe and include early hepatic iron loading, cardiac failure and arrhythmias, diabetes, hypogonadism resulting from pituitary involvement, and occasionally joint disease.<sup>39</sup> Most JH patients do not survive if they are not treated using phlebotomy, with death usually due to cardiomyopathy.

Recent advances in the understanding of JH have been due to the detection of mutations in new key proteins of iron metabolism. Two subtypes of JH have been identified that are due to mutations in the *HJV* gene or the *HAMP* gene.<sup>40-42</sup> *HJV*-related JH occurs more frequently than *HAMP*-related JH, but both are rare. The severe nature of JH indicates that both *HJV* and hepcidin have a critical role in the regulation of iron metabolism.

Type 3 or transferrin receptor 2 (*TFR2*)-related HH is an iron overload disorder with autosomal recessive inheritance that manifests in the third to fourth decade of life.<sup>40</sup> It was originally described in southern Italy and results from mutations in the *TFR2* gene.<sup>43-46</sup> *TFR2* mutations confer a very similar clinical phenotype to that of *HFE*-HH, although these patients exhibit greater variation in the severity of symptoms. *TFR2* mutations have been reported in Italian, Portuguese, French, and Japanese fam-

ilies.<sup>43-49</sup> Treatment is accomplished using phlebotomy therapy to reduce iron stores.

Type 4 or *FPN*-related HH is inherited in autosomal dominant fashion. Age of clinical onset for this disease is in the fourth to fifth decade of life.<sup>40</sup> Two groups independently reported that mutations in the *FPN* gene<sup>50,51</sup> were responsible for this form of HH. *FPN*-related HH has been reported in a number of groups such as French, Solomon Islander, African, African-American, Spanish, and Indian persons.<sup>52-56</sup> Some of these subjects may develop hepatic, cardiac, and pancreatic complications similar to that described in other types of HH.

There is now a consensus that there are two categories of *FPN* mutations (Table 1). The first category includes "loss-of-function" mutations that reduce the cell surface localization of *FPN*, reducing its ability to export iron. This results in iron deposition primarily in macrophages,<sup>40,50,57</sup> and this disorder is sometimes termed "ferroportin disease". Treatment of iron overload in subjects with this type of HH is problematic because the nature of the underlying disorder limits the ability of phlebotomy therapy to mobilize iron stores. The second category includes "gain-of-function" *FPN* mutations that do not alter cell surface expression but rather abolish hepcidin-induced *FPN* internalization and degradation.<sup>58-60</sup> Distribution of iron is similar to *HFE*-HH, being primarily parenchymal. Treatment of individuals with this disorder is similar to that for *HFE*-HH.

Secondary causes of iron overload are common (Table 1) and are reviewed elsewhere.<sup>61</sup> Other iron overload disorders such as aceruloplasminemia, atransferrinemia, and neonatal hemochromatosis are extremely rare conditions.<sup>62,63</sup> While rare, there have been significant advances in the understanding of the pathogenesis of neonatal hemochromatosis. Many cases are now considered to be caused by congenital alloimmune hepatitis<sup>64</sup>; immune-mediated liver injury in the fetus may be associated with the development of iron overload, potentially via loss of hepcidin secretion or redistribution of iron due to hepatocellular necrosis. Iron appears to play little or no role in the pathogenesis of the liver injury. Treatment with intravenous immunoglobulin during pregnancy has been shown to markedly slow or prevent the development of neonatal hemochromatosis.<sup>64</sup>

## Pathogenesis of Hereditary Hemochromatosis

In all types of HH, iron overload results from the impairment of the hepcidin-*FPN* regulatory pathway. In humans and mice, mutations or the absence of *HFE*, *HJV*, *HAMP*, and *TFR2* genes, which cause HH types 1, 2A, 2B, and 3, respectively, reduce hepcidin expres-



sion.<sup>41,65-67</sup> Furthermore, “gain-of-function” mutations in *FPN*, which cause HH type 4B, prevent the hepcidin-mediated degradation of *FPN*.<sup>58</sup> In all types of HH (except type 4A), reduced hepcidin expression or activity results in increased expression of *FPN* protein, thereby enhancing iron export from enterocytes and macrophages (Fig. 1B). The up-regulation of both iron absorption and mobilization elevates plasma transferrin saturation and NTBI levels leading to iron loading of hepatocytes and other parenchymal cells, while macrophages remain relatively spared of iron.<sup>68</sup>

