Hereditary Hemochromatosis: Genetics, Pathogenesis, and Clinical Management

Concise Review

Hereditary Hemochromatosis:
Genetics, Pathogenesis, and Clinical Management

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Abstract

Recent findings have led to major advances in our understanding of genetics and pathophysiology of hereditary hemochromatosis. Many crucial genes and molecules have come to light, and the complex interrelationships between them are being studied. However, several questions still remain unanswered. Availability of genotyping has changed the approach to diagnosis, and serum markers hold promise for prognostication. However, the effectiveness of population screening continues to be an area of controversy. Finally, there is a promise of development of newer therapeutic modalities based on our understanding of the mechanism of iron absorption. In this review, we describe the current state of understanding in the clinical features, pathophysiology and treatment of hereditary hemochromatosis.

Key words: HFE, iron overload, fibrosis, cirrhosis, phlebotomy.

Introduction

The discovery of the HFE gene in 1996 by Feder et al1 was a landmark event in the history of hereditary hemochromatosis (HH). This has lead to a paradigm shift in our understanding of the disease in the last one decade. It is now recognized that HFE mutations are very common among populations of Northern European descent and that HFE mutations are responsible for the majority of cases of phenotypic hemochromatosis among Caucasians. We have also come to appreciate that many, if not most, patients identified to have the homozygous C282Y mutation in the HFE gene in population screening studies do not have significant end-organ damage. Conversely, the recognition that up to 20% of patients with phenotypic hemochromatosis lack the C282Y+/- mutation has led to the identification of mutations in several novel genes involved in iron transport. The identification of a large number of individuals with HFE mutations but minimal disease has led to active debate about the utility of population screening.

Genetics

Mutations of at least 5 different genes, including HFE, Hemojuvenilin (HJV), HAMP (the gene encoding Hepcidin), Transferrin receptor 2 (TfR2) and Ferroportin (FP) - have been identified to cause hereditary hemochromatosis. Therefore, an attempt has been made to classify hereditary hemochromatosis (HH) based on the type of genetic mutations. One such classification is shown in table I.2 Most forms of HH are inherited in an autosomal recessive pattern, except for HH associated with the Ferroportin mutation, which can be inherited in an autosomal dominant pattern. The Type 1 HH constitutes the vast majority of cases and the other Types of HH are found in a small minority.

