EXPERT REVIEWS

Diagnosis and treatment of hereditary hemochromatosis: an update

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Pushpjeet Kanwar and Kris V Kowdley*

Liver Center for Excellence, Digestive Disease Institute, Virginia Mason Medical Center, Seattle, WA, USA
**Author for correspondence: kris.kowdley@vmmc.org*

Hereditary hemochromatosis is an inherited iron overload disorder caused by inappropriately low hepcidin secretion leading to increased duodenal absorption of dietary iron, most commonly in C282Y homozygous individuals. This can result in elevated serum ferritin, iron deposition in various organs and ultimately end-organ damage, although there is incomplete biochemical and clinical penetrance and variable phenotypic expression of the *HFE* mutation in hereditary hemochromatosis. An elevated SF <1000 µg/l is associated with an increased risk of cirrhosis and mortality in C282Y homozygotes. Conversely, a SF <1000 µg/l is associated with a very low likelihood of cirrhosis, making liver biopsy unnecessary among C282Y homozygotes in the absence of concomitant risk factors for liver disease. Phlebotomy remains the mainstay of treatment and new treatments being studied include erythrocytapheresis and 'mini-hepcidins'. Iron overload is being recognized to play a carcinogenic role in hepatocellular carcinoma and other cancers, possibly supporting iron depletion in these patients.

Keywords: C282Y mutation • ferritin • hemochromatosis • hepcidin • *HFE* • iron overload • penetrance

Introduction

Hereditary hemochromatosis (HH) is an inherited autosomal recessive iron overload disorder resulting in the failure of the normal hepcidin response to body iron stores, leading to increased duodenal absorption of dietary iron [1–3]. The increased iron enters the plasma and may be deposited in various target organs and may lead to clinical signs and symptoms [4].

Our understanding of this condition has grown significantly since the initial description of advanced HH as 'bronze diabetes' by Trousseau [5] in 1865, to the discovery of the role of iron metabolism in its pathogenesis by Sheldon [6] in 1935, to the identification of the C282Y mutation in *HFE* as responsible for most cases of HH in 1996 [7] and most recently to the recognition of a central role of hepcidin in the regulation of iron absorption and pathogenesis of HH [2,3,8]. The current classification system for HH has categorized this disorder into four types [301]. The most common is type 1 or classical HH which is associated with a homozygous cysteine to tyrosine missense mutation in *HFE* gene. Since *HFE*-associated hemochromatosis (type 1) is the most common form of inherited iron overload, we will focus primarily on type 1 but will also review the current status of the understanding of types 2–4 HH.

Epidemiology & pathophysiology

Evidence from multiple studies has shown that the prevalence of C282Y homozygosity is approximately one out of 250 in populations of predominantly Northern European descent [9–11]. The presence of mutations in a large proportion of patients with phenotypic HH was initially reported by Feder *et al.* in 1996 [7]. These authors reported that the C282Y and H63D mutations in the *HFE* gene (located on the short arm of chromosome 6) were present in most patients (especially those of North European origin); the most common pattern was homozygosity for the C282Y mutation with a small portion carrying the C282Y/H63D compound heterozygous genotype. These missense mutations are characterized by replacement of cysteine with tyrosine at position 282 (C282Y) and histidine with aspartic acid at position 63 (H63D) respectively. The mean prevalence of the C282Y allele varies based on multiple screening studies and is approximately 6% [12]. Among Irish individuals, the prevalence of C282Y is >10%, while the frequency varies from 5–10%

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in most other Northern European countries [13,14]. It is less frequent (~1–5%) in Southern Europe [14,15] and extremely rare in nonwhite populations [16]. The H63D allele carrier frequency is between 10–20% [14] and has lower geographical variability.

The C282Y homozygosity prevalence among Caucasian populations in Northern Europe is approximately 1:200–1:300 [17] while the prevalence of C282Y/H63D compound heterozygotes, based on multiple studies, is approximately 2% [18]. Approximately 90% of individuals with HH are C282Y homozygotes while 5% or less are C282Y/H63D compound heterozygotes [19,20].

The widespread prevalence of the C282Y mutation in populations in Northern Europe has led to much speculation as to the possible survival advantage that may have been conferred to heterozygote carriers (similar to the hypothesis suggesting that carriage of the sickle-cell trait may have provided resistance against malaria in endemic areas) [21]. It has been proposed that *HFE* mutations may have resulted in increased efficiency of iron absorption and may have reduced the severity of iron deficiency in Northern Europe during a period of malnutrition as populations made the transition from 'hunter-gatherer' societies to more sedentary, agrarian cultures with resultant turmoil and conflict [22,23]. However, such theories remain largely speculative. From an evolutionary genetic point of view, the age of the C282Y mutations has been estimated to be older than 4000 B.C. [24].

The central mechanism for iron overload in HH is deficient hepatic hepcidin response to body iron stores which results in excessive iron absorption in the duodenum [4]. The gene encoding hepcidin is highly expressed in hepatocytes and is regulated at the transcriptional level in response to body iron stores [25,26]. The gene product, hepcidin, is a 25 amino acid peptide which is cleaved from prohepcidin and released into the circulation, where it controls iron metabolism by binding to ferroportin, the major cellular iron exporter [27]. Following internalization of ferroportin, it is degraded via a lysosomal pathway, thus reducing iron efflux from cells, which include hepatocytes and macrophages [28]. Hepcidin-mediated loss of ferroportin in the enterocyte leads to a decrease in intestinal absorption of inorganic iron and consequently, reduced body iron stores [19]. Since the human body does not have a primary mechanism for mobilizing or removing excess iron, hepcidin-mediated reduction of iron absorption represents the major mechanism by which the body regulates iron stores. Therefore, under conditions of iron deficiency, hepcidin expression is reduced leading to increased iron export (and subsequently import) of iron into cells secondary to increased ferroportin activity [29]. However, under conditions of iron excess, which is sensed by the hepatocytes via increased serum TS, hepcidin production is increased leading to iron withholding within cells, thus ultimately reducing iron efflux from iron storage sites and intestinal iron absorption [30].

