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Hereditary hemochromatosis: update for 2003

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1. Introduction

The term 'hemochromatosis' was first used by von Recklinghausen, a German pathologist in the late 1800s [1]; he determined that the pigmentation seen in patients with advanced hemochromatosis was due to iron. In 1935, Joseph Sheldon, a British geriatrician, published a monograph describing over 300 patients with hemochromatosis [2]. He concluded that the disorder was an inherited defect with all of the pathology caused by excess iron in involved tissues. In the mid-1970s, Simon and colleagues in northern France determined definitively that hereditary hemochromatosis (HH) was inherited as an autosomal recessive disorder linked to the short arm of chromosome 6 in the region of HLA-A3 [3]. Finally, in 1996, a team of molecular geneticists at a small biotechnology company called Mercator Genetics in California used a positional cloning technique to discover the gene responsible for HH [4]. It was first called *HLA-H* and was later renamed *HFE*. Since that important discovery, tremendous advances in our understanding of the pathophysiologic mechanisms that occur in HH have taken place; further, our ability to diagnose patients, screen families, and evaluate whole populations has been enhanced.

2. Classification of iron overload syndromes

Iron overload is a common problem found in clinical practice and can be classified as shown in Table 1. It is important to distinguish whether iron overload is primary (i.e. as a result of an inherited metabolic disorder) or whether it is secondary to another problem that results in an increase in iron absorption. The most common form of hereditary hemochromatosis is *HFE*-related HH that affects between one and 200 and one in 400 individuals of northern European descent [5,6]. *HFE*-related HH is characterized by increased gastrointestinal iron absorption with subsequent tissue iron deposition in the liver, heart, pancreas, other endocrine organs, joints and skin. The *HFE* gene encodes a major histocompatability complex (MHC) class 1-like protein that may modulate cellular iron transport by binding to transferrin receptor-1 (TfR-1) [7–9]. Recent advances in the genetics of HH have allowed us to identify patients who have mutations in the *HFE* gene: most cases of *HFE*-related HH are caused by the C282Y mutation. Accordingly, we are no longer confined to the old definition of HH which identified patients who had abnormal iron studies with stainable iron in hepatocytes on liver biopsy and/or patients who had symptomatic disease and who developed end organ damage including cirrhosis, heart failure, diabetes, arthritis, or skin pigmentation. Presently, if both alleles for *HFE* have the C282Y mutation and if the individual has direct or indirect markers of iron overload, they should be considered to have *HFE*-related HH.

With the advent of *HFE* genotyping, population studies have revealed that a substantial proportion of C282Y homozygotes do not have an increase in iron stores [10]. These individuals do not show evidence of phenotypic expression of the disorder and their long-term prognosis is not clear. The sub-classification of patients based on their genetics and phenotype has focused on three characteristics: (1) their genetic susceptibility, (2) their iron study results, and (3) their symptoms. Individuals with inherited iron overload syndromes can be sub-classified into four distinct groups: (1) genetic predisposition with no other abnormalities, (2) iron overload without symptoms, (3) iron overload with early symptoms, and (4) iron overload with organ damage. This classification system has become increasingly popular for patients with *HFE*-related HH.

Over the last several years, it has become increasingly recognized that there are other inherited forms of iron overload that are not caused by *HFE* mutations. These include recognition of families with mutations in the genes for ferroportin 1 [11,12] and transferrin receptor-2 (TfR-2) [13,14]. A mutant mouse model for TfR-2-related HH has recently been developed [15]. Juvenile hemochromatosis (linked to a locus on chromosome 1q) [16] and African iron overload [17,18] are also considered to be inherited disorders. African iron overload is caused by increased absorption of iron that occurs in some individuals from sub-Saharan Africa and in some African–Americans.

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Secondary iron overload occurs when there is a stimulus to absorb increased amounts of iron from the gastrointestinal tract that is independent from HH [19]. Examples include the various iron-loading anemias such as thalassemia major, sideroblastic anemia, and certain hemolytic anemias. Some patients with chronic liver disease such as non-alcoholic steatohepatitis, hepatitis C, and alcoholic liver disease have mild degrees of secondary iron overload [20–24]. These patients usually have iron deposition within sinusoidal lining cells and is not limited to hepatocytes as is seen with *HFE*-related HH.

Parenteral iron overload is caused by transfusions of red blood cells or injections of iron–dextran given by physicians to patients who are anemic [25]. Some patients with ineffective erythropoiesis (e.g., thalassemia) have increased iron loading from both the stimulus caused by anemia and from receipt of blood transfusions [19,25]. Neonatal iron overload is a rare disorder that causes serious liver injury in infants and is almost always fatal [26,27].