HFE is a major histocompatibility class I–like protein that forms a complex with  $\beta_2$ -microglobulin.<sup>34</sup> The HFE C282Y mutation prevents the interaction between these two proteins, impairing intracellular trafficking and abrogating the surface expression of the complex.<sup>69</sup> HFE is highly expressed in hepatocytes where it plays an essential role in the regulation of iron metabolism, as evident by hepatocyte-specific knockout of the *HFE* gene that causes iron overload.<sup>70</sup> HFE has been shown to bind to TFR1 and more recently also to TFR2.<sup>71,72</sup> The binding sites for HFE and diferric transferrin on TFR1 overlap, and HFE competes with diferric transferrin for binding to TFR1.<sup>73</sup> HFE has also been shown to modify the cellular uptake of transferrin-bound iron.<sup>74</sup> HFE binds to TFR2 at a site that differs from the site of HFE and TFR1 interaction, and is independent of the TFR2-transferrin binding domain.<sup>75</sup> Both HFE and diferric transferrin independently increase TFR2 expression,<sup>75,76</sup> and HFE increases the affinity of diferric transferrin for TFR2 and enhances cellular uptake of transferrin-bound iron.<sup>77</sup>

A BMP-dependent signaling pathway is now considered to play a key role in iron-induced regulation of hepcidin expression.<sup>28,78-80</sup> BMPs bind to specific receptors on hepatocytes, and this triggers SMAD-dependent activation of hepcidin expression. Selective inhibition of BMP signaling abrogates iron-induced up-regulation of hepcidin.<sup>80</sup> Interestingly, HJV is a BMP coreceptor, facilitating the binding of BMP to its receptor: knockout of *HJV* markedly decreases BMP signaling and hepcidin expression and causes iron overload.<sup>78</sup> The molecular mechanisms by which HFE and TFR2 influence iron-dependent regulation of hepcidin remain unclear. Both HFE and TFR2 may be able to interact with HJV, suggesting that a complex of HFE and TFR2 may play a regulatory role on BMP signaling.<sup>78</sup> One proposed model suggests that the complex of TFR1 and HFE acts as an iron sensor at the cell membrane of the hepatocyte: as the transferrin saturation increases, diferric transferrin displaces HFE from TFR1, thereby making HFE available to bind to TFR2. The complex of HFE and TFR2 is then postulated to influence hepcidin expression.<sup>71,78,81</sup> It is

also possible that TFR2 may serve as an iron sensor, and that HFE modulates TFR2 signaling between plasma diferric transferrin levels and hepcidin expression.<sup>77</sup> Further investigations are required to elucidate the details of the iron regulatory signaling pathway that controls hepcidin expression and how it is impaired in HH.

## Hepatic Pathology in HFE-HH

The progressive deposition of iron in the liver in *HFE*-HH can result in fibrosis and ultimately cirrhosis.<sup>82</sup> In early HH, iron remains localized within hepatocytes along the pericanalicular axis of the cell.<sup>83</sup> Iron is distributed within the hepatic lobule in a decreasing gradient from periportal to centrilobular regions.<sup>83</sup> Increasing levels of hepatic iron increase the risk of cirrhosis,<sup>84</sup> and age and duration of iron loading have been shown to play a significant role in the development of advanced fibrosis.<sup>85</sup> There is a threshold of hepatic iron concentration associated with the development of cirrhosis.<sup>86,87</sup> Substantial hepatocyte and Kupffer cell iron loading is required before fibrosis becomes evident.<sup>88,89</sup> Unlike many common liver diseases, hepatic injury in *HFE*-HH develops in the absence of significant necrosis or marked inflammation.<sup>90</sup> Mild foci of liver inflammation have been described along with sideronecrosis,<sup>91,92</sup> the latter usually occurring at extreme levels of hepatic iron loading.<sup>92</sup> Sideronecrosis is thought to be responsible for macrophage activation, which may lead to both the development of fibrosis and redistribution of iron toward nonparenchymal cells. It has been hypothesized that leukocytes and activated Kupffer cells may release proinflammatory and profibrogenic cytokines in the absence of an overt inflammatory infiltrate leading to hepatic fibrogenesis.<sup>93,94</sup> Although a potential role for inflammatory mediators in the pathogenesis of iron-induced fibrosis has been proposed, the sources of these mediators remain to be elucidated.