The complete mechanism whereby the presence of the mutant HFE protein in hepatocytes leads to HH has not been completely elucidated. However, it is now recognized that a complex set of interactions within the hepatocytes in response to circulating iron between hemojuvelin (*HJV*), bone morphogenic protein (BMP), SMAD 4, transferrin receptor 1 (*TfR1*), transferrin receptor 2 (*TfR2*) and transmembrane protease serine 6 (TMPRSS6) appears necessary to maintain appropriate iron mediated-hepcidin expression [31]. Most inherited disorders of iron overload (in particular HH type 1–3) are caused by decreased hepcidin production, which leads to unopposed iron hyperabsorption and, ultimately, excessive deposition of iron in certain tissues leading to end-organ damage and disease [4]. Type 4 HH or 'ferroportin disease' may be caused by mutations in ferroportin (*FPN*) resulting in inability to bind or resistance to interact with hepcidin [32,33]. The various possible mutations in iron regulatory genes and the resulting type of HH are shown in (Table 1). It is also likely that other genetic, hormonal and environmental factors modulate the amount of body iron stores in HH, given the wide variability in penetrance and expressivity of this disorder.

Table 1. Types of hemochromatosis.

Type	Gene, inheritance mode	Pathogenesis	Main clinical feature	Iron studies	Severity
Type 1 (classic HH)	*HFE*, AR	Decreased hepcidin	Onset in the fourth or fifth decade, liver fibrosis, cirrhosis	↑TS, ↑ferritin	Variable
Type 2A (juvenile HH)	*HJV*, AR	Inhibition of hepcidin expression	Cardiomyopathy, hypogonadism, onset before 30 years of age	↑TS, ↑ferritin	Severe
Type 2B (juvenile HH)	*HAMP*, AR	Decreased hepcidin	Cardiomyopathy, hypogonadism, onset before 30 years of age	↑TS, ↑ferritin	Severe
Type 3 (*TfR2*)	*TfR2*, AR	Abnormal sensing of iron, decreased hepcidin	Onset in the fourth or fifth decade, liver fibrosis, cirrhosis	↑TS, ↑ferritin	Variable
Type 4 (ferroportin disease)	*SLC40A1*, AD	Decreased iron export from macrophages and enterocytes, rarely hepcidin resistance in non-classical type	Anemia and thus lower tolerance to phlebotomies	None or ↓TS	Mild

↓: Decrease; ↑: Increase; AD: Autosomal dominant; AR: Autosomal recessive; HH: Hemochromatosis; TS: Transferrin saturation.

Alcohol, obesity and viral hepatitis have been shown to accelerate progression of hepatic fibrosis to cirrhosis among patients with type 1 HH. *In vitro* studies have suggested that both hepatitis C virus and alcohol reduce hepcidin expression and lead to increased hepatic iron stores [34,35]. There is also a growing body of literature suggesting that iron loading in the liver may be associated with advanced fibrosis and that iron excess in reticuloendothelial system (RES) cells in the liver may accelerate liver injury and fibrosis in nonalcoholic steatohepatitis (NASH) [36].

Diagnosis

Type 1 (or *HFE*-HH) represents the majority of cases of HH and most of the published literature on the clinical features, diagnosis and testing relates to this form of the disease. In fact, routine clinical genotypic testing is only available for type 1 HH at the present time. Furthermore, since large case series and natural history studies are primarily based on cohorts of patients with type 1 or *HFE*-HH, the discussion of diagnosis, prognosis and treatment will be largely limited to this type of HH.

Clinical features

The clinical features of HH are highly variable, ranging from presence of the homozygous C282Y mutation with a normal TS and SF, to elevations in serum TS with or without hyperferritinemia or evidence of end-organ damage [37].

HH is associated with iron loading of liver, pancreas, heart, pituitary, skin and joints leading to fibrosis, cirrhosis and hepatocellular carcinoma, diabetes, cardiomyopathy, impotence, hypogonadotrophic hypogonadism, abnormal increased skin pigmentation and arthritis involving the second and third metacarpophalangeal (MCP) joints respectively [38,39]. Owing to greater awareness of the disease and widespread use of screening tests, the classical presentation of 'bronze diabetes' is no longer typically seen [40]. Rather, the most common manifestations leading to further evaluation of patients for HH include arthralgia, lethargy and malaise [16,23,41]. On physical exam, signs attributed to HH include hepatomegaly, bronze pigmentation of skin, cardiac abnormalities, signs of cirrhosis, testicular atrophy and swelling and thickening of the second and third MCPs [38,39]. The most common sign on presentation is hepatomegaly [4].

Arthralgia is a nonspecific symptom, but it is not specific to HH given the high prevalence of this symptom in the general population. Nevertheless, C282Y homozygosity is related to a higher rate of unilateral and bilateral hip replacement [42]. Arthropathy of the second and third MCP joints has been shown to be specific to HH patients and increases with age, SF levels and presence of C282Y homozygosity, suggesting that iron overload is associated with MCP arthropathy [43]. The prevalence of cirrhosis in C282Y homozygotes varies in different studies, and the frequency varying from 0.66–6% according to the differences in ascertainment in various studies [44–46]. Cardiac iron loading is infrequent in type 1 HH and is more commonly seen in *HJV* and *TfR2* mutations (types 2 and 3 HH) [4]. Diabetes is commonly associated with HH, especially in advanced disease [47]. The association could be due to iron deposition in beta cells of the pancreas or possibly insulin resistance [48]. The presence of diabetes before hepatic iron overload occurs may increase the progression of hepatic fibrosis [49]. The presence of signs of hypogonadism in HH has been well documented, although age and presence of cirrhosis are confounding factors [50]. The underlying pathophysiology could be multifactorial as hypogonadism can be caused by hypothalamic, pituitary or gonadal dysfunction [51]. The prevalence of association of hypogonadism has decreased in HH to around 6.4%, presumably due to earlier diagnosis [50].