3. *HFE* gene and protein

In the 1970s, Marcel Simon and colleagues identified HH as a disorder that is linked to a genetic locus on the short arm of chromosome 6 in the region of HLA-A3 and that is inherited in an autosomal recessive fashion [3]. About 20 years later, Feder and colleagues used a positional cloning technique to identify a novel gene called *HFE* in the HLA region of chromosome 6 [4]. This gene encodes a MHC class 1-like protein that binds to β_2-microglobulin (β_2M) (like other MHC class 1 molecules) and that interacts with TfR-1 [7–9,28]. HFE protein has a large extracellular domain, a single transmembrane region, and a short cytoplasmic tail. In the initial study published in 1996, two missense mutations were identified in *HFE* [4]. One results in the change of a cysteine to a tyrosine at amino acid position 282 (C282Y). This amino acid substitution prevents the binding of HFE protein to β_2M by disrupting a disulfide bridge in the extracellular domain and thereby decreases the amount of HFE protein expressed on the cell surface [7–9]. The second mutation in *HFE* results in a change in histidine to aspartate at amino acid position 63 (H63D) and is found in 15–20% of the general population [29,30]. This abnormality does not inhibit the binding of HFE protein to β_2M, and individuals who are homozygous for the H63D mutation have only mild iron accumulation [31]. However, some individuals who carry both the C282Y and H63D mutations (compound heterozygotes) have clinically significant iron overload [29,30]. More recently, another mutation has been identified in *HFE* which results in a change of serine to cysteine at amino acid position 65 (S65C), which is very close to the H63D mutation. This mutation occurs infrequently but some C282Y/S65C individuals have mild iron loading [32]. In the original work by Feder and colleagues, 83% of 178 phenotypic HH patients were found to be homozygous for the C282Y mutation and 4% were compound heterozygotes (C282Y/H63D) [4].

Numerous other genotyping studies have confirmed the findings of Feder et al. and demonstrate that approximately 85% of typical HH patients from around the world are homozygous for C282Y [29,30] although there is some regional variation [33]. The development of mice with targeted disruption of the *HFE* gene (*HFE* knockout mice) validated the importance of *HFE* in the development of iron overload [34–36]. These *HFE* knockout mice have increased iron absorption, elevated transferrin saturation levels and increased hepatocellular iron storage similar to humans with *HFE*-linked HH [34–37].

4. Pathophysiology of hemochromatosis

The primary defect in *HFE*-related HH is an increase in intestinal iron absorption relative to body iron stores [29,30,38,39]. Recent advances have provided a better understanding of the proteins involved in maintaining iron homeostasis and their potential involvement in the pathophysiology of HH.

Table 1
Iron overload conditions

- Hereditary hemochromatosis
  - *HFE*-related
    - C282Y/C282Y
    - C282Y/H63D
    - Other *HFE* mutations
  - Non-*HFE*-related
    - Juvenile hemochromatosis (*HFE* 2)
    - Transferrin receptor-2 mutations (*HFE* 3)
    - Ferroportin 1 mutations (*HFE* 4)
    - African iron overload
- Secondary iron overload
  - Acquired iron overload
    - Iron-loading anemias
      - Thalassemia major
      - Sideroblastic anemia
      - Chronic hemolytic anemia
      - Aplastic anemia
      - Pyruvate kinase deficiency
      - Pyridoxine-responsive anemia
    - Parenteral iron overload
      - Red blood cell transfusions
      - Iron–dextran injections
      - Long term hemodialysis
    - Chronic liver disease
      - Porphyria cutanea tarda
      - Hepatitis C
      - Hepatitis B
      - Alcoholic liver disease
      - Non-alcoholic steatohepatitis
      - Following portocaval shunt
    - Dysmetabolic iron overload syndrome
  - Miscellaneous
    - Neonatal iron overload
    - Acerulopasminemia
    - Congenital atransferrinemia