## Mechanisms of Iron-Induced Tissue Injury

The excessive absorption of iron leads to cellular injury through the Fenton and Haber-Weiss reactions which generate oxyradicals; these, in turn, promote lipid peroxidation of organelle membranes.<sup>82,95</sup> This damage to hepatocellular lysosomes and mitochondria has been hypothesized to contribute to local hepatocyte injury, necrosis, and apoptosis, as demonstrated in animal models of iron overload (reviewed in Philippe et al.<sup>95</sup>). However, there is little definitive evidence showing that this process occurs in individuals with *HFE*-HH. When present, excessive alcohol consumption,<sup>96</sup> chronic infection with hepatitis C virus,<sup>97</sup> and obesity-related steatosis<sup>98</sup> have been shown to act as cofactors in the development of

fibrosis and cirrhosis in *HFE*-HH.<sup>95,99</sup> In non-HH liver disease, iron may be a cofactor in exacerbating liver injury.<sup>100</sup> Recently, it has been reported that iron-dependent oxidation of uroporphyrinogen produces uroporphomethene, an inhibitor of uroporphyrinogen decarboxylase, that causes porphyria cutanea tarda.<sup>101</sup>

Hepatic stellate cells (HSCs) are critical for the development of hepatic fibrosis following their transformation to myofibroblasts.<sup>82,95</sup> Numerous studies have assessed the potential stimuli associated with iron-induced hepatocellular injury causing HSC activation in *HFE*-HH.<sup>95</sup> Lipid peroxidation leads to the production of malondialdehyde and 4-hydroxynonenal, which form adducts with cellular DNA and proteins leading to cellular injury. While there is some evidence for a role of lipid peroxidation in inducing HSC activation,<sup>102,103</sup> it is generally thought that oxidative stress-derived events perpetuate, rather than initiate, the activated HSC phenotype.<sup>104,105</sup> A direct role of chelatable iron in the induction of HSC activation is controversial.<sup>106-109</sup>

Injury to hepatocytes and subsequent phagocytosis by Kupffer cells<sup>110</sup> may lead to the generation of a variety of different growth factors and cytokines which promote HSC activation.<sup>95,111</sup> The iron-binding proteins transferrin<sup>112</sup> and ferritin<sup>113</sup> have been shown to play a direct role in altering the phenotype of activated HSCs. Highly specific receptors for these molecules have been characterized on the activated stellate cell,<sup>112,114</sup> and recent preliminary evidence suggests a role for a specific ferritin-induced intracellular signaling pathway which regulates inflammatory gene expression in these cells.<sup>115</sup>

## Key Developments in *HFE*-HH

Clinical expression and penetrance of *HFE*-HH is not as high as previously thought. The classical progression of iron accumulation to iron overload is not invariable.<sup>116</sup> Iron stores can vary as much as 10-fold between patients, and this variability is likely governed by genetic as well as environmental factors such as diet, alcohol, blood loss, and blood donation.<sup>95,99</sup> Although large, systematic, population-based cross-sectional studies have shown that the majority of C282Y homozygotes have increased transferrin saturation and serum ferritin levels, approximately 25%-35% of homozygous individuals have normal serum ferritin levels and may not develop iron overload.<sup>36,117,118</sup> The majority of C282Y homozygotes do not have iron overload-related disease. Iron overload-related disease is defined as documented iron overload associated with well-accepted clinical criteria of hepatocellular carcinoma, hepatic fibrosis or cirrhosis, elevated alanine aminotransferase, or arthritis of the second and/or third metacarpophalangeal joints. Cross-sectional studies indi-