The HFE gene, the mutation of which results in type 1 HH, is located in the short arm of chromosome 6, and encodes a nonclassical major histocompatibility (MHC) class I protein. The overwhelming majority of patients with Type 1 HH have the homozygous C282Y mutation; approximately 5-7% is compound heterozygous for the C282Y and H63D mutation. The homozygous H63D mutation is uncommon and is not associated with phenotypic hemochromatosis. It is generally thought that this mutation results in significant iron overload only when associated with other diseases such as alcoholic liver disease or hepatitis C. Another polymorphism in the HFE gene is S65C; the clinical significance of this mutation when present in compound heterozygous form with C282Y or
<table>
<thead>
<tr>
<th>Feature</th>
<th>HFE-Related hereditary hemochromatosis†</th>
<th>Juvenile hereditary hemochromatosis</th>
<th>HFE2-Related hereditary hemochromatosis</th>
<th>Ferroportin-related iron overload‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMIM classification</td>
<td>Type 1</td>
<td>Type 2, subtype A</td>
<td>Type 2, Subtype B</td>
<td>Type 3</td>
</tr>
<tr>
<td>Implicated gene and its chromosomal location</td>
<td>HFE, 6p21.3</td>
<td>HJV (originally called HFE2), 14q21</td>
<td>HAMP, 19q13.1</td>
<td>TfR2.7q22</td>
</tr>
<tr>
<td>Gene product Name</td>
<td>HFE</td>
<td>Hemojuvelin</td>
<td>Hepcidin</td>
<td>Transferin receptor 2</td>
</tr>
<tr>
<td>Known or postulated function§</td>
<td>Interaction with transferring receptor 1, probably facilitating uptake of transferrin-bound iron; possibly modulation of hepcidin expression</td>
<td>Unknown; possibly modulation of hepcidin expression</td>
<td>Down-regulation of iron release by enterocytes, macrophages, or placental cells</td>
<td>Possibly uptake of iron by hepatocytes</td>
</tr>
<tr>
<td>Pattern of inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Evidence or expanded plasma iron compartment (high transferrin saturation)</td>
<td>Earliest detectable biochemical anomaly</td>
<td>Earliest detectable biochemical anomaly</td>
<td>Earliest detectable biochemical anomaly</td>
<td>Only in advanced stages</td>
</tr>
<tr>
<td>Main organs accumulating iron</td>
<td>Liver, endocrine glands, heart</td>
<td>Liver, endocrine glands, heart</td>
<td>Liver, endocrine glands, heart</td>
<td>Liver, spleen</td>
</tr>
<tr>
<td>Predominant cell distribution of iron accumulation</td>
<td>Parenchymal</td>
<td>Parenchymal</td>
<td>Parenchymal</td>
<td>Reticuloendothelial</td>
</tr>
<tr>
<td>Potential for organ damage</td>
<td>Variable</td>
<td>High</td>
<td>High</td>
<td>Variable</td>
</tr>
<tr>
<td>Anemia</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>May be seen in menstruating women or after phlebotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to therapeutic phlebotomy</td>
<td>Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia</td>
<td>Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia</td>
<td>Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia</td>
<td>Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia</td>
</tr>
<tr>
<td>Fair: rapid decrease in transferrin saturation with persistently high serum ferritin; substantial risk of anemia with aggressive phlebotomy regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decade of onset of symptomatic organ disease</td>
<td>4th or 5th</td>
<td>2nd or 3rd</td>
<td>2nd or 3rd</td>
<td>4th or 5th</td>
</tr>
</tbody>
</table>

*OMIM denotes Online Medelian Inheritance in Man.
† Other common names are hereditary hemochromatosis, classic hemochromatosis, and HLA-linked hemochromatosis.
‡ Other common names are autosomal dominant hemochromatosis, ferroportin disease, and autosomal dominant reticuloendothelial iron overload.
§There may be other, as yet unknown, functions related to iron overload. The listed functions do not, at least at this time, always account for the known pathophysiological features associated with the gene mutation.
H63D is controversial. The C282Y mutation is present in 80-100% of hemochromatosis patients of northern European origin, and in 60-80% of southern European origin. The distribution of this mutation is more or less restricted to Caucasians, whereas H63D mutation is also present in other populations.

Mutations in the genes encoding Hemojuvelin (HJV) or Hepcidin (HAMP) result in type 2 HH. These mutations are characterized by more severe iron accumulation and earlier onset of disease than Type 1 HH. Mutations in HAMP, the gene encoding hepcidin, result in Type 2 HH, a form of juvenile hemochromatosis; these mutations result in lack of functional hepcidin and are associated with greatly increased iron loading earlier in life than in patients with Type 1 HH. G71D and R59G, the most common HAMP mutations, have been found only in heterozygous form, and cause iron overload only when co-inherited with HFE mutations. Other HAMP mutations such as C78T, 93delG, G-to-A transition at nucleotide +14, C70R and C78T, which are found only in homozygous form, and R56X which is found in both homozygous and heterozygous forms, are all extremely uncommon. In addition, 13 homozygous and 9 heterozygous HJV mutations have been identified, which are much more common than HAMP mutations. All HAMP and HJV mutations cause severe iron loading, resulting in earlier clinical presentation (usually before age 30), advanced fibrosis or cirrhosis at the time of diagnosis and more severe extracellular involvement than adult forms of HH.

Mutations in the gene for transferrin receptor 2 (TfR2), result in Type 3 HH, which is phenotypically similar to Type 1 HH. Six TfR2 mutations have been described as of now, all of which are extremely rare and found mostly among southern Europeans and Japanese. These mutations cause more rapid iron accumulation than HFE mutations, but not as severe as HAMP and HJV mutations.