SF levels >1000 µg/l at diagnosis are associated with a higher likelihood of hepatomegaly, elevated serum aminotransferases, cirrhosis and increased mortality risk. This increased mortality risk is not eliminated by therapeutic phlebotomy [52]. Allen and colleagues from Australia compared 102 C282Y homozygotes with 291 wild-type genotype subjects and found that the prevalence of HH-related clinical symptoms including fatigue, diabetes, hepatomegaly, elevated aminotransferases and self-reported liver disease was similar in both groups and did not differ whether SF levels were normal or moderately elevated (300–1000 µg/l). The only significant difference was the prevalence of second and third MCP arthropathy which was 11% higher in C282Y homozygotes with elevated SF compared to the wild-type genotype group [53].

Clinical penetrance

The definition of iron overload-related disease varies in different studies and therefore the penetrance rate also varies based on these studies. Allen and colleagues reported that among 203 C282Y homozygotes, 28.4% of men and 1.2% of women by age 65 developed iron overload-related disease [45]. Women not only have a lower incidence of iron overload based on biochemical elevation of iron studies [54,55] but also have a much lower risk of iron overload related disease. In the past, this observation has been attributed solely to menstrual blood losses in women preventing significant iron overload. However, recent research has shown that certain HLA haplotypes are more common in women and are possibly related to increased iron indices [56]. Also, animal studies have shown that hepcidin gene expression in liver is higher in female mice [57].

The clinical penetrance of *HFE* C282Y/H63D compound heterozygote HH genotype may depend on environmental factors or presence of another liver disease. Cheng *et al.,* showed that liver biopsy specimens from patients with the HH phenotype who were C282Y/H63D compound heterozygotes were more likely to show concomitant hepatitis or steatosis compared to C282Y homozygous patients [58]. However, the vast majority (98%) of C282Y/H63D compound heterozygotes identified via screening will never develop iron-overload related morbidity [59,60].

Initial testing

The initial tests to be performed while evaluating a patient with suspicion of iron overload disease are SF and TS [61]. Two consecutive measurements should be done for both tests. Both TS and unsaturated iron binding capacity (UIBC) are equally effective initial tests along with SF [62,63]. Moreover, UIBC testing is somewhat less costly. Calculation of TS is performed by dividing

serum iron with total iron-binding capacity (TIBC). Both random and fasting levels have been shown to have similar sensitivity and specificity for detection of C282Y homozygotes [64]. Multiple studies have used different cutoffs for TS and SF but TS >45% has higher sensitivity (although lower specificity and positive predictive value) compared to higher cutoffs [12]. But, being a screening test, higher sensitivity is a priority. If the TS is >45% especially along with elevated SF (>300 µg/l for men and 200 µg/l for women), *HFE* genotyping should be performed [23].

SF and TS have much higher sensitivity and specificity when used together rather than individually [65]. If testing shows increased SF with normal TS, common causes of raised SF such as kidney disease, infection, inflammation or connective tissue diseases should be ruled out. If there is suspicion of liver disease, common causes of liver diseases such as viral hepatitis, alcoholic liver disease and nonalcoholic steatohepatitis should also be evaluated for. *HFE* genotyping should be considered if the SF is elevated with normal TS, but is likely to be negative for the homozygous C282Y mutation. In the converse situation (elevated TS but normal SF) *HFE* genotyping should be considered but may not be of clinical benefit given the absence of increased body iron stores.

A common cause of elevated SF is dysmetabolic iron overload syndrome (DIOS). This disorder involves a constellation of features which are closely linked to each other. These include genetic predisposition, steatosis, subclinical inflammation and insulin resistance [66]. An increase in SF has been shown to be associated with these individual factors such as insulin resistance and the related syndromes such as metabolic syndrome and type 2 diabetes, even independent of inflammation [67–69]. Patients with HH, especially C282Y homozygotes, usually lack these features of metabolic syndrome, insulin resistance and the non-parenchymal or mixed pattern of iron staining on liver biopsy.

Biochemical penetrance

Two large population studies have evaluated the penetrance rate of the HH biochemical phenotype in C282Y homozygotes. In the HEIRS study, approximately 77% of men and 47% of women had elevated SF and TS values on initial evaluation [45]. However, the HEIRS data did not have long term follow up in patients (only a mean of 112 days) to evaluate for long-term variability in these biochemical tests and possibility of a chance to predict outcomes [70].

The HealthIron study was a population-based study wherein subjects underwent *HFE* genotyping from baseline samples, repeat samples were tested for biochemical progression and subjects were examined for iron overload symptoms and complications after 12 years [45]. Approximately 78% of men and 52% of women with the C282Y homozygous genotype had elevated SF and TS at baseline. Approximately 37% of males and 3% of females in the study had SF >1000 µg/l at baseline. As noted elsewhere, a SF >1000 µg/l at diagnosis has been shown to be associated with an increased risk of cirrhosis and mortality [52]. The predicted probability of advancing to SF >1000 µg/l after 12 years among individuals with baseline SF between 300–1000 µg/l was 13–35% in males and 16–22% in females. The investigators found that one third of the cohort with SF 300–1000µg/l and markedly elevated TS progressed to SF >1000 µg/l, the majority by 55 years of age; based on these data, it appears reasonable to recommend therapeutic phlebotomy to such individuals [55]. Thus, although iron indices at a particular point of time are a 'snap-shot' in the clinical course of disease; they may predict the chance of progression to cirrhosis.