cate that cirrhosis occurs in 1%-10% of C282Y homozygotes.<sup>119-121</sup> Recently, the clinical penetrance of *HFE*-HH has been characterized in a large Australian longitudinal study of 31,000 adults for a 12-year period.<sup>120</sup> *HFE* genotyping identified 203 individuals who were C282Y homozygotes, and careful clinical assessment was used to define iron overload-related disease in comparison with appropriately matched controls. A total of 28% of men and 1% of women developed definite iron overload-related disease. There were no differences in the prevalences of diabetes or mortality between C282Y homozygotes and the control population, similar to other large population studies.<sup>119</sup> In men, the most common clinical features were fatigue (24%), arthritis (52%), and liver disease (17%) while in women, the most common clinical feature was arthritis (35%). Men with a serum ferritin level of greater than 1000 ng/mL had an increased risk of fatigue and liver disease compared with men who had serum ferritin levels less than 1000 ng/mL. The presence of arthritis was not related to the severity of iron overload. The prevalence rates of hepatic fibrosis and cirrhosis in male C282Y homozygotes were 14% and 3%, respectively.<sup>120</sup> It is possible that the risk of cirrhosis was underestimated because only 43% of C282Y homozygotes underwent liver biopsy. These values are slightly lower than those reported in other *HFE* genotype screening studies, probably because of population differences.<sup>86,122,123</sup>

It is clear from published studies that other genetic and environmental factors are involved in modifying the clinical and biochemical penetrance of C282Y homozygosity.<sup>99</sup> The increased prevalence of iron overload-related disease in C282Y homozygous men versus women is often explained on the basis of recurrent physiological blood loss in women. However, the observation of frequency disparities in human leukocyte antigen haplotypes A\*03-B\*07 in men and women may point to other genetic factors which regulate clinical expression in a gender-selective manner.<sup>124</sup>

Environmental factors such as blood loss, diet, and the presence of other liver injury processes may also contribute to the development of iron overload-related disease. It is recognized that dietary iron intake, alcohol consumption, and more recently noncitrus fruit intake, can all modify the degree of iron accumulation independently of *HFE* status.<sup>98,99,125,126</sup>

Given the clinical sequelae of heavy iron overload that occur in some patients with *HFE*-HH, early detection is important because it may result in earlier treatment with phlebotomy.<sup>127</sup> Although there have been no randomized studies demonstrating the clinical benefits of phlebotomy, it is standard of care to institute therapy when elevated iron stores and symptoms indicate a requirement to

do so.<sup>128,129</sup> It is assumed on the basis of the known pathogenesis of iron-induced liver injury that phlebotomy therapy may prevent or minimize the severity of progression of liver disease. Life expectancy is reduced in patients with HH and cirrhosis, but not in those without cirrhosis, compared to an age-matched and sex-matched normal population. Compared to the normal population, liver cancer is 100-fold to 219-fold more frequent, cardiomyopathy is 306-fold more frequent, and cirrhosis is 13-fold more frequent in patients with HH.<sup>130</sup> However, given the variability of phenotypic expression and nonspecific nature of symptoms such as lethargy, arthralgia, or abdominal pain, the diagnosis can be difficult to make.<sup>118</sup> Clinicians should have a high index of suspicion in individuals with a family history of *HFE*-HH, and individuals with hepatomegaly, abnormal iron studies, abnormal liver function tests, porphyria cutanea tarda, peripheral arthritis, early onset impotence, infertility, or dilated cardiomyopathy. The most useful biochemical tests of phenotype are serum transferrin saturation and ferritin level. A raised transferrin saturation is the earliest phenotypic marker for *HFE*-HH.<sup>36,131</sup> Biochemical testing is most beneficial when undertaken in individuals aged 40 years or older, because individuals who are homozygous for C282Y may have relatively normal iron stores in their third and fourth decades, yet may progress and develop significant iron overload and organ injury in their fifth decade or later.<sup>132</sup> Sequential measurements of transferrin saturation and serum ferritin may be required over a period of many years to monitor or confirm nonexpression in younger adults who are homozygous for C282Y.<sup>132</sup>

Serum ferritin levels correlate with total body iron stores but are not as sensitive as transferrin saturation in detecting early disease. Ferritin levels can vary significantly over time and may fluctuate in the presence of inflammation, hepatocellular necrosis, and malignancy due to increase in its induction via inflammatory cytokines. Most subjects with significant fibrosis have serum ferritin levels greater than 1000 ng/mL.<sup>132,133</sup> Given that the main reason for early diagnosis is the prevention or identification of treatable iron overload-related disease, a recent study by Waalen et al.<sup>134</sup> has argued that evaluation using only serum ferritin to detect those subjects with substantially elevated ferritin levels (>1000 ng/mL) is clinically appropriate, and subjects who do not have high ferritin levels are not at risk for iron overload-related disease. This recommendation was based on the observation that serum ferritin levels do not rise over long periods of time in the majority of adults homozygous for C282Y.<sup>134</sup> However, caution should be taken in applying this observation because previous studies in subjects with biopsy-documented significant fibrosis or cirrhosis due to