Normally, total body iron content ranges from 3 to 5 g, and 20–30 mg of iron are recycled daily, mainly through phagocytosis of senescent erythrocytes by macrophages [38]. Only 1–2 mg of iron per day are typically lost from the body, and females have an increased physiologic loss of iron through menstruation. Accordingly, 1–2 mg of iron are absorbed daily from the diet to maintain normal iron homeostasis [38,39]. Homeostasis of iron is achieved through a complex array of biochemical processes within the crypt cell of the duodenum, the enterocyte on the duodenal villus, the hepatocyte, and the cells of the reticuloendothelial system. Iron absorption is regulated by two principal factors, the 'store regulator' and the 'erythroid regulator' [38,40]. The store regulator is responsive to the amount of iron stored in the body and one component of this regulator may be circulating diferric transferrin. Another component of the store regulator may be hepcidin, a peptide expressed primarily in the liver, that may function as a humoral factor linking the level of hepatic iron stores to the rate of iron absorption in the duodenum [41,42]. Hepcidin expression in the liver is induced by iron, and circulating hepcidin may act to downregulate iron absorption. When erythropoiesis is stimulated, iron absorption is enhanced via the erythroid regulator, whose identity is currently unknown [38,40].

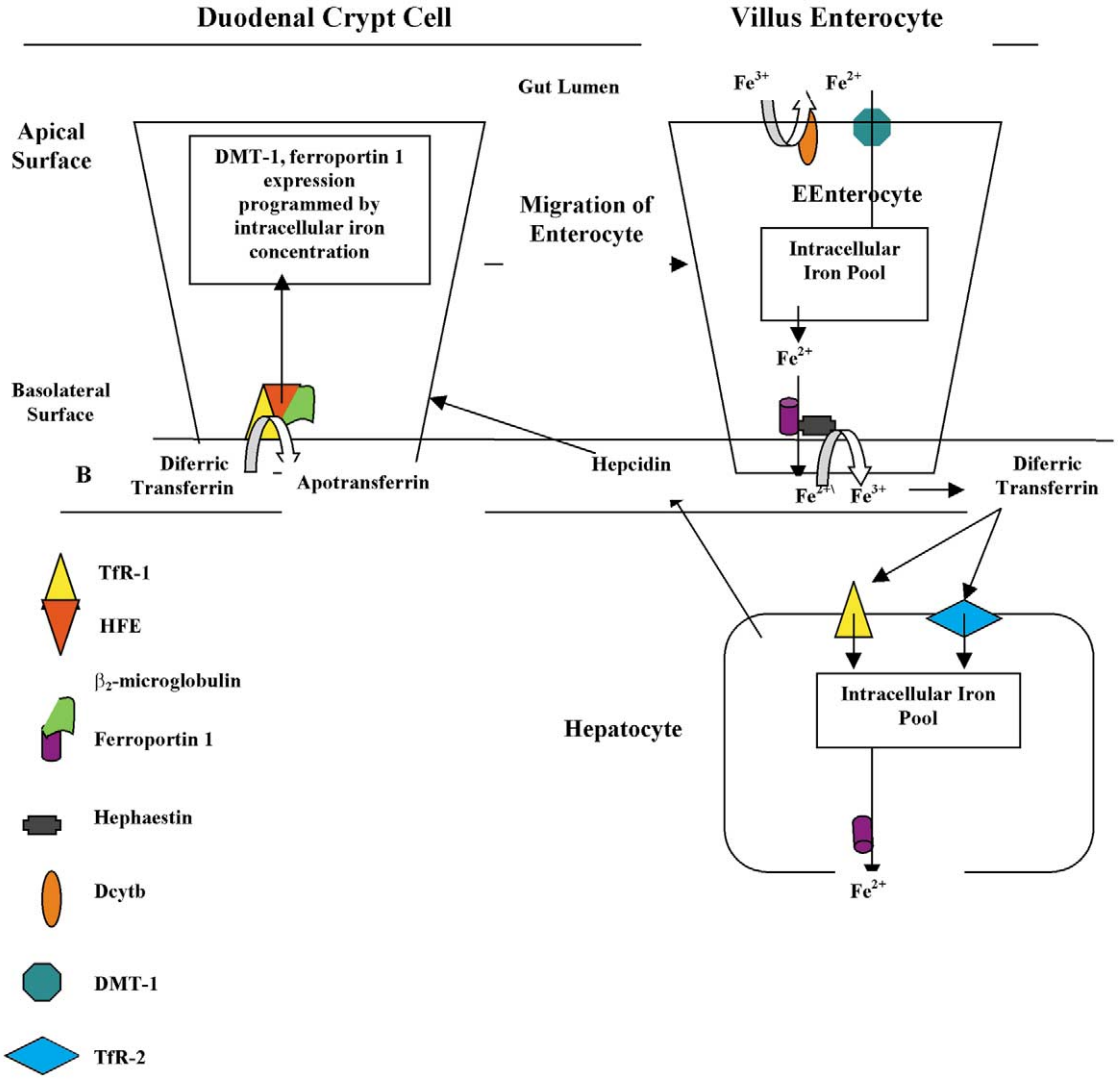

Fig. 1. Dietary iron absorption occurs mainly in duodenal villus cells, and these cells become mature absorptive cells during migration from the crypts to the apex of the villus. The regulation of iron absorption is thought to occur at the level of the duodenal crypt cell that expresses both TfR-1 and HFE protein. Body iron stores are sensed by crypt cells through the action of the store regulator that may consist of circulating diferric transferrin and hepcidin. HFE protein may act to facilitate the uptake of transferrin-bound iron by crypt cells. Ionic iron in the diet requires reduction from the ferric to the ferrous state prior to uptake by villus enterocytes. This is performed by the ferric reductase, Dcytb, located on the apical surface of duodenal enterocytes and the ferrous iron is then transported into these cells via DMT-1. Iron can be exported across the basolateral membrane of the cell via ferroportin 1, and during this process it is oxidized by hephaestin to the ferric state and binds to apotransferrin, forming diferric transferrin. Both TfR-1 and TfR-2 receptors may mediate the uptake of transferrin-bound iron by hepatocytes, while iron may be transported out of hepatocytes via ferroportin 1. Hepcidin, a peptide whose expression is induced by iron in hepatocytes, may function as a humoral factor, linking hepatocyte iron stores to the rate of iron absorption by the duodenum. Patients with *HFE*-related HH have increased duodenal expression of DMT-1 and ferroportin 1.

Iron is absorbed primarily by villus enterocytes of the duodenum (Fig. 1). Lumenal ferric iron is reduced to ferrous iron by the ferric reductase, Dcytb [43], and is then transported across the apical membrane by a protein called divalent metal transporter-1 (DMT-1) [44,45]. Iron is subsequently exported from the basolateral surface of the cell by another iron transporter called ferroportin 1 [46,47]; during this process, iron is converted back to its ferric form by a ferroxidase called hephaestin [48] and is subsequently bound to apotransferrin in the portal blood stream. The levels of DMT-1 and ferroportin 1 expressed in villus enterocytes influence how much ionic iron is absorbed from the diet.

