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REVIEW

Changing aspects of *HFE*-related hereditary haemochromatosis and endeavours to early diagnosis

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ABSTRACT

***HFE*-related hereditary haemochromatosis (HH) is an iron overload disease attributed to the highly prevalent homozygosity for the C282Y mutation in the *HFE* gene. The pathophysiology of this error in iron metabolism is not completely elucidated yet, although deficiency of the iron regulatory hormone hepcidin appears to play a role. Ways of diagnosing iron overload include measurement of the serum iron parameters, i.e. serum transferrin saturation and serum ferritin, by a liver biopsy or by calculating the amount of mobilisable body iron withdrawn by phlebotomies. Clinical signs attributed to *HFE*-related HH include liver failure, arthralgia, chronic fatigue, diabetes mellitus and congestive heart failure. Organ failure can be prevented by phlebotomies starting before irreversible damage has occurred. Therefore, screening to facilitate early diagnosis is desirable in individuals at risk of developing *HFE*-related iron overload. Over time it appeared that the clinical penetrance of the *HFE* mutations was much lower than had previously been thought. This changed the opinion about a suitable screening modality from case detection, via population screening, to family screening as the most appropriate method to prevent *HFE*-related disease. However, before the implementation of family screening it is vital to have thorough information on the relevance of the specific health problem involved, on the clinical penetrance of C282Y homozygosity and on the effectiveness of the screening approach.**

KEYWORDS

Diagnosis, family, hereditary haemochromatosis, *HFE*, screening

INTRODUCTION

Classical hereditary haemochromatosis (HH) is a disease related to iron overload with an increase in physical symptoms over time, leading to organ failure and poor survival. Treatment is relatively simple: removing iron overload by phlebotomies, thereby preventing disease and increasing survival. After the discovery of its prime gene mutation, the C282Y mutation of the *HFE* gene, large-scale screening for *HFE*-related HH became feasible. However, along the years it became clear that the traditionally low prevalence of patients with HH could not be fully ascribed to the ignorance of the medical staff, but was likely to be due to the limited penetrance of the *HFE* gene mutation. This review describes new insights into pathophysiology, diagnosis and penetrance of *HFE*-related HH, and its implications for secondary prevention and early treatment of the clinical disease.

HISTORY

One of the first to describe a clinical syndrome characterised by cirrhosis of the liver, diabetes mellitus

and bronze skin pigmentation was Trousseau.[1] The name haemochromatosis was first used by von Recklinghausen (1889), describing post-mortem findings in patients who had died from 'bronzed diabetes'.[2] In 1935, Sheldon suggested a familial form of haemochromatosis,[3] but it was not until 1975 that Simon *et al*. described an autosomal recessive form of idiopathic haemochromatosis related to the HLA-A3 allele in the major histocompatibility complex (MHC) on chromosome 6. In 1996 Feder *et al*. were able to isolate the HH gene in 85% of HH patients.[4] It was initially called HLA-H, as its organisation and structure were similar to genes in the HLA region that coded for HLA-class I heavy chains. However, as a HLA-class I pseudo gene had already been named HLA-H, the newly identified haemochromatosis gene was renamed *HFE* (the abbreviation of HFE being surprisingly not otherwise specified) as proposed by the Genome Databank.[5]
Until now, more than 30 allelic variants of the *HFE* gene have been reported.[6] The most common mutation is C282Y that results from a transition at nucleotide 845 (845G→A), leading to substitution of tyrosine for cysteine. This alters the HFE protein and its association with β2-microglobulin, resulting in a decreased presentation of the HFE protein on the cell surface.[7-9] A second, although less important, HH-associated mutation occurs at nucleotide 187 of the *HFE* gene, with a substitution of histidine for aspartate at nucleotide 63 (63H→D).[4] Several other *HFE* mutations, some of unknown significance, have been reported.