Data from the HealthIron study also showed that C282Y/H63D compound heterozygotes have higher baseline SF levels and TS levels when compared to wild-type genotypes; however these levels do not change much with time in men and postmenopausal women. In premenopausal women, these levels do increase, possibly due to lack of menstrual iron losses with the onset of menopause [59].

In summary, based on the HealthIron Study in a population-based sample, not all patients with a genotypic susceptibility for HH, develop elevated iron indices (biochemical phenotype). Similarly, not all patients with genetic propensity and biochemical phenotype develop iron overload symptoms (clinical phenotype) and only one third of patients with iron overload finally develop end organ damage causing iron overload-related disease.

Patients with other *HFE* genotypes such as H63D homozygous may develop significantly elevated iron indices, both TS and SF, compared to wild-type genotype. However, they do not develop clinically significant iron overload except when associated with concomitant risk factors such as alcohol and steatosis [19].

The incomplete clinical and biochemical penetrance of the *HFE* mutation could be related to multiple genetic and environmental factors. Environmental factors such as alcohol, viral hepatitis and liver steatosis act as cofactors for liver injury and are implicated in accelerated hepatic fibrosis [71–75]. Presence of beta thalassemia trait has long been debated as a risk factor for iron overload, with different studies showing contradictory results. A recent study in 142 beta thalassemia carriers by Lopez-Escribano *et al.* did not show any significant difference in iron stores in those with and without HFE mutations [76]. The lack of significant increase in iron overload can be explained by the fact that the geographical distribution of beta thalassemia corresponds with that of H63D and most studies involved mutation of this allele only. An Italian study showed that presence of beta thalassemia trait in C282Y homozygous patients is associated with worsened clinical expression of the phenotype; this was not seen with single C282Y or H63D mutations [77].

The genetic factors that may affect penetrance include sex, multiple single-nucleotide polymorphisms (SNPs) in different iron-related genes involved in iron metabolism that may regulate the expression of the disease in C282Y homozygous individuals. These SNPs have been shown to be associated with increased iron indices such as SF and TS [78–81]. There are also genetic modifiers located in genomic regions unrelated to iron metabolism, which have been shown to be associated with phenotypic expression in HH patients [82–85]. However the most important variables enhancing penetrance are male sex and alcohol use [86].

Genetic testing

Prior to the availability of *HFE* genotyping, confirmation of the diagnosis of HH was performed by liver biopsy. Confirmation of

the diagnosis of type 1 HH can now be done with identification of the homozygous C282Y mutation (or less often, the C282Y/H63D compound heterozygous mutation). Currently C282Y, H63D and S65C (the latter is generally not clinically useful) testing is clinically available. Testing for the more rare mutations (Table 1) is only available in a few laboratories around the world and is not likely to be clinically useful due to the rarity of these types of HH.

Genetic testing is most useful in patients of northern European descent. It should also be considered in first degree relatives given the 25% likelihood of homozygosity in a sibling of a C282Y homozygous proband.

Evaluation of hepatic iron overload

Liver biopsy

Liver biopsy with measurement of hepatic iron concentration has historically been the cornerstone of diagnosis for HH. The characteristic pattern of increased stainable iron in hepatocytes with a decreasing peri-portal to peri-central 'iron gradient' and absence of iron in reticuloendothelial system (RES) cells are features of type 1 HH; a similar pattern is observed in type 3 HH [68,69]. type 2 HH is characterized by much heavier iron staining which may be pan-lobular and without sparing of RES cells [87,88]. Type 4 HH has a significantly different iron staining pattern with a preponderance of iron in RES cells and involvement of hepatocytes as the disease progresses [87,88]. Iron staining on liver biopsy samples is graded in a semi-quantitative manner (0–4+). Biochemical measurement of hepatic iron concentration (HIC) and calculation of the hepatic iron index (HII) were introduced in the 1980s to increase the accuracy of liver biopsy in the diagnosis of HH [89,90]. HIC >4000 µg/g was found in most patients with phenotypic HH. Hepatic iron index (HII) is HIC (in micromoles/gm dry weight) divided by patient's age in years. The concept of the HII was introduced prior to the era of genetic testing to differentiate putative HH homozygotes that would be expected to continue to absorb iron at a high rate over time from those with mild to moderate siderosis due to alcohol or heterozygosity [89,90]. However it is now recognized that many *HFE* C282Y homozygous patients may have HII <1.9 and patients with liver disease (especially in end-stage cirrhosis) due to other causes may have HII >1.9 [91,92]. Removal of >4 g of iron by quantitative phlebotomy (since each unit of blood removed contains approximately 250 mg of iron) has also been proposed as a diagnostic criterion for homozygous HH, although this method, similar to the HII, has become largely irrelevant as a mean to diagnose HH [93,94].

Liver biopsy remains the most accurate means of identifying the presence or absence of cirrhosis. Following the advent of *HFE* gene testing, biopsy is no longer necessary to confirm the diagnosis if the patient is found to be C282Y homozygous on gene testing. Therefore, at present liver biopsy is needed mainly for prognostic value to determine the presence or absence of cirrhosis in type 1 HH. With the recognition that SF is a useful predictor of the absence of cirrhosis if <1000 µg/l, at present this procedure is only necessary in patients who have a SF >1000 µg/l at time of diagnosis or if another liver disease is suspected [95].