Duodenal crypt cells are thought to be responsible for the sensing of body iron status, and this process influences the level of iron absorption upon the differentiation of these cells into absorptive enterocytes and migration to the villus tip [38,39]. Circulating diferric transferrin can be taken up by crypt cells after binding to TfR-1 on the basolateral surface. Crypt cells also express HFE protein, and the complex of HFE protein and β_2M binds to TfR-1 [49]. Once diferric transferrin is bound to TfR-1, the complex undergoes endocytosis in a clatharin-coated pit that forms an endocytic vesicle within the cell [7]. The internal milieu of this vesicle becomes acidic, and iron is released from transferrin and transported to the cytoplasm. The complex of TfR-1 and apotransferrin then recycles back to the basolateral surface where apotransferrin is released at the higher extracellular pH [7]. The concentration of intracellular iron in duodenal crypt cells may play an important role in programming the eventual expression of DMT-1 and ferroportin 1 in daughter enterocytes [50,51]. This regulation may occur at the level of mRNA translation through the action of the iron regulatory protein/iron responsive element system, or at the level of gene transcription [51].

There has been intense interest in investigating the cell biology of HFE protein. A physical association between HFE protein and TfR-1 has been demonstrated in human duodenum, cultured cells and in vitro [7–9,28,49]. HFE protein and diferric transferrin have overlapping binding sites on TfR-1 [52], but the physiological consequences of the competitive binding of these two proteins to TfR-1 are still unclear. There have been many investigations on the effect of HFE protein on TfR-1-mediated iron uptake and cellular iron homeostasis, and divergent results have been reported (reviewed in [7,8,50]). For example, overexpression of normal HFE protein in HeLa cells leads to a decrease in transferrin-mediated uptake of iron by these cells. The decrease in transferrin-mediated iron uptake is, in turn, associated with a decrease in the intracellular labile iron pool, an increase in cellular iron regulatory protein binding activity, an increase in TfR-1 expression, and a decrease in intracellular ferritin levels. These observations are all consistent with a comparatively iron-deficient phenotype in HeLa cells overexpressing HFE protein. It should be noted that these studies were performed without overexpression of β_2M. It is thus possible that much of the HFE protein was abnormally processed in these cells, leading to otherwise unanticipated secondary consequences on iron homeostasis. This possibility is supported by the recent findings that overexpression of both HFE protein and β_2M in Chinese hamster ovary (CHO) cells results in an increase in TfR-1-dependent iron uptake, while overexpression of HFE protein alone results in a decrease in TfR-1-dependent iron uptake [53]. Interestingly, Montosi et al. found that uptake of iron from diferric transferrin is lower in macrophages from patients with *HFE*-related HH, and that this is reversed by approximately 50% after transfection with *HFE* [54]. Therefore, in human macrophages and in CHO cells co-expressing β_2M, wild-type HFE protein enhances transferrin-mediated iron uptake and, by inference, functional loss of HFE protein would be expected to cause a decrease in cellular iron levels.