PREVALENCE OF C282Y *HFE* GENE MUTATION

The prevalence of the C282Y *HFE* gene mutation varies throughout the world. The overall prevalence of homozygosity and heterozygosity for the C282Y mutation in European countries is 0.4 and 9.2%, respectively, with heterozygosity ranging from 1% in the Southern European countries to 24.8% in Ireland.[10] In North America an overall frequency of C282Y heterozygosity, regardless of the ethnical roots, was reported as 9.0%, whereas in the Indian subcontinent, and African, Middle Eastern and Australian populations prevalences of 0 to 0.5% were found.[10] For the Netherlands the percentages of C282Y homozygosity and heterozygosity are calculated at 0.2 and 12.0%, respectively.[11]

PATHOPHYSIOLOGY

The exact role of the mutated *HFE* in the pathophysiology of iron overload is still unclear. It has been suggested that the HFE protein modulates uptake of transferrin-bound iron by undifferentiated intestinal crypt cells, thereby programming the absorptive capacity of enterocytes derived from these cells.[12] However, over the years, this 'crypt model' as the sole explanation of unneeded iron entering the circulation became controversial. Indeed, recently a normal iron metabolism was described despite the lack of *HFE* gene expression in the duodenum.[13] In 2003, mice studies by Nicolas *et al*. suggested that it is mainly the failure of hepcidin induction that contributes to the pathogenesis of HH.[14] Hepcidin has been shown to regulate iron homeostasis by internalisation and subsequent degradation of ferroportin, a major cellular iron exporter protein in the duodenal villi cells and macrophages, thereby suppressing iron uptake and release, respectively.[15] Absent or very low hepcidin concentrations lead to a juvenile onset of the clinical iron overload disease, whereas moderately decreased hepcidin concentrations, in case of mutations in the *HFE* gene, lead to relatively low and late onset of iron overload disease.[16-19]

CLINICAL SIGNS AND SYMPTOMS

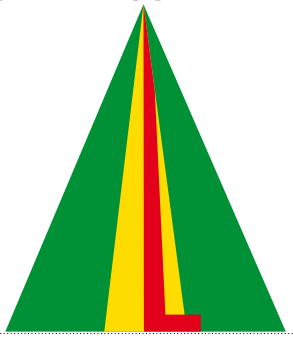
In 2000 an expert group described *HFE*-related HH as follows: 'HH is an inherited disorder resulting from an inborn error of iron metabolism which leads to progressive loading of parenchymal cells in the liver, pancreas and heart. In its fully developed stage organ structure and function are impaired'.[20] Early clinical symptoms are described to encompass weakness, joint pain, palpitations and abdominal pain, whereas massive iron overload will ultimately lead to arthritis, severe fatigue, chronic abdominal pain, liver enzyme elevations, liver cirrhosis, primary liver cancer, diabetes mellitus, hypopituitarism, hypogonadism, congestive heart failure, cardiac dysrhythmias, increased skin pigmentation and an increased risk of certain bacterial infections.[20-27] All symptoms are relatively nonspecific, making it difficult to recognise them as being related to iron overload. In addition the clinical penetrance of the *HFE* gene mutations is very variable.[28-30] Until now searches for additional gene mutations that may identify patients at increased risk of developing clinical manifestations of haemochromatosis have not been successful.

DIAGNOSIS OF IRON OVERLOAD

Elevated iron parameters in the serum, i.e. serum transferrin saturation (TS) and serum ferritin (SF) are a strong indication for altered iron metabolism (*figure 1*). In the literature various reference ranges are mentioned, probably due to differences in the populations examined and lack of standardisation of especially serum ferritin analysis. A serum transferrin saturation above 45%, in combination with an elevated SF level, is highly suggestive

Figure 1. *Schematic view of the five stages of HFE-related hereditary haemochromatosis (HH) together with the various strategies for screening on HH*[34]

Five stages	Composition of the population
C282Y homozygosity with increased transferrin saturation, increased serum ferritin, and clinical symptoms manifesting organ damage predisposing to early mortality	IV
C282Y homozygosity with increased transferrin saturation, increased serum ferritin, and clinical symptoms affecting the quality of life	III
C282Y homozygosity with both increased transferrin saturation, increased serum ferritin, but no clinical symptoms	II
C282Y homozygosity with increased transferrin saturation, but normal serum ferritin values and no clinical symptoms	I
C282Y homozygosity without biochemical or clinical symptoms (normal plasma transferrin saturation and serum ferritin)	O



Green = population screening strategy; yellow = family screening strategy; red = individuals who develop clinically important HH, targeted screening; horizontal red bar = individuals with C282Y homozygosity.