Type 4 HH results from mutations in the gene for ferroportin (FP), in which at least a dozen pathogenic mutations and several nonpathogenic polymorphisms have been identified. This type of iron overload is quite distinct from Type 1 HH. The serum transferrin-iron saturation is lower than in most patients with Type HH and the ferritin is usually disproportionately elevated. The pattern of iron distribution in the liver is altered with the presence of iron in reticuloendothelial cells. These patients may not tolerate phlebotomy well. It has been proposed that mutations in FP may represent an important cause of iron overload among African-Americans.

Pathogenesis

Despite the recent advances, our understanding of iron homeostasis and pathogenesis of hemochromatosis remain incomplete. Though the roles of proteins which are abnormal in hemochromatosis have been identified, much remains unknown about intracellular iron homoeostasis, and the communication between the intestine, liver and bone marrow in regulating the level of body of iron stores.

HFE is a transmembrane protein involved in the uptake of iron from plasma into macrophages and intestinal epithelial cells. Normal iron absorption appears to require interaction between HFE and beta2 microglobulin and transferrin receptor (Tf R-1) to facilitate entry of transferrin-bound iron into cells. It has been proposed that the intestinal crypt cells ‘sense’ the body’s iron status from circulating iron in plasma, and modulate iron absorption via up or down-regulation of divalent metal transporter-1 (DMT1) and/or FP, which are expressed on the mucosal and basolateral surface of the enterocytes respectively. The mutated HFE protein is trapped intracellularly and fails to traverse the cell membrane; it has been suggested that this results in impaired HFE-mediated entry of transferrin into crypt cells and results in a state of “intracellular iron deficiency”. Consequently, there is increased expression of the proteins responsible for iron absorption resulting in increased inorganic iron absorption from these cells after they have differentiated into villus enterocytes. Absorption of iron from a normal diet may be 2-4 times normal and may reach or exceed 8-10 gm per day. Over time, there is increased deposition of iron in tissues including the liver, heart, skin, joints, anterior pituitary, and pancreas.

The recent discovery of hepcidin has further complicated our understanding of iron metabolism but also established the role of the liver as essential to the maintenance of iron homeostasis. (See Figure 1). Hepcidin is a small antimicrobial peptide which was serendipitously found to inhibit iron absorption. This protein is secreted into the circulation by the liver and appears to block iron absorption from the intestine. The accelerated clinical course of Type 2 HH is thought to reflect the central role for hepcidin, compared to HFE, TfR2 or Ferroportin, in iron homeostasis. Hepcidin inhibits the release of iron from intestinal epithelial cells and from macrophages into plasma possibly via binding to ferroportin. Hepcidin expression is decreased in the setting of iron deficiency and increased in the setting of iron overload. Reduction in dietary iron in rodents leads to a prompt decrease in hepatic expression of hamp, the gene encoding hepcidin. Hepcidin expression is increased in the presence of inflammation and decreased in the setting of hypoxia. However, the mechanisms which coordinate hepcidin expression to body iron status are not clearly known. It is possible that some cell other than the hepatocyte detect body iron stores and signals hepcidin production.

Type 3 HH results from mutations in the gene for transferrin receptor 2 (TfR2), which like the classical transferrin receptor (TfR1), mediates hepatic uptake of transferrin bound iron. It is not known how mutations which cause loss of function of this receptor paradoxically lead to hepatic iron accumulation.
Type 4 HH results from mutations in the gene for Ferroportin (Fpn).\textsuperscript{9} Ferroportin mutations can be classified into 2 groups: one resulting in a loss of iron export function, and a second which is characterized by decreased sensitivity to the effect of hepcidin on ferroportin.\textsuperscript{9} The former leads to accumulation of iron in macrophages and enterocytes, with a depleted plasma iron pool. This is reflected by high serum ferritin levels and low to normal transferrin-iron saturation. It is paradoxical that loss of function of the iron export protein results in excessive iron absorption. It has been proposed, but not proven, that iron-restricted erythropoiesis resulting from reduced supply of iron to the bone marrow by macrophages, somehow stimulates enterocytes to increase iron absorption in spite of defective ferroportin function and increased iron in enterocytes.\textsuperscript{19} The mutation which causes hepcidin resistance produces a situation analogous to hepcidin deficiency, and results in a phenotype similar to Type 1 HH, with inappropriately high duodenal absorption of iron, increased transferrin saturation, and iron deposition in hepatocytes. Both these types of mutations however are unique in that hepcidin production is normal, in contrast to all other forms of HH.