A key question concerns the effect of HFE protein on iron sensing by duodenal crypt cells because these cells play a pivotal regulatory role in iron absorption [38,39]. It has been proposed that wild-type HFE protein acts to facilitate TfR-1-mediated iron uptake from plasma into crypt cells, and that this action of HFE protein is abrogated in *HFE*-linked

Table 2
Clinical features of patients with hereditary hemochromatosis

Symptoms
Asymptomatic
Abnormal serum iron studies on routine screening chemistry panel
Evaluation of abnormal liver tests
Identified by family screening
Identified by population screening
Non-specific, systemic symptoms
Weakness, fatigue, lethargy, apathy, weight loss
Specific, organ-related symptoms
Abdominal pain secondary to hepatomegaly
Arthralgias
Diabetes
Amenorrhea
Loss of libido, impotence
Congestive heart failure, arrhythmias
Signs
Asymptomatic
Hepatomegaly
Symptomatic
Liver
Hepatomegaly
Cutaneous stigmata of chronic liver disease
Splenomegaly
Portal hypertension (ascites, encephalopathy)
Joints
Arthritis
Joint swelling
Heart
Dilated cardiomyopathy
Congestive heart failure
Skin
Increased pigmentation
Endocrine
Testicular atrophy
Hypogonadism
Hypothyroidism

HH in which there is functional loss of HFE protein caused by the C282Y mutation [39,49,53,55]. This hypothesis predicts that when the C282Y mutation is present, there will be: (1) decreased uptake of diferric transferrin into crypt cells, (2) a decreased intracellular iron pool, and (3) increased expression of DMT-1 and ferroportin 1 in daughter villus enterocytes, leading to increased iron absorption. Thus, in the face of whole body iron overload, increased iron absorption will occur. Supporting this hypothesis are the observations that there is increased expression of DMT-1 and ferroportin 1 in the duodenum of patients with *HFE*-linked HH [56–58]. In addition, *HFE*-knockout mice models of HH show impaired duodenal uptake of circulating transferrin-bound iron [59] and increased duodenal expression of DMT-1 [60,61] and ferroportin 1 [61] These latter effects are modulated by the genetic background of the mice [61] suggesting the existence of genes that can modulate the phenotype of *HFE*-related HH.

Since the discovery of *HFE* in 1996, considerable new understanding into the physiology of normal iron absorption, cellular uptake and egress has emerged. New knowledge about the altered iron homeostasis that occurs in *HFE*-linked HH has become apparent. However, the reasons for the phenotypic variability in *HFE*-related HH are still not known and the mechanisms underlying the effects of HFE protein on duodenal iron absorption are not completely understood.

5. Iron-induced hepatotoxicity

Excess accumulation of iron within tissues can lead to significant cellular damage. Within the liver, injury may result from oxidative stress generated by iron-stimulated, free radical production within hepatocytes [62–66]. During states of persistent iron overload, there may be an increase in the low-molecular-weight intracellular pool of iron. Such low-molecular-weight iron chelates can be catalytically active in producing oxyradical species such as the hydroxyl radical and lipid peroxyl and alkoxyl radicals [62,63,66]. Oxidative stress, including lipid peroxidation, can result in mitochondrial injury, lysosomal fragility and impaired cellular calcium homeostasis [62,63]. In addition, oxidative stress (perhaps with the involvement of Kupffer cells) may stimulate hepatic stellate cells to become activated and thereby increase their production of collagen and other components of the extracellular matrix [64]. Increased fibrogenesis and ultimately cirrhosis can occur. Excess iron may also result in damage to hepatic DNA which in the presence of cirrhosis may be responsible for the increased risk of hepatocellular cancer in HH [63].

