HFE-associated hereditary hemochromatosis

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EJ Eijkelkamp, TR Yapp, LW Powell. *HFE*-associated hereditary hemochromatosis. Can J Gastroenterol 2000;14(2): 121-125. Hereditary hemochromatosis is a common inherited disorder of the iron metabolism. Screening studies indicate that it has a prevalence of one in 200 to 400, depending on the population studied, and a carrier rate of about one in seven to one in 10.

Feder et al identified the hereditary hemochromatosis gene (*HFE*) in 1996 and two candidate mutations; the C282Y mutation has been shown to be responsible for the majority of the hereditary hemochromatosis cases worldwide. The gene discovery has led to rapid advances in the field of iron metabolism. Although the basic defect is still not fully understood, much is known about the sequence of events leading to iron overload.

Hereditary hemochromatosis is a major candidate for population screening and meets the screening criteria of the World Health Organization, and Wilson and Jungner. It is one of the most prevalent genetic diseases in white populations, and, importantly, early diagnosis and simple effective treatment allow normal life expectancy.

The discovery of the *HFE* gene and the frequency of the single C282Y mutation as a cause of most cases of hereditary hemochromatosis allow the possibility of widespread genetic testing. However, the logistics, and the psychological and social consequence of this, coupled with incomplete expression of the genotype, necessitate further studies before population screening can be justified.

Key Words: *Hereditary hemochromatosis;* HFE*; Iron metabolism*

Hémochromatose héréditaire associée à l'*HFE*

RÉSUMÉ : L'hémochromatose héréditaire est une maladie héréditaire courante du métabolisme du fer. Les études épidémiologiques révèlent que sa prévalence est de 1 pour 200 ou 400 selon la population étudiée, et le taux de porteurs serait d'environ 1 pour sept ou dix.

Feder et coll. ont identifié le gène de l'hémochromatose (*HFE*) en 1996 et deux mutations candidates; la mutation C282Y s'est révélée responsable de la majorité des cas d'hémochromatose héréditaire à l'échelle mondiale. La découverte de ce gène a pavé la voie à de rapides progrès dans le domaine du métabolisme du fer. Bien que l'anomalie de base ne soit pas encore entièrement élucidée, on comprend bien maintenant la séquence de phénomènes qui conduit à la surcharge ferrique.

L'hémochromatose héréditaire est une importante maladie à dépister auprès des populations et elle répond aux critères établis à cet égard par l'Organisation mondiale de la santé et par Wilson et Jungner. Il s'agit de l'une des maladies génétiques les plus prévalentes au sein de la population de race blanche et, fait à noter, le diagnostic précoce suivi d'un traitement simple et efficace donne accès à une espérance de vie normale.

La découverte du gène *HFE* et la fréquence de la mutation C282Y comme cause de la plupart des cas d'hémochromatose héréditaire nous permet d'entrevoir la possibilité de procéder à un dépistage génétique élargi. Par contre, pour des questions d'ordre logistique, psychologique et social, alliées à l'expression incomplète du génotype, il faudra poursuivre les recherches avant de pouvoir justifier le dépistage des populations.

Iron overload can result from several different etiological disorders, some inherited and some acquired. The term 'hemochromatosis' was first used by von Recklinghausen (1) and has been widely used to describe the clinicopathological consequences of iron overload, of whatever etiology, ever since. Sheldon (2) was the first to suggest that hemochromatosis was an inborn error of iron metabolism with a familial association. For this reason, we believe that the term 'hemochromatosis' should be reserved for primary inherited forms of iron overload (synonymous with hereditary hemochromatosis [HHC]).

HHC, the inherited form of iron overload, is the most common autosomal recessive disorder in white populations. The deposition of iron in the parenchymal cells of the liver, joints, pancreas, heart, skin and pituitary gland can eventually lead to fibrosis and organ failure, such as hepatic cirrho-

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sis, diabetes mellitus, cardiac dysfunction, arthritis and hypogonadism (3-8).