**Clinical features and evaluation**

The clinical manifestations of HH are the result of iron accumulation in parenchymal tissues and subsequent tissue damage. The extent and severity of tissue damage depends both on the magnitude and duration of iron overload.\textsuperscript{20} Type 1 HH most commonly presents in middle age and the phenotypic presentation occurs later in life and is milder in women. The clinical manifestations of HH include to various degrees, hepatomegaly, cirrhosis, hepatocellular carcinoma, diabetes mellitus, hypogonadotropic hypogonadism, impotence, hypothyroidism, restrictive cardiomyopathy, arrhythmias, heart failure, destructive arthropathy and hyperpigmentation. However, it is becoming increasingly uncommon to identify patients with severe manifestations of disease because many, if not most patients are now being identified before the development of advanced organ damage. The classic manifestations are therefore uncommonly seen in Type 1 HH. More common are vague, nonspecific symptoms such as fatigue, arthralgias and abdominal pain.\textsuperscript{21} In contrast, type 2 HH is characterized by onset of iron overload at a young age, equal occurrence in both sexes and more severe extrahepatic involvement, especially endocrine and cardiac.\textsuperscript{22} In summary, the diagnosis of hemochromatosis should be suspected in middle aged adults with fatigue, malaise, arthralgia, hepatomegaly or elevated aminotransferase levels, and children or adolescents with liver disease, hypogonadotropic hypogonadism or unexplained heart failure.

The appropriate initial screening tests for most cases of HH include the serum-transferrin-iron saturation and ferritin.\textsuperscript{23} Together, these tests have a negative predictive value of 97% for Type 1 or HFE-associated hemochromatosis.\textsuperscript{24} Hence, hemochromatosis can be ruled out, for all practical purposes, if both these tests are normal. Increased transferrin saturation (>45%) indicates increased plasma iron, and is the earliest detectable biochemical abnormality in hereditary hemochromatosis. However, it is important to remember that the transferrin-iron saturation

![Figure 1. Proposed model for control of intestinal iron absorption.](image-url)
can also be increased due to excessive alcohol consumption and decreased due to decreased transferrin synthesis associated with liver dysfunction. Transferrin is also a negative acute phase reactant and may be decreased in inflammatory conditions. Circadian and postprandial variations of transferrin saturation are a potential source of error, but this can be excluded by overnight fasting. It has recently been shown that unsaturated iron-binding capacity (UIBC) is both less expensive than, and at least as reliable as, transferrin-iron saturation as a screening test.25 The serum ferritin level is a marker of tissue iron stores. However, ferritin is also an acute phase protein, and levels may be elevated in several inflammatory conditions and malignancies. Ferritin level also increases with age, and is dependent on gender.12 Hence, interpretation of an elevated serum ferritin among patients with suspected iron overload should take into account the presence of all these confounding factors in addition to transferrin saturation.