6. Clinical features of hereditary hemochromatosis

In the past, HH was recognized by a constellation of symptoms and physical findings that are related to significant iron loading in the liver, pancreas, heart, skin, and pituitary (Table 2) [25]. In recent times, many HH patients are being identified due to (1) screening laboratory data obtained as part of a routine health physical, (2) identification of *HFE* mutations in individuals as part of family screening, and (3) prospective population screening studies. When individuals are identified in these ways, the vast majority are asymptomatic and phlebotomy treatment can be started prior to the development of any significant manifestations of the disease [6]. Recent large population studies using *HFE* genotyping have observed that the incidence of symptoms in C282Y homozygotes does not differ substantially from a matched control population [67], and that cirrhosis is infrequent in C282Y homozygotes [68].

Characteristic iron studies that occur in patients with typical HH are an elevation in transferrin saturation (>45%) and an elevation in serum ferritin level [5,6]. In the absence of any other underlying disease states, serum ferritin levels are reflective of tissue iron stores. However, ferritin can be influenced by numerous other conditions such as inflammatory diseases, other chronic liver diseases, or malignancies and thus, an elevated serum ferritin in these disorders does not necessarily reflect increased iron stores [6]. Conversely, some individuals who are homozygous for the C282Y mutation do not have evidence of phenotypic expression (elevated ferritin or elevated transferrin saturation) [10,67]. The reasons for this lack of phenotypic expression are not yet known but probably relate to the existence of modifying genes. Alcohol and chronic hepatitis C are potentiating factors in the development of hepatic fibrosis in HH patients [69,70]. For example, C282Y homozygotes who drink more than 60 g of alcohol per day have a 9-fold increase in the prevalence of cirrhosis [69].

7. Diagnosis of hemochromatosis

The inclusion of iron studies during routine clinical visits coupled with the availability of *HFE* genotyping for family and population studies has facilitated the detection of HH [5,6]. It has also led to identification of C282Y homozygotes who do not have phenotypic expression. In the past, liver biopsy provided important information to determine whether or not a patient had HH; liver biopsy is no longer considered essential for the diagnosis of HH. Nonetheless, it still provides an important assessment of liver fibrosis and it can give the clinician a qualitative assessment of iron loading that can be useful in determining the amount of phlebotomy that may be required to eliminate excess iron stores. Therefore, liver biopsy is generally restricted to those HH patients suspected of having significant fibrosis or cirrhosis. Recent studies have examined possible criteria for liver biopsy in the era of genetic diagnosis. Guyader and colleagues in a French study of 197 C282Y homozygotes showed that none of 94 patients with a serum ferritin <1000 ng/ml, with a normal aspartate aminotransferase (AST) level, and without hepatomegaly on physical examination had severe

fibrosis [71]. Similar findings have been reported by Fletcher et al. for Australian C282Y homozygotes [69]. In addition, our group has observed that significant fibrosis is not present in HH patients when liver enzymes are normal and the patient is under 40 years of age[72]. Thus, it is currently recommended that liver biopsy is not necessary in C282Y homozygotes, if they are under 40 years of age, if liver enzymes are normal, and if serum ferritin is <1000 ng/ml.

While liver biopsies are less frequently being performed to confirm a diagnosis of HH, it is still useful to understand the findings that are apparent on biopsies. Using the Perls' Prussian blue stain for storage iron, iron can be found in an acinar gradient with the highest amount in periportal hepatocytes [73]. Iron is typically in hepatocytes rather than in sinusoidal lining cells (Kupffer cells). The fibrosis that occurs in HH is portal-based and, in the absence of complicating factors, cirrhosis is uncommon at hepatic iron concentrations below 16,000 μg/g dry wt [69,74]. The hepatic iron index (HII) is the ratio of the hepatic iron concentration (in μmols/g dry wt) divided by the age of the patient (in years) [5]. The development of the HII was based on the concept that with increasing age there was an increase in hepatic iron concentration in HH. With the advent of genetic testing, the diagnostic utility of the HII is much diminished. However, when liver biopsy is done in a suspected HH patient, it is important to measure the hepatic iron concentration to determine the relative degree of iron loading.