Feder et al (9) identified the HHC gene and the two candidate mutations in 1996. A single missense mutation, a guanine to adenine transition, leading to a cysteine to tyrosine substitution at position 282 (C282Y), is responsible for the majority of the HHC cases worldwide (6,9-11).

PATHOPHYSIOLOGY OF IRON OVERLOAD

Iron balance is regulated by iron absorption (12), which in turn is reciprocally regulated by the level of body iron stores (13). In HHC, the enhanced net absorption of 3 to 4 mg/day results in the accumulation of 500 to 1000 mg of iron/year during adult life. Increased absorption in HHC occurs in the context of normal dietary intake and normal levels of erythropoiesis, and continues parallel with progressively accumulating iron stores (14). The process of iron absorption involves uptake from the intestinal lumen into the enterocyte, storage of iron in several pools within the enterocyte and transfer from the enterocyte into the portal circulation (3). Cellular uptake of iron is both dependent on, and independent of, transferrin and the transferrin receptor (TfR), located on plasma membranes. TfR is a key protein in iron transport, and modulation of its expression regulates iron uptake. It has been demonstrated that the normal HFE protein binds to the TfR, which may attenuate its capacity to transport iron into the cell (15). With the mutated protein, this binding does not occur, which may lead to increased iron transport into the cell (3).

Independent of transferrin and TfR, an iron transporter gene (*Nramp2* or *DCT-1*) has been defined in animal studies. Expression of *Nramp2* or *DCT-1* (also called divalent metal transporter 1) is inversely regulated by cellular iron concentration; thus, this protein is an ideal candidate for a regulated brush border iron transporter. The role of this transporter in human diseases is a subject of major research interest (3).

The basolateral transport of iron to the circulation is also an important step in the iron absorption pathway and is probably the rate-limiting step (16,17). The recent isolation of a component of this transfer step, a ceruloplasmin homologue known as hephaestin, may hold the key to the understanding of this component of the iron absorptive pathway and to the precise defect in HHC.

How iron leads to tissue damage is still unknown, but possible mechanisms include iron-induced peroxidative injury to phospholipids of organelle membranes, in particular, of lysosomes and mitochondria (18,19); activation of iron-loaded Kupffer cells, which leads to the release of profibrogenic cytokines (20); and altered matrix degradation (21). The evidence is overwhelming that it is the tissue iron concentration per se that is primarily responsible (22,23).

HFE AND ITS MUTATIONS

Gene discovery: Feder et al (9) identified a candidate HHC gene in 1996, which has been termed '*HFE*'. The gene lies 4.5 Mb telomeric to human leukocyte antigen and shows similarity with major histocompatability complex class I genes. One of the two known mutations is a guanine to adenine transition that results in a substitution of a cysteine with tyrosine at position 282 (C282Y) (11). Several studies have confirmed that the C282Y mutation is responsible for the majority of the phenotypically diagnosed HHC cases (6,9-11), ie, groups of investigators from several areas of the world showed that 60% to 100% of white patients with clinically diagnosed HHC were homozygous for the C282Y mutation (9,10,24-27).

Mapping of the whole *HFE* gene revealed a second mutation – a cysteine to guanine change, causing a histidine to aspartic acid substitution at position 63 (H63D). However, the relationship between the H63D mutation and HHC has not been completely elucidated (11,28), though clearly some compound heterozygotes (heterozygous for both C282Y and H63D) show significant iron accumulation but rarely at the level of C282Y homozygotes (28).

Prevalence and mode of inheritance: HHC has an autosomal recessive mode of inheritance. Screening studies using serum ferritin (SF) and transferrin saturation (TS) indicate a prevalence of one in 200 to 400 persons, depending on the population (4,10,11,29,30), though many patients are asymptomatic (30). HHC has a carrier rate of about one in seven (23) to 10 (31,32) individuals. These carriers (heterozygotes) of the C282Y mutation may have an increased iron absorption, but in isolation, this has no effect, and they are unlikely to develop any clinical manifestations (29).