Patients with wild genotype or those who have single alleles of C282Y and H63D or even H63D homozygous are unlikely to develop phenotypic HH. In these patients, further work up is not needed if the iron indices are normal. If they do have elevated ferritin and transferrin saturation, further management should include ruling out secondary causes of iron overload especially if MRI T2* suggests iron overload (Figure 1). These secondary causes include but are not limited to hematological disorders like thalassemia, porphyria cutanea tarda, sickle cell disease, hereditary spherocytosis, sideroblastic anemia and liver diseases like hepatitis C and NASH. Liver biopsy should be done if risk factors for liver disease such as alcohol abuse, obesity, metabolic syndrome, viral hepatitis are present [96].

Imaging

MRI T2* is now increasingly used to confirm iron overload in the liver and has largely replaced liver biopsy as a method to estimate hepatic iron concentration [97]. The presence of iron in the liver leads to alteration in the magnetic field, in turn altering the relaxation time of protons in the field when a high-energy radiofrequency pulse is applied, as with MRI. The relaxation time of protons in iron-loaded tissues can be quantified and is inversely proportional to the iron content [98]. The loss of signal intensity caused by relaxation of the spins is divided by the signal intensity of a reference tissue such as paraspinal muscles, thus quantifying the hepatic iron content [98,99]. MRI T2* and FerriScan® (a similar commercially marketed technique) [201] use software and post-processing techniques to provide an estimate of liver concentration. They may have several advantages over liver biopsy including the ability to monitor HIC over time, the noninvasive nature of these tests and the ability to adjust for sampling and biologic variability in HIC observed with liver biopsy as long as the precision of the estimation of HIC is adequate [100].

Multiple combinations of noninvasive biochemical markers have been used to predict cirrhosis in C282Y homozygous patients. Two studies used a test and validation cohort comprising elevated AST, platelets <200K, ferritin >1000 µg/l (77% in the Canadian arm, 90% in the French arm predicted cirrhosis) [101] and a combination of serum hyaluronic acid and serum ferritin (100% sensitivity and specificity) to predict presence or absence of cirrhosis [102].

Treatment

The main treatment for HH is iron removal by therapeutic phlebotomy (also referred to as venesection). Phlebotomy increases erythropoiesis which subsequently results in removal of iron from the liver, the major storage site for iron, until iron stores are depleted. The amount of blood removed to deplete excess body iron stores is highly variable, although on an average SF decreases by 30 µg/l for every phlebotomy [103]. Most patients with type 1 HH expressing the phenotype have >4g of total body iron stores, which would require approximately 15 therapeutic phlebotomies of 500 cc of blood (each unit of blood contains approximately 250 mg of elemental iron) [4].