for increased body iron levels. However, abnormal values can be found in the presence of other pathology, including liver diseases and alcohol abuse.[31-35] Homozygosity for the C282Y mutation or the combined C282Y/H63D genotype in the *HFE* gene analysis confirms the HH diagnosis.[34,36] The traditional gold standard for diagnosing iron overload is a liver biopsy, although it is generally only required for diagnosis in the presence of comorbidities and for prognosis and management when serum ferritin levels exceed 1000 µg/l.[37,38] Hepatic magnetic resonance imaging (MRI) provides a noninvasive approach to semi-quantify the amount of liver iron.[39-41] The severity of iron overload can also be calculated from the number of phlebotomies required to deplete iron stores.[20,22]

TREATMENT OF HEREDITARY HAEMOCHROMATOSIS

The treatment of HH consists of venesection, as described by Davis.[42] It is safe, inexpensive, and appears to be effective, although this has never been proved. With the removal of 500 ml of blood, about 200 to 250 mg of iron is withdrawn from the body. Venesection is started when the SF levels are consistently above the upper limit of the reference range, pointing to body iron excess. Meanwhile, other causes of increased SF must be eliminated.[31-35] Weekly phlebotomies are performed to withdraw excessive amounts of iron, followed by yearly measurement of the serum ferritin and when necessary maintenance phlebotomies to maintain low body iron stores.[20,32,43] Erythrocytapheresis might be an attractive alternative but more studies are awaited to assess its (cost) effectiveness in comparison with venesection. Next to venesection, dietary advice has been described to be beneficial, including moderation of alcohol intake and avoidance of iron, vitamin C supplements and uncooked seafood.[43-47] Consumption of black tea with meals has been reported to decrease iron absorption by formation of nonabsorbable iron complexes.[48]

FROM EARLY DIAGNOSIS AND TREATMENT TO DEATH PREVENTION

Despite the high frequency of the C282Y mutation and the obvious iron overload in a subset of patients, the clinical diagnosis of HH is easily overlooked and delayed until irreversible organ damage has developed, as early symptoms are relatively nonspecific. Even more advanced complications are not always recognised as symptoms of HH, unless specifically looked for. This is underlined by the recent findings of Powell *et al.*[49] Through assessment of disease manifestation by clinical examination and liver biopsy in their population of asymptomatic C282Y homozygous subjects, they found that hepatic iron overload was already present in 56% of the males and 35% of the female subjects. Moreover, one or more unrecognised HH-related disease conditions (arthropathy, diabetes mellitus, hepatomegaly, hypogonadism or cardiac arrhythmia) were present in 30% of the males and 12% of the females.[49] This supports the statement that screening is mandatory for early detection of *HFE*-related iron overload to prevent organ failure and death.

To reappraise in general terms the indication for and attitude to screening Whitby restated the principles of early disease detection set up by Wilson and Jungner (*table 1*).[50,51] Many reports have been written on the feasibility of early screening on HH in the general population.[52-61] Indeed, *HFE*-related HH meets important criteria as described by Wilson & Jungner, and Whitby: A recognisable latent or early stage, a suitable test for examination, facilities for diagnosis and treatment and an accepted treatment.[50,51,53,62]

Table 1. *Restatement of the Wilson and Jungner principles for mass screening programmes (World Health Organization, 1968)*[50]

1. The condition being sought should be an important health problem, for the individual and the community
2. There should be an acceptable form of treatment for patients with recognisable disease
3. The natural history of the condition, including its development from latent to declared disease, should be adequately understood
4. There should be a recognisable latent or early symptomatic stage
5. There should be a suitable screening test or examination for detecting the disease at the latent or early symptomatic stage, and this test should be acceptable to the population
6. The facilities required for diagnosis and treatment of patients revealed by the screening programme should be available
7. There should be an agreed policy on whom to treat as patients
8. Treatment at the presymptomatic, borderline stage of a disease should favourably influence its course and prognosis
9. The cost of case finding (which would include the cost of case finding and treatment) needs to be economically balanced in relation to possible expenditure on medical care as a whole
10. Case finding should be a continuing process and not a 'once and for all' project

One important question that remains unanswered: Is HH indeed an important health problem, for the community, and for the individual?[50,51,63] At first it was assumed that all C282Y homozygous individuals would eventually develop iron overload resulting in tissue damage and disease.[31] But selection bias, differences in case definition and population characteristics led to different findings. Some authors found haemochromatosis-related disease in a high percentage of C282Y homozygous individuals, whereas others barely found any penetrance of the *HFE* gene mutations.[21,49,64-67] Some large and controlled studies reported that a significant proportion of the C282Y homozygotes had no symptoms of disease at all, questioning the importance of the health problem.[29,30,68-71]

Another principle of screening still not profoundly resolved is statement 8 added by Whitby (*table 1*): Treatment at the presymptomatic, borderline stage of a disease, early treatment, should favourably influence the course and prognosis of the disease. In other words it should be more effective started early than started later in the disease development and/or clinical phase.