As with any autosomal recessive disorder, equal numbers of men and women are affected, though in all clinical series men have outnumbered women, often by a substantial margin. A possible explanation for this sex difference is loss of iron through menstruation and pregnancy (4).

Role of mutation in the pathophysiology: In the normal human intestine, HFE binds to beta-2 microglobulin and migrates to the cell surface, where it interacts with the TfR. In hemochromatosis patients with the characteristic mutation (C282Y), HFE undergoes a conformational change resulting in an inability to bind beta-2 microglobulin. As a result, the HFE protein is trapped within the cell rather than being expressed on the cell surface. It has been demonstrated (15) that the normal HFE protein binds to TfR, which may attenuate its capacity to transport iron into the cell. With the mutated protein, this binding does not occur, which may lead to increased iron transport into the cell (3).

CLINICAL ASPECTS

The symptoms of HHC are often nonspecific and can be attributed to other causes, so a high index of clinical suspicion is required in making the diagnosis. The most frequent early symptoms are lethargy and arthralgia, though patients also present with abdominal pain, loss of libido or potency, and secondary amenorrhea. Frequent signs and complications are hepatomegaly, pigmentation of the skin, arthritis, hypogonadism, diabetes mellitus and changes in cardiac function (7,8), though their presence usually reflects advanced disease.

Particular attention when taking a history should focus on previous oral and parenteral iron administration, and causes of blood loss (blood donations, excessive menstrual blood loss and/or multiple pregnancies). A hematological examination should be performed for evidence of anemia and abnormal erythropoiesis to exclude iron loading secondary to a hematological disorder such as thalassemia major or sideroblastic anemia.

With the widespread use of biochemical and genetic screening tests, HHC is being recognized earlier in the natural history of the disease and before symptoms and/or physical signs develop. Of interest is the large population screening study performed in Australia (33), which showed that the prevalence of homozygotes for the C282Y mutation was 0.05%, but of these, 30% were asymptomatic and without biochemical abnormalities, 20% had hepatic iron overload without symptoms and only 25% were cirrhotic.

The relevant biochemical abnormalities of iron metabolism suggesting iron overload due to HHC are as follows.

- TS reflects the basic iron abnormality and is, therefore, the most sensitive single test for phenotypic identification of HHC. The normal TS is 30% to 40% (34,35).
- SF can reflect the amount of tissue iron excess, though, as an acute phase reactant, it can be elevated by infection, inflammation and malignancy. The normal SF range is 10 to 300 µg/L (34,35).
- The measurement of the serum iron level is of limited value because of numerous variables. The normal serum iron level is 20 µmol/L (34,36,37).

Liver biopsy is the only practical means of assessing organ damage (6-8,38). Its role in the diagnosis of HHC was recently redefined by Guyader et al (39). The liver biopsy provides a semiquantitative evaluation of iron excess using the hepatic iron index as described by Bassett et al (40) (hepatic iron concentration/age), which indicates iron overload of the degree seen in homozygous HHC when greater than 1.9 or 2.0 (35,40,41). Liver biopsy also allows the assessment of the degree of fibrosis and/or cirrhosis. However, Guyader et al (39) showed that, though the diagnosis of severe fibrosis relies on liver biopsy, the absence of severe fibrosis can be accurately predicted in most patients by using simple clinical and biochemical variables. There is virtually no risk of severe fibrosis in a C282Y homozygous patient with the following: SF level less than 1000 µg/L, normal aspartate transaminase values and no hepatomegaly (39). New techniques of computed tomography and magnetic resonance imaging scanning show potential but are too insensitive for the detection of fibrosis, especially early in the disease process.

Body iron stores may be assessed accurately by careful quantitative phlebotomy, though this is retrospective.