Patients found to have repeatedly elevated transferrin saturation (especially in the presence of an elevated serum ferritin) should undergo HFE genotyping. The diagnosis of hereditary hemochromatosis can be confirmed if a hemochromatosis-associated genotype is present (C282Y/C282Y or C282Y/H63D). A suggested algorithm for evaluation and management of patients with elevated serum transferrin-iron saturation is described in Figure 2. At present, testing for mutations in Tf R2, FP and HAMP are not currently available in routine clinical practice. Furthermore, these mutations, particularly in the case of Type 4 HH, are frequently “private” mutations (i.e., mutations unique to an individual); therefore, gene testing for these mutations would not be useful for screening. However, since 80-90% of cases of hereditary hemochromatosis among Caucasians are associated with either C282Y homozygosity or C282Y/H63D compound heterozygosity, analysis of these HFE mutations is sufficient to establish the diagnosis in the vast majority of cases. If a patient with suspected hemochromatosis does not carry either of these mutation profiles, liver biopsy should be performed to confirm the presence and pattern of increased hepatic iron stores, to identify the presence or absence of significant hepatic fibrosis, and to exclude other causes for increased iron stores such as fatty liver disease, viral hepatitis or alcoholic liver disease.

**Role of liver biopsy**

Prior to the era of HFE gene testing, liver biopsy was considered the “gold standard” for the confirmation of the diagnosis of HH. Commonly accepted diagnostic criteria for HH on liver biopsy include.26

1. Grade 4 stainable iron in hepatocytes, with a periporal distribution, and a paucity of stainable iron in Kupffer cells

2. Hepatic Iron Conc. (HIC) > 80 µmol (4,500 mg)/gm dry wt

3. Hepatic Iron Index (HII = HIC in µmol/g ÷ age in years) > 1.9

The role of liver biopsy for the diagnosis of HH has become much less important after the discovery and widespread use of HFE mutation analysis. Liver biopsy for the diagnosis of HH is now primarily used in patients who are not C282Y homozygotes. In such patients, it is important to consider the possibility of other confounding factors that might contribute to an elevated serum transferrin saturation and ferritin, such as alcohol consumption, fatty liver disease, and viral hepatitis. Liver biopsy is helpful to exclude such coexistent or alternate diagnoses. Among C282Y homozygotes, liver biopsy is only necessary to evaluate for the presence or absence of advanced hepatic fibrosis. The presence of elevated liver enzymes and a serum ferritin >1,000 µg/L significantly increases the risk of cirrhosis.27 By contrast, cirrhosis has been found to be present in only 7% of patients with an initial serum ferritin < 1,000 µg/L, even among patients older than 40 years, and those with elevated liver enzymes.27 Therefore, it has been recommended that liver biopsy is not necessary in C282Y homozygotes who have a serum ferritin <1,000 µg/L at the time of diagnosis, especially if they have normal liver enzyme levels and no hepatomegaly.23

There has been growing interest in the past few years in development of non-invasive markers of cirrhosis or hepatic fibrosis. Panels of multiple noninvasive markers of hepatic fibrosis have now become commercially available. One such panel consists of γ-glutamyltransferase, α-2-macroglobulin, haptoglobin, apolipoprotein A1 and total bilirubin (marketed as FibroTest in Europe; Fibrosure in North America), and another consists of hyaluronic acid, tissue inhibitor of metalloproteinase (TIMP)-1, and α-2-macroglobulin (marketed as FibroSpect II).28 The European Liver Fibrosis Group has recently proposed an algorithm combining age, hyaluronic acid, amino-terminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1 for detecting liver fibrosis.29 If these noninvasive markers can be proven to have consistently good positive and negative predictive value for hepatic fibrosis, then liver biopsy may be obviated in even more C282Y homozygotes in the future.

Magnetic resonance imaging (MRI) may be another attractive technique for diagnosis and prognosis of patients with HH. Specialized MRI techniques such as the “R2” measurement utilize the paramagnetic properties of iron in the liver to provide an estimation of HIC. An excellent correlation between HIC estimated by R2 MRI and biochemical HIC from liver biopsy was shown in one study; this technique is now approved by the US FDA.30 A combination of age and estimated HIC by MRI has been recently reported to have a sensitivity and specific-
ity of 100% and 86%, respectively, for diagnosis of high-grade fibrosis. Genetic markers like glutathione S-transferase P1 polymorphism, myeloperoxidase promoter polymorphism and TGF-beta1 codon 25 gene polymorphism have also been reported to predict progression to cirrhosis among patients with HH.