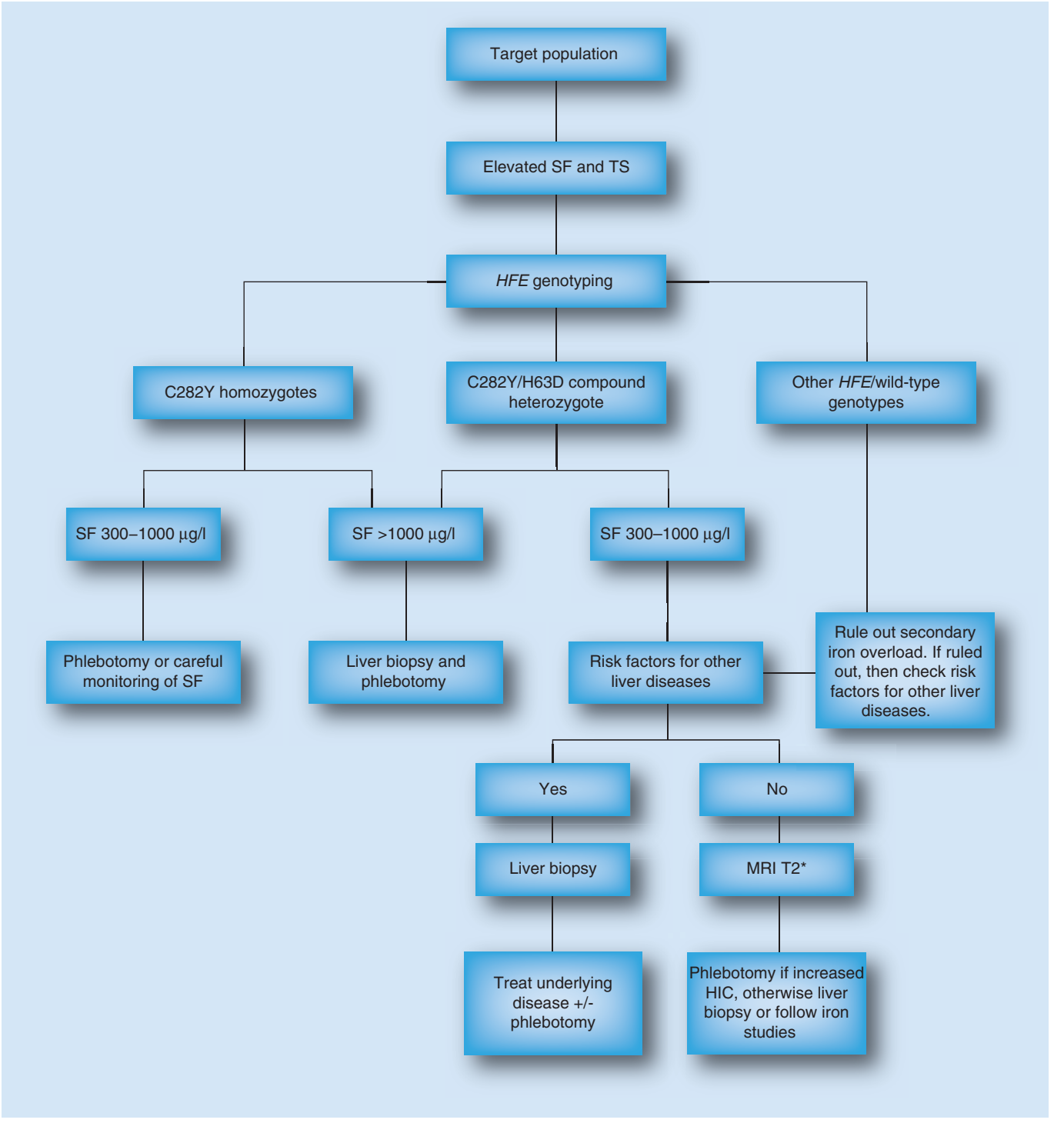

Figure 1. Management of iron overload in *HFE* hemochromatosis.
HIC: Hepatic iron concentration; SF: Serum ferritin; TS: Transferrin saturation.

The frequency of phlebotomy can be decreased to every 2 weeks if a hemoglobin decrease to less than 12 g/dl is noted. The traditional recommendations by most experts for the initial goals of therapy are to achieve a reduction in serum hematocrit to 75% of the baseline level or a SF of <50 µg/l [40]. Lynch and colleagues showed that the non heme iron absorption is 12 and 42% if the mean SF is 538 and 14 µg/l respectively [104]. Consequently, excessive iron depletion to iron-deficient levels may be counterproductive and significantly increase iron absorption and thus the need for maintenance phlebotomy may also further increase. Most experts now suggest that the initial target SF should be between 50 to 100 µg/l and subsequently more frequent monitoring of SF should be done [96]. Depending upon the stability or increase in SF levels, further requirement of maintenance phlebotomy is

decided. The rate of increase in SF influences the frequency of maintenance phlebotomy which can vary from every 1 month to every 3 months.

Phlebotomy treatment may reverse skin pigmentation changes, fatigue and some degree of fibrosis, but MCP arthropathy, hypogonadism, diabetes and cirrhosis are not reversed with iron depletion [105,106]. Some experts have reported improvement in arthropathy after phlebotomy [P Kanwar & KV Kowdley, Pers. Comm.].

Upcoming treatment options

Use of iron chelators has been reserved for patients who are either unable or unwilling to tolerate phlebotomy [107]. Deferasirox is a recently approved oral iron chelator that is safe, effective and usually used for iron overload due to dyserythropoietic anemia. Phatak and colleagues studied deferasirox in *HFE*-related HH in a Phase II study and showed that 10 mg/kg/day dosing for 48 weeks was able to decrease median SF levels by approximately 75% [108]. The main toxicities of deferasirox that are clinically relevant include gastrointestinal symptoms and nephro-and hepatotoxicity. Given these side effects and the cost, deferasirox is not likely to replace phlebotomy as a first line therapy but is an effective option among patients who cannot undergo therapeutic phlebotomy.

Erythrocytapheresis, which constitutes removal of only red blood cells, has been recently compared with phlebotomy and has been shown to significantly decrease the number of treatment sessions and thus the overall treatment duration. Rombout-Sestrienkona *et al.* from The Netherlands showed that the number of sessions of erythrocytapheresis needed to reach a goal SF of 50 µg/l was 33% of the number of phlebotomy sessions needed to reduce SF down to the same level. After they adjusted for the initial SF and body weight, there was still a significant reduction to 43% of the number of sessions. Furthermore, there was no significant difference in treatment costs. However, it is possible that erythrocytapheresis may be more expensive in other countries [109].

Hepcidin, as a peptide hormone may have potential as a therapeutic agent in the treatment of HH. However, large scale production of synthetic hepcidin is not feasible at present and clinical use is limited by a short half-life, low oral absorption and potential risk from excessive dosing. A recent study by Preza *et al.* evaluated mini-hepcidins, which are 7 to 9 N-terminal amino acid peptides acting as the active moiety of hepcidin. Intraperitoneal treatment in mice led to a significant decrease in serum iron levels as compared to controls. The decrease in serum iron was similar to full length hepcidin. There was a significant decrease in HIC when intraperitoneal injections were given to hepcidin-1 knockout mice for 2 weeks with similar efficacy when these mini-hepcidins were conjugated with fatty or bile acids and given orally as gavage. If it is possible to introduce similar oral agents with safety and efficacy, these may be a major improvement to the current treatment whether used alone or in combination with current strategies [110].

Dietary recommendation

Patients with HH should try to abstain from alcohol especially if advanced fibrosis is present [94]. They should also minimize vitamin C use and supplements containing iron tablets [94,111]. Vitamin C can lead to increased intestinal iron absorption and release of iron stores [112]. Concomitant risk factors for liver disease should be evaluated for and managed as needed. Thus, patients with metabolic syndrome should be advised to lose weight with appropriate diet and exercise. Non-citrus fruits may be beneficial [113]. Similarly, proton pump inhibitors such as omeprazole also can be helpful as they decrease the acidity of duodenal contents, thus decreasing absorption of iron [114]. There is increased absorption of dietary iron, especially heme iron in HH patients, which is associated with higher SF concentration in some patients [113,115]. Food fortified with inorganic iron may worsen the iron overload severity in HH patients [116]. Although, it is not clear whether use of supplemental iron may cause clinical symptoms in undiagnosed HH patients, their use is prohibited as reports of iron overload with their intake has been described in literature [117,118]. Similarly, there have been reports of *Vibrio vulnificus* infection with raw shellfish or uncooked oyster ingestion [119] and HH patients should be educated about it. Iron homeostasis is also related to other metals such as copper. An Austrian study showed that copper deficiency was associated with increased SF and hepatic iron levels [120], possibly due to copper involvement in the enzymes involved in iron transport such as copper containing ferroxidases [121].