8. Treatment of hemochromatosis

Once the diagnosis of HH is confirmed, the therapeutic approach is relatively simple and quite effective. Weekly therapeutic phlebotomy should be initiated with the goal of removing 250 mg of iron with each phlebotomy (each unit of blood contains between 200 to 250 mg of iron depending on the hemoglobin content) [6]. The goal should be to continue weekly phlebotomy until the patient's serum ferritin level is <50 ng/ml and the transferrin saturation is <50% [6]. In the uncomplicated patient, each unit of blood removed will result in a decrease in the serum ferritin level by about 30 ng/ml. This can be used as a rough guideline to predict phlebotomy requirements to deplete excess iron stores. It must be remembered that the goal of treatment is not to make patients iron deficient and/or anemic, but rather to deplete excess iron stores and to achieve serum iron values in the low normal range. Once initial therapeutic phlebotomy has been accomplished, maintenance phlebotomy should be performed with one unit of blood being removed every 2 to 4 months with subsequent assessment of iron status by measuring serum ferritin and transferrin saturation [6]. Most patients will require maintenance phlebotomy but occasionally there are some patients who do not reaccumulate excess iron. The reasons for this are unknown.

9. Family screening for hemochromatosis

Once a proband has been identified and treated, it must be remembered that this is a familial disorder and all first degree relatives should be offered the opportunity to be screened for HH [5,6,75]. Generally, this is done by measuring fasting transferrin saturation and ferritin in all first-degree relatives. Genetic testing can also be performed. If elevated iron studies are found, and/or if any relatives are C282Y/C282Y or C282Y/H63D, then therapeutic phlebotomy can be initiated, using the guidelines mentioned above. For evaluation of children of a proband, it is often appropriate to first perform *HFE* mutation analysis in the other parent [75]. This may obviate the need to do blood testing in the children. For example, if the proband is a C282Y homozygote and the other parent does not carry either the C282Y or H63D mutation, then it is known that the children will be obligate C282Y heterozygotes and will not be at any increased risk for iron loading. This avoids 'labeling' the child with a genetic diagnosis which may have some consequences in terms of genetic discrimination from both an insurance standpoint or a job standpoint. This is an issue in the United States and other countries that have not yet prohibited insurance discrimination on the basis of a genetic diagnosis.

10. Population screening for hemochromatosis

After the original discovery of *HFE*, it was suggested that population screening using genetic testing might be ideal for *HFE*-related HH. This was because the disorder is known to be common, there is a long latent phase before the development of disease manifestations, treatment is simple and effective, and tests of phenotypic markers are available. However, it has become apparent that not all C282Y homozygotes have phenotypic expression and this raises questions about the advisability of large-scale population screening. Table 3 summarizes the results of some studies that have utilized *HFE* genotyping for population screening (excluding studies of blood donors), indicating that 24–58% of C282Y homozygotes have a normal serum ferritin concentration [67,76–79].

Phenotypic testing based on serum iron studies has traditionally been the standard for detecting iron overload. An elevated transferrin saturation has usually been considered the main test for detecting iron overload given its excellent sensitivity, acceptable specificity, relatively low cost, and widespread availability [80]. Methodologic issues need to be taken into consideration and some investigators have suggested using the unbound iron-binding capacity as a screening tool given its reproducibility, low cost, and high sensitivity and specificity [81–83]. As mentioned previously, the serum ferritin level is often elevated in iron overload and its sensitivity is quite high. Unfortunately, it has a low specificity and it is best utilized in conjunction with transferrin saturation. Population screening for HH using a combination of phenotypic testing followed by

Table 3
Selected population studies using *HFE* genotyping

Population sample	Country	Sample size	Prevalence of C282Y homozygotes	C282Y homozygotes with a normal ferritin (%)
Electoral roll [76]	New Zealand	1064	1 in 213	40
Epidemiological survey [77]	Australia	3011	1 in 188	25
Primary care [78]	USA	4865	1 in 405	58
Health clinic [67]	USA	41,038	1 in 270	24 (males) 46 (females)
Primary care [79]	USA/Canada	20,130	1 in 322	50

focused *HFE* genotyping may be feasible. However, the cost-effectiveness of screening remains a key factor when considering public health recommendations and population screening for HH requires further evaluation [80,84–86].

11. *HFE* mutations and iron in other liver diseases

Abnormalities in serum iron study results are common in patients with porphyria cutanea tarda (PCT), non-alcoholic steatohepatitis (NASH), chronic hepatitis C, and alcoholic liver disease [20–24,87]. Up to 50% of patients with these disorders will have abnormal serum markers of iron metabolism. The hepatic iron concentration is typically normal or slightly increased [6,25].