HHC can be confidently diagnosed based on genetic testing for the C282Y mutation, particularly if there are increases in TS and/or SF levels or a family history of HHC (6). However, especially in Southern Italy, non-*HFE*-associated familial hemochromatosis has been described (25,34,42).

Family screening: Following the diagnosis of HHC in a C282Y homozygous proband, it is possible to evaluate the hemochromatosis risk among family members by assessing their C282Y status. Two points, however, need to be considered. The first concerns the chance of lack of clinical expression in cases of C282Y homozygosity, ie, the degree of penetrance of this genotypic profile. It is increasingly acknowledged that some homozygous individuals will not develop significant iron overload during their life (3,34,43). The second point concerns children. In so far as there is no expected therapeutic decision to be taken during childhood, screening for HHC, particularly at birth, is not justified. This is reinforced by the fact that penetrance of the homozygous status is incomplete (34).

TREATMENT

Removal of iron by phlebotomy therapy is the mainstay of treatment of HHC (3,44). Once or twice weekly, if tolerated, 500 mL of blood (equivalent to 250 mg of iron) should be removed until the storage iron pool is depleted. In practice, this may take two to three years for individuals with a very heavy body iron burden. The SF can be expected to decline progressively during therapy with the greatest fall/mg of iron removed (1 ng/mL of ferritin per 2 mg of iron) occurring in the early phlebotomies. Serum iron and TS levels usually remain elevated until iron depletion has occurred. Usually, they do not fall until the SF level falls below 30 to 50 ng/mL. Estimation of hemoglobin concentration is an adequate guide to the therapeutic 'endpoint'; phlebotomy should continue at weekly intervals until the hemoglobin concentration falls below 11 g/dL and does not immediately recover, indicating the development of mild iron deficiency. The frequency of phlebotomy therapy can then be reduced to three to six/year to maintain TS at under 30% to 50% and the SF at less than 50 to 100 µg/L. Lifelong phlebotomy therapy is required, preferably on a regular rather than intermittent basis. Measurement of SF and TS periodically (every three to six months) is useful to predict phlebotomy requirements and is helpful to encourage patients to continue with therapy (44).

There are no dietary restrictions, but patients are advised to avoid oral iron therapy, alcohol abuse and vitamin C intake (which enhances iron uptake) (3). Chelation therapy with desferroxamine is not routinely recommended (3) and is usually reserved for patients who cannot tolerate phlebotomy because of heart failure, hypoproteinemia or anemia. For patients with HHC, phlebotomy not only is more effective but also is less expensive, more convenient and safer.

PROGNOSIS

The prognosis of symptomatic untreated HHC is poor. Several studies show that there is a significant difference in survival time between untreated and treated patients. The mean survival of untreated patients was 1.5 to 4.9 years, whereas the mean survival time of treated patients was 5.3 to 8.2 years (2,7,8,45).