How to decide which population is to be screened? Searching for individuals with an elevated risk of HH can be performed at three population levels: i) clinical examination of individuals with symptoms pointing to HH, i.e. targeted screening or case detection; ii) screening the families of patients in whom the clinical diagnosis of HH has been made; and iii) population screening (*figure 1*).

Ad i) Case detection

Medical examination of individuals with symptoms pointing to HH is a very direct way of detecting patients with HH. However, despite the high frequency of C282Y homozygosity in Northern European countries, it can be assumed that the clinical disease is under diagnosed, possibly due to the misunderstanding on the part of physicians that the diagnosis should only be considered if skin bronzing / hyperpigmentation, diabetes mellitus and hepatic cirrhosis are present. Furthermore, unfamiliarity with the existence of the disease and scepticism about the prevalence are a serious barrier to accepting an effective screening for HH.[35,72,73] Therefore, it is important to make physicians more aware of the nature of *HFE*-related HH, e.g. the gene mutation frequency, its clinical penetrance and phenotypic expression, and also of the diagnostic pathway and therapeutic options when choosing this type of screening.[74] Implementation of a guideline for physicians on the targeted detection of HH in an early, symptomatic, stage could be beneficial.[72] Jacobs *et al.* studied the impact of such a guideline. It led to an increased awareness for HH, but at the cost of an increased rate of false-positive newly diagnosed HH patients. Of the patients eligible for HH, 70% were still not tested.[75]

Taken together, this screening strategy of case detection has its shortcomings for early disease detection.

Ad ii) Family screening

In family screening first-degree relatives of C282Y homozygous patients with clinically detected *HFE*-related HH are screened for HH. After all, these family members are at relatively high risk: there is a 25% risk of siblings being homozygous.[28] They are likely to share genetic and environmental factors with the clinically positive proband, which may engrave phenotypic expression of HH. From a theoretical point of view this screening strategy has a potentially increased detection rate as well as higher effectiveness of early intervention.[76-79]

Ad iii) Population screening

In comparison with family screening, population screening offers the possibility of an even earlier and larger-scale detection of *HFE*-related HH. However, health-threatening symptoms have been shown to occur in only a minority of C282Y homozygotes, making population screening not the first option of HH screening given the low penetrance for cirrhosis of the liver of 2% found by Beutler and 5% found by Powell.[29,49,63]

FUTURE INTERVENTION

HFE-related HH is a recognised clinical entity, with variable clinical penetrance. Screening and detecting those

individuals at high risk of iron overload, before irreversible damage evolves, is likely to prevent organ detriment and death. From all the mentioned screening options, family screening is likely to be the most appropriate approach. However, before starting screening programmes questions remain to be answered: Do C282Y homozygous individuals have a relevant health problem? Which individuals are at risk to develop *HFE*-related iron overload and its accompanied disease? Is screening for these individuals cost-effective? To get an answer to these questions the Dutch HEmochromatosis FAmily Study (HEFAS) was initiated. From 224 probands homozygous for the C282Y mutation and presenting with clinically recognised symptoms of HH and 735 of their first-degree family members a large set of data has been collected, with regard to demographics, lifestyle (smoking, use of alcohol, diet), health, disease, and family structure, including familial death rate. Additionally iron parameters and *HFE* genotype were collected or determined. These data are currently being analysed; preliminary results are reported in an accompanying paper in this issue (80). They can give instrumental answers on how to prevent disease in as yet unidentified individuals at risk for *HFE*-related HH.

In conclusion, there are changing views concerning the penetrance of *HFE* mutations. The need for diagnosing HH early is a challenge to develop appropriate screening strategies for prevention of iron overload-related tissue damage in individuals at risk.

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