11.1. Porphyria cutanea tarda

Mild to modest degrees of iron accumulation are frequently seen in patients with type 1 (sporadic) PCT [87]. These patients have a defect in uroporphyrinogen decarboxylase that predisposes this enzyme to reversible inactivation induced by various environmental factors including iron, alcohol, and hepatitis C. Approximately 40% of PCT patients in the United Kingdom, Australia and the USA are either homozygous or heterozygous for the C282Y mutation, while this enrichment in C282Y is not found in Italian PCT patients [87]. Thus, whenever encountering a patient with PCT, it is recommended that *HFE* mutation analysis be performed. Patients with PCT should also be tested for hepatitis C and then treatment should be initiated with phlebotomy using the same guidelines as are used above in patients with typical HH. Skin manifestations of PCT respond to phlebotomy therapy and alanine aminotransferase (ALT) levels often decrease into the normal range. If patients also have hepatitis C, then appropriate antiviral therapy should be considered.

11.2. Non-alcoholic steatohepatitis

Serum iron studies are often abnormal in NASH patients [20,21,88–91] and stainable hepatic iron is often evident. These observations led to studies focusing on the prevalence of *HFE* mutations in NASH and the potential role of hepatic iron in disease severity. In three studies, it has been observed that NASH patients have a higher prevalence of the C282Y mutation (usually heterozygotes)[90–92], and one of these studies also reported a higher prevalence of the H63D mutation [91]. Not all NASH patients with elevated hepatic iron levels have the C282Y mutation, indicating that other factors are involved. Two of these three studies observed an increase in hepatic fibrosis in NASH patients who carried the C282Y mutation [90,91]. Some investigations have reported an association of increased hepatic iron with the development of fibrosis in NASH [90,91], but other studies have not found this association [92,93]. Regardless of iron levels and *HFE* mutations, several small trials indicate that phlebotomy therapy decreases ALT levels in NASH patients [21,94]; the potential effects on hepatic histopathology have not been evaluated.

11.3. Chronic hepatitis C

It has been known for quite some time that many patients with chronic hepatitis C have abnormal iron studies [22,23]. In different series, 22–62% of patients have elevated serum ferritin values, and 18–32% have elevated transferrin saturation levels [23]. Evidence suggests that an elevated hepatic iron concentration is associated with increased hepatic fibrosis in patients with chronic hepatitis C [95–98]. However, the frequency of *HFE* mutations is no greater than the general population [96,97,99–101]. Some studies have reported that the presence of *HFE* mutations (especially C282Y) in hepatitis C patients is associated with increased fibrosis and cirrhosis [96,97,102] but other studies have not found this association [98,101,103]. While the potential contribution of *HFE* mutations remains uncertain, many studies have demonstrated that iron reduction therapy by phlebotomy decreases ALT levels in hepatitis C patients without affecting circulating viral RNA levels [23]. In addition, two recent reports indicate that long-term phlebotomy therapy in hepatitis C patients may have beneficial effects on hepatic histopathology [104,105].

11.4. Alcoholic liver disease

It has been known for many years that some patients with alcoholic liver disease have modest degrees of iron overload [24]. When examined for *HFE* mutations, no increased

enrichment was found in these patients, and possession of a single copy of either C282Y or H63D did not influence hepatic iron levels or the risk of fibrotic disease [106]. Thus, although some patients with alcoholic liver disease have increased hepatic iron levels, it appears that mechanisms other than mutations in *HFE* are responsible.

12. Summary

HH should be distinguished from the other syndromes of iron overload. Many patients with *HFE*-related HH have abnormal serum iron values before the development of any significant symptoms or clinical findings, and liver biopsy is less important in the evaluation of these patients. Treatment by phlebotomy is safe and effective, and prevents the sequelae of iron overload. Hepatic iron may play a contributory role in the pathogenesis of PCT, NASH, and chronic hepatitis C. *HFE* genotyping for the C282Y and H63D mutations has strengthened our ability to diagnosis HH accurately and it is very useful in family studies. It is now clear that a substantial proportion of C282Y homozygotes do not have evidence of phenotypic expression, suggesting the existence of genetic modifiers. An increase in the duodenal expression of the iron transporters, DMT-1 and ferroportin 1, appears to be responsible for increased iron absorption in *HFE*-related HH, but the exact mechanisms underlying this effect are still being elucidated. However, our understanding of the normal physiology of iron absorption is growing and the ability to decipher the contribution of inter-individual genetic differences on this process will be enhanced in the future.

Declaration

The authors who have taken part in this study have not a relationship with the manufacturers of the drugs involved either in the past or present and did not receive funding from the manufacturers to carry out their research.

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