Orthotopic liver transplantation

Orthotopic liver transplantation (OLT) is considered a curative treatment for patients with end stage liver disease and can also be used for patients with HH and decompensated cirrhosis or HCC. However, historically post-OLT survival in HH patients has been poor compared to other indications for OLT. This is because of multiple perioperative infections, especially within a year after transplant, and cardiomyopathy after the first year [122,123]. A recent study suggested that post-OLT outcomes may have improved in HH and are comparable to other indications for OLT although the latter study did not confirm the diagnosis of HH using objective criteria but rather used data reported from transplant center diagnosis codes [124].

Survival

The mortality rate in HH patients with phenotypic clinical expression is doubled when compared to those without HH, and is even higher in HH patients with cirrhosis [125,126]. First degree relatives of HH individuals have a minimal or no increase in mortality when compared to control populations or spouses of HH patients [126]. Most studies evaluating survival or mortality in HH included only patients with clinical HH or C282Y homozygotes. Studies evaluating C282Y heterozygosity have not shown any increase in mortality with this genotype [127,128]. HH patients with phenotypic clinical expression have a higher mortality risk if not treated [125,129,130]. HH patients without cirrhosis or diabetes have similar prognosis as the general population once they are adequately iron depleted [131,132]. However, there have been no randomized controlled trials to evaluate survival improvement with phlebotomy, likely due to ethical concerns. Therefore, it is difficult to predict survival

benefit in HH patients with mildly abnormal iron indices especially since their risk of cirrhosis and iron-overload related disease is low.

Cancer risk

Patients with cirrhosis due to HH as with other causes of cirrhosis are at increased risk of hepatocellular carcinoma (HCC) and should undergo HCC surveillance every 6 months. Although the mean risk of HCC in HH patients, especially those with cirrhosis was thought to be approximately 8–10% based on multiple studies [133–136], recent studies have reported that the risk is probably lower. These studies suggest that approximately 5–6% of men and 1.5% women with HH will develop HCC [133,137,138]. Moreover, HCC in the absence of cirrhosis has been reported in HH [133,139–141] suggesting a role of iron in carcinogenesis. Ko *et al.* showed that hepatic iron loading in various end stage liver diseases was associated with increased risk of HCC [142]. Even after adjusting for the underlying cause of liver disease, iron loading independently increased the risk of HCC. This supports the hypothesis that patients with HH-related cirrhosis who are at higher risk for HCC and even those with already diagnosed HCC should undergo iron depletion to decrease the carcinogenic effect of iron and thus decrease the risk of developing HCC.

HH patients, especially C282Y homozygotes, have also been shown to have increased risk of breast and colorectal cancers and age appropriate screening should be strongly advised in such individuals [143]. An association between TS, TIBC or SF and increased risk of all cancers has been also shown in multiple studies [144,145]. These studies have therefore renewed interest in iron and its role in carcinogenesis.

Screening & evaluation of family members

Studies have shown that although HH is one of the commonest genetic disorders, its incomplete penetrance and variable expressivity makes genetic screening less attractive [16]. Concerns have been raised by some authors that screening may cause psychological and social stress and can be expensive considering the relatively low prevalence and incomplete penetrance. However recent studies have shown that there are minimal psychological effects of screening [146–149]. Additionally, studies outside the United States where insurance and health care access and coverage are less of a concern have suggested that practical reasons such as lack of awareness and access to screening programs are the major reasons for non-participation in screening programs [150]. We believe that screening persons of Northern European descent may be useful; with phenotypic screening with TS preferred over *HFE* genotyping although TS screening does have limitations due to the performance characteristics of this test [64,151].

All first-degree relatives of family members with HH should undergo genotypic and phenotypic (TS and SF) testing [77]. Children of a proband do not need to undergo genotyping if the other parent has the *HFE* wild-type genotype as this is an autosomal recessive disorder [152]. Given the rarity of complications prior to adulthood in type 1 HH, screening can be deferred in children until adulthood [40].

Expert commentary & five-year view

Many significant advances have been made in our understanding of HH in recent years. It has become clear that the primary physiologic defect in this disorder is in the liver, and is the result of inadequate hepcidin response to iron sensing in the hepatocytes due to the homozygous C282Y mutation in the *HFE* gene. Other genetic forms of HH have been identified due to mutations in other iron regulatory genes such as *HJV* and *HAMP* (hepcidin). Liver biopsy has been largely replaced by *HFE* gene testing and the application of noninvasive tools has helped further define which patients with type 1 HH (*HFE*-HH) are at increased risk of cirrhosis. Population-based studies in the past 10 years have shown us that many individuals (especially women) with the C282Y homozygous mutation do not demonstrate iron overload and even fewer have end-organ damage. In the last 5 years we have seen evidence that most patients with SF <1000 µg/l do not develop iron overload over time and do not have an increased mortality risk. Based on these observations, the need for therapeutic phlebotomy among patients with type 1 HH who have minimal or mildly increased iron overload is unclear and it is possible that aggressive iron depletion may become less common. By contrast, it is clear that those with type 1 HH and SF >1000 µg/l have an increased risk of mortality and end-organ damage.

Hepcidin levels can now be measured by the ELISA method and it is therefore plausible that this assay may become useful during initial evaluation as one of the risk factors for patients to undergo further genetic evaluation [153]. Due to incomplete penetrance in HH, the hepcidin assay may have a role as a diagnostic marker where it is unclear whether a patient will show full clinical expression of the disease.