**Treatment**

Phlebotomy remains the safest, most effective, and most economical iron lowering therapy for hemochromatosis. The target of phlebotomy is to achieve and maintain the serum ferritin level <50 µg/L. This is usually accomplished initially by phlebotomies of 500 mL (containing approximately 250 mg of iron) once or twice a week until the serum ferritin level is ≤50 µg/L. The duration of initial induction treatment might last to two or three years. The hematocrit should be checked prior to each phlebotomy to avoid significant anemia. The serum ferritin should be monitored once in every 10 to 12 phlebotomies. However, as the serum ferritin approaches 50µg/L, more frequent monitoring is appropriate. Phlebotomy should be temporarily withheld if the hematocrit decreases to below 32% and may be resumed every other week. Once the serum ferritin is ≤50 µg/L, weekly phlebotomy can be discontinued and serum ferritin should be monitored every 3-4 months. The required frequency of is variable, usually ranging from once in 2 to 4 months to once every month, reflecting the individual variability in iron absorption, although some patients will not require further treatment for an extended period.

The clinical features which respond well to phlebotomy include malaise, fatigue, increased skin pigmentation, insulin requirement in patients with diabetes mellitus and abdominal pain. Arthropyathy, hypogonadism and cirrhosis respond less well to iron depletion. Several studies have shown that life expectancy is normal if phlebotomy is initiated in the pre-cirrhotic stage. However, once cirrhosis has developed, life expectancy is shortened and the risk of liver cancer is high, even after complete iron depletion. Overall, 30% of the deaths in patients with Type 1 HH are caused by hepatocellular carcinoma, with other complications of cirrhosis contributing another 20%. Sudden death is most commonly due to cardiac arrhythmias. Since vitamin C may increase the risk by making free iron available in blood during periods of iron mobilization and also because ascorbic acid may enhance duodenal iron absorption, it should be completely avoided by patients with HH, especially during phlebotomy.

Patients with hemochromatosis should be advised to abstain from alcohol and educated about the risk factors of viral hepatitis. Vaccinations against Hepatitis A and Hepatitis B should be done if there is no evidence of previous exposure. HCC surveillance should be carried out using alpha fetoprotein and ultrasonography or CT scan.

![Figure 2. Proposed algorithm for management of Hereditary Hemochromatosis.](image-url)
ning as liver transplantation may offer the only chance for a cure.\textsuperscript{23} Liver transplantation is also the only viable therapy once hepatic decompensation has developed. However, survival after liver transplantation is worse among patients with Type 1 HH compared to patients undergoing liver transplantation for other indications.\textsuperscript{37} Most of the post-transplant deaths are due to cardiac complications or infection.\textsuperscript{38}

Though iron chelators are the treatment of choice in iron overload associated with hemolytic anemias, they are generally not used in HH because of the increased risk of toxicity and need for parenteral administration. The recent approval of ICL670 (EXJADE), an oral iron chelator, may prove to be of benefit for cirrhotic HH patients who have comitant anemia or who do not tolerate phlebotomy.\textsuperscript{39} Another novel approach for treatment of experimental HH used antisense gene therapy directed against divalent metal transporter-1 (DMT-1) which transports iron from the intestinal lumen across the apical membrane into the intestinal epithelial cells.\textsuperscript{40}

**Conclusion**

In the years since the discovery of HFE gene, our understanding of iron metabolism as well as hemochromatosis has improved vastly. Several new concepts of iron regulation have emerged, but the crucial steps that are required for the 'sensor' mechanisms of iron stores in hepatocytes and enterocytes remain unclear. Clear guidelines have been formulated for diagnosis of cases and screening of first degree relatives, but there are divergent views regarding population screening for the disorder. Liver biopsy and measurement of hepatic iron concentration has become less important for diagnostic purposes among patients with Type 1 HH, but remains important for prognosis and for diagnosis among patients without the classic HFE mutations. Phlebotomy remains as the treatment of choice, its safety and efficacy having withstood the test of time.

**Acknowledgements**

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**References**