In a study by Niederau et al (8) in 1996, the nonspecific

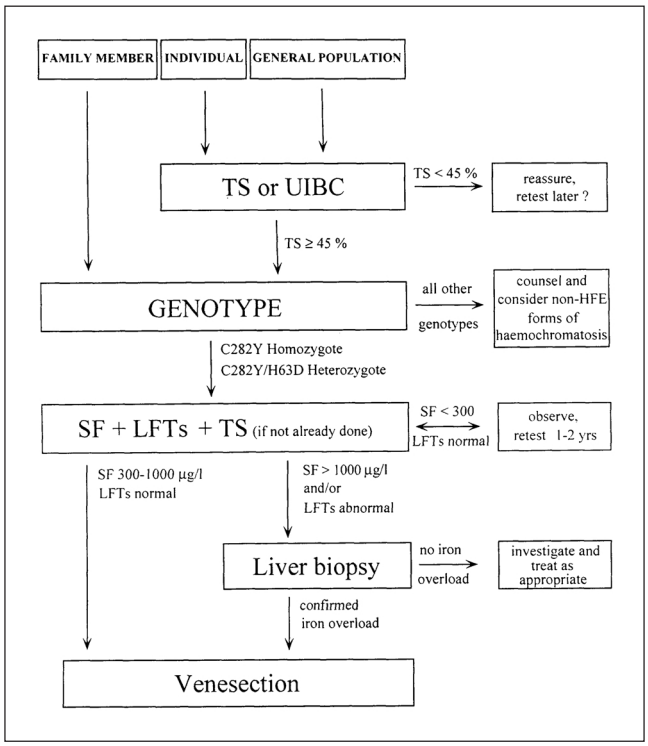

Figure 1) *Diagnostic algorithm for family members of known cases, individuals and general population screening. LFTs Liver function tests; SF Serum ferritin; TS Transferrin saturation; UIBC Unsaturated iron binding capacity*

symptoms of weakness, lethargy and abdominal pain improved after the initial removal of iron in the majority of patients. More specific signs of liver disease decreased in more than 70% of patients, whereas endocrine and joint changes improved in only 19% to 30% of patients after iron removal. Insulin dependency could not be eliminated by iron removal, although the daily dose of insulin was reduced in 41% of patients.

Survival in noncirrhotic and nondiabetic patients was significantly better than that of cirrhotic and diabetic patients, and was virtually identical to that expected for a sex- and age-matched normal population (8,46,47).

The major causes of death in HCC patients are hepatocellular carcinoma, cirrhosis and cardiomyopathy (7,8). In HHC, the risk seems to be mainly restricted to cirrhotic patients (8), but rare cases without cirrhosis but with fibrosis and architectural distortion have been described (48-50). The relative risk of hepatocellular carcinoma in patients with HHC and cirrhosis is 200-fold (7,38). Men are much more susceptible to hepatocellular carcinoma than woman (51,52). For all patients with cirrhosis, the risk of hepatocellular carcinoma increases with time (50). Screening of cirrhotic patients for hepatocellular carcinoma with interval (usually six monthly) ultrasound and alfa-foetoprotein is recommended, although not conclusively shown to be cost effective.

The presence of cirrhosis is the single most important prognostic feature. Cirrhosis is irreversible and not affected by phlebotomy. For patients with end-stage liver disease due to HHC, liver transplantation is a therapeutic possibility (44).

GENERAL POPULATION SCREENING

HHC is a major candidate for population screening (53) because it is an important health problem. With its high frequency, it is one of the most prevalent genetic diseases in white populations (more frequent than, for example, phenylketonuria, which is routinely screened for at birth) (4). Most importantly, early diagnosis and treatment can improve the patient's prognosis to normal life expectancy (8,47,53). A suitable and accepted test for early diagnosis is available, and so are the facilities for the safe and effective treatment of HHC. There is an agreed policy on whom to treat. The natural history and clinical course of HHC are well established in a patient with iron overload. Screening for HHC would be cost effective and could be a continuing process (32,54-56). These characteristics meet the screening criteria of the World Health Organization (3), Wilson and Jungner (57), and others (4,53-55). The optimal timing for phenotypic screening is when the patient is under 30 years of age, at a time when HHC is evident from iron studies in most patients but before serious organ damage has occurred (6) (Figure 1).

In considering population screening with the DNA test, one should be aware of the lack of information about the penetrance of the genotype, because nonexpressing homozygotes are common, even in elderly subjects (3,4,54). There is also a lack of information on other, undetected genetic mutations (so called 'non-*HFE*-associated HHC'), and about psychological and social consequences (such as the possibility of medical and genetic discrimination, and stigmatization) (4,53).

Current debate centres on the relative roles of screening tests. More information is required on the natural history of the disease and the penetrance of the genetic mutation before widespread genetic tests can be recommended. However, early diagnosis and removal of excess iron result in normal life expectancy and prevent the complications of iron overload. Thus, family screening is important, and iron measurements should be part of standard health checks.

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