It is also clear from recent large scale screening studies that there is a continued need to educate clinicians about the proper use of diagnostic tests to screen for and confirm the diagnosis of HH rather than rely on symptoms of the classic presentation of the disease. Those patients found to have elevated TS and SF, should undergo *HFE* genotyping. All patients with known or suspected HH (and not only C282Y homozygotes) should be offered liver biopsy to evaluate for cirrhosis if SF <1000 µg/l.

Novel methods for estimating HIC such as T2* and FerriScan® [201] may be more widely employed in the coming years and noninvasive tools to estimate liver fibrosis may become commonplace and reduce the use of liver biopsy.

It has become more apparent that many if not most C282Y/H63D compound heterozygous individuals who have moderately elevated SF and TS may have another liver disease such as hepatitis or fatty liver and so liver biopsy should be considered in these patients even if the SF <1000 µg/l especially if liver enzymes are elevated.

By contrast, C282Y homozygous individuals with SF between 300–1000 µg/l and normal liver tests may undergo phlebotomy without a liver biopsy. However, recent studies suggest that many, if not most of these patients,will never develop significant iron overload-related morbidity, especially if they are female and do not have other risk factors such as excess alcohol use. Therefore treatment guidelines may change to recommend watchful waiting

or volunteer blood donation alone for those with SF within the normal range or even if minimally elevated.

Patients who are C282Y and H63D heterozygotes, H63D homozygotes or those who have *HFE* wild-type genotype are unlikely to develop phenotypic expression of HH. In these patients, further work up is not needed if the iron indices are normal. If they do have elevated SF and TS, further management should include ruling out secondary causes of iron overload such as hematological disorders and other chronic liver diseases. Liver biopsy should be considered if risk factors for liver disease such as alcohol abuse, obesity, metabolic syndrome, viral hepatitis are present.

Phlebotomy remains the core treatment strategy for iron depletion in HH patients. The target value for initial iron depletion needs more homogeneity as current guidelines from different international societies are somewhat arbitrary. New algorithms such as those utilizing the hepcidin:SF ratio may become useful [154]. Although, it is difficult to predict exactly how much SF will decrease with each phlebotomy session, a definite range of target values need to be outlined, where the hepcidin levels are high enough to prevent further iron absorption and the ferritin is low enough to decrease the iron stores and prevent further morbidity. Therefore, the therapeutic goal may be less restrictive in the future.

Oral iron chelators and erythrocytapheresis may be used more often in the coming years if the cost, safety and efficacy become competitive to standard phlebotomy. Hepcidin or other small molecules used to prevent iron overload may have a role in the future but appear unlikely to be implemented in clinical practice in the next 5 years. Finally, although population-based genetic or phenotypic screening for *HFE*-HH have not generated enthusiasm, we hope that phenotypic-based screening strategies for iron overload using TS, UIBC or ferritin will be more widely adopted as part of 'point of care' or preventive health maintenance 'targeted screening' strategies especially among men of Northern European descent.

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Key issues

- Hereditary hemochromatosis (HH) is an iron overload disorder classified into four types based on different mutations. The most common is autosomal recessive, type 1, which is associated with *HFE* mutations, predominantly C282Y homozygous and rarely C282Y/H63D compound heterozygous. The highest prevalence of C282Y homozygosity is 1:250, seen in populations of North European ancestry.
- Low circulating hepcidin due to decreased hepatic expression of hepcidin resulting from *HFE* mutations is the central mechanism of the disease and, therefore, the role of serum hepcidin as a marker for expression of disease and even a possible treatment option in the future needs to be further studied.
- HH has incomplete penetrance; although a majority of the C282Y homozygotes develop elevated serum ferritin (SF) and transferrin saturation (TS), less than half develop any symptoms and about 28% of men develop end organ damage, while women rarely develop end organ damage.
- The variable expressivity of *HFE*-HH is related to sex and dietary factors; however, other mutations, and environmental factors such as steatosis and viral hepatitis may also contribute to clinical expression.
- C282Y homozygous patients with SF <1000 µg/l especially in the presence of normal serum aminotransferases are at low risk to develop cirrhosis. On the contrary, SF >1000 µg/l is associated with a substantial risk of cirrhosis and therefore a liver biopsy is recommended for staging.
- C282Y homozygous patients with normal or mildly elevated ferritin can undergo frequent monitoring of SF and TS and treatment should not be initiated if their levels remain stable.
- C282Y/H63D compound heterozygous individuals may develop clinical and biochemical iron overload but frequently do so in the presence of co-morbid factors.
- Liver biopsy has become less frequent with the emergence of *HFE* genotyping. With the advent of noninvasive techniques to gauge iron overload such as MRI T2* and evaluation for cirrhosis by techniques such as transient elastography, serum hyaluronic acid levels, liver biopsy will be less frequently used; however, it will remain an important diagnostic method especially where other hepatic causes of iron overload are involved.
- Phlebotomy remains the mainstay of treatment in HH; however erythrocytapheresis and novel chelators along with phlebotomy may become the treatment options in the future.
- The target SF levels during the initial iron depletion therapy need to be better defined. They need to be in a range where optimal hepcidin levels and iron absorption intersect. Some of the guidelines recommend a target SF level of 50 µg/l, which may be too low,
- Due to iron's role in carcinogenesis, venesection for individuals with cirrhosis and/or hepatocellular carcinoma with hepatic iron overload should be considered. Even though these are irreversible conditions, iron depletion may improve their prognosis.
- Although HH has incomplete penetrance and variable expressivity, it has significant prevalence in Northern European populations and screening of these populations should be considered due to the potential for significant morbidity, the availability of inexpensive diagnostic and confirmatory tests and safe and effective therapy.

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