*HFE*-HH have also shown that ferritin does not always rise in these subjects.<sup>132</sup> Also, in the setting of chronic liver disease, ferritin levels may not accurately reflect iron load and are influenced by the presence of inflammation.<sup>61,100,135</sup> Most physicians would elect to determine the hepatic iron concentration or total body iron burden by noninvasive or invasive methods, or implement therapeutic phlebotomy if there was any doubt as to iron status in *HFE*-HH.

Liver biopsy has been the “gold” standard for the diagnosis of hepatic fibrosis and cirrhosis as well as the quantitation of hepatic iron content. Liver biopsy should be considered in addition to genotyping if patients have a serum ferritin level of greater than 1000 ng/mL or abnormal liver enzyme levels.<sup>133,136,137</sup> Utilization of these criteria will identify the vast majority of C282Y homozygotes who are at risk of advanced fibrosis or cirrhosis, allowing liver biopsy to be used more selectively. Serum levels of collagen type IV have been proposed as a surrogate marker of progressive hepatic fibrosis in *HFE*-HH.<sup>138</sup> In a large population screening study that did not include liver biopsy, 25% of C282Y homozygotes had elevated serum collagen type IV levels, suggesting the presence of fibrosis.<sup>121</sup> This value is similar to other studies which demonstrated that biopsy-proven fibrosis is present in approximately 15%-25% of individuals with *HFE*-HH.<sup>86,87,120,139</sup> Other novel serum-based assessments of fibrosis have also been reported but have yet to be validated in the clinic.<sup>138,140</sup> There is an emerging role for the use of magnetic resonance imaging (MRI) as an accurate, noninvasive measurement of hepatic iron content and the possible detection of fibrosis. There is a high correlation between the mean liver proton transverse relaxation rates measured using MRI and the biochemical liver iron content. MRI provides a high degree of sensitivity and specificity for measurement of liver iron concentration with an area under the receiver operating characteristic curve greater than 0.98.<sup>141,142</sup> Furthermore, MRI can provide information regarding the stage of fibrosis.<sup>141,142</sup> In a recent study of 18 subjects with HH with high-grade fibrosis and 42 subjects with HH with low-grade fibrosis, hepatic iron concentration alone had a high sensitivity (100%) but low specificity (67%) in the diagnosis of high-grade fibrosis. However, the product of (hepatic iron concentration  $\times$  age) had a sensitivity and specificity of 100% and 86%, respectively, for diagnosis of high-grade fibrosis, and patients were accurately assigned to fibrosis groups via the use of MRI.<sup>85</sup>

Currently, the American Association for the Study of Liver Diseases (AASLD) guidelines recommend *HFE* genotyping in individuals who have abnormal serum iron studies (transferrin saturation, ferritin) and in first-degree



relatives of those identified with C282Y homozygosity.<sup>129</sup> There is general consensus that genetic screening for *HFE*-HH in the general asymptomatic population is not warranted.<sup>128,129,143</sup>

## Concluding Remarks

Since the discovery of *HFE* in 1996, there have been profound advances in the understanding of the regulation of iron metabolism and in the molecular basis of several forms of HH. The discovery of the iron-regulatory hormone, hepcidin, was a landmark event. Excess iron has deleterious effects on the liver, heart, and endocrine organs: progress has been made in understanding the mechanisms of iron-induced cellular injury and hepatic fibrosis. Large population screening studies using *HFE* genotyping revealed that C282Y homozygosity is common in persons of northern European ancestry, but that biochemical and clinical penetrance is lower than once thought. In addition to environmental factors that contribute to the variability in penetrance in C282Y homozygotes, it seems clear that there are important genetic modifiers that remain to be discovered. Future investigations are anticipated to provide new insights into the regulation of iron metabolism, and to elucidate the key genetic and environmental modifiers which underlie the variability in clinical expression in HH.

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