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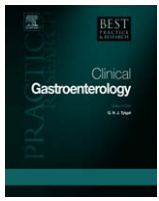

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Hereditary haemochromatosis

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Haemochromatosis should currently refer to hereditary iron overload disorders presenting with a definite and common phenotype characterised by normal erythropoiesis, increased transferrin saturation and ferritin and primarily parenchymal iron deposition related to innate low (but normally regulated) production of the hepatic peptide hormone hepcidin. Since the discovery of the haemochromatosis gene (*HFE*) in 1996, several novel gene defects have been detected, explaining the mechanism and diversity of iron overload diseases. Overall, at least four main types of hereditary haemochromatosis (HH) have been identified. This review describes the systematic diagnostic and therapeutic strategy and pitfalls for patients suspected for HH and their relatives.

Introduction

Haemochromatosis can be broadly defined as any disorder characterised by iron deposition and tissue injury in multiple organs. Hereditary haemochromatosis (HH) is one of the most common inherited disorders, with a frequency of 0.4–1.0% in people of Northern European origin [1]. It exhibits an autosomal recessive inheritance pattern and leads to excessive absorption of dietary iron inappropriate to body iron store, leading to iron deposition in the liver, heart, joints, pancreas, and other endocrine organs [2]. Early diagnosis and therapeutic phlebotomy can prevent the development of tissue damage, reducing morbidity and mortality and providing long-term survival similar to the general population [3]. Table 1 demonstrates the differential diagnosis of iron overload in humans.

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Table 1
Differential diagnosis of iron overload in humans.

Hereditary haemochromatosis
HFE-associated hereditary haemochromatosis (type 1)
C282Y homozygosity
Or C282Y/H63D compound heterozygosity
Non-HFE-associated hereditary haemochromatosis
Type 2A haemojuvelin mutations
Type 2B hepcidin mutations
Type 3 transferrin receptor-2 mutations
Type 4 ferroportin mutations
Other
H-ferritin IRE (HHCS)
Hemoxygenase deficiency
Neonatal iron overload
Aceruloplasminaemia
Congenital atransferrinaemia or hypotransferrinaemia
DMT1 mutations
Secondary iron overload
Iron loading anaemias
Ineffective erythropoiesis
Thalassaemic syndromes
Sideroblastic anaemia
Myelodysplastic syndrome
Congenital dyserythropoiesis
Increased erythropoiesis
Chronic haemolytic anaemia
Parenteral iron overload (including multiple blood transfusions)
Other
Metabolic syndrome
Obesitas
Hypertension
Insulin resistance
Chronic liver disease
Hepatitis
Alcohol abuse
Non-alcoholic steatohepatitis (NASH)
Porphyria cutanea tarda
Iron overload in sub-Saharan Africa

In 1996, Feder et al identified the haemochromatosis (*HFE*) gene [4,5]. They attributed the most common form of HH to homozygosity for the C282Y sequence variation of this gene. Since then it rapidly became clear that the situation was much different from that previously thought: despite its remarkably high prevalence, C282Y homozygosity was characterised by relatively low clinical penetrance. It has also become obvious that other genetic entities can cause clinical pictures identical to that of the *HFE* gene defect. On the other hand, some of these new gene abnormalities are associated with markedly different clinical and biochemical pictures of iron overload.

Recent discoveries have been fruitful, resulting in a better understanding of iron metabolism with the discovery of new iron homeostasis related proteins including the hepatic peptide hormone hepcidin which plays a critical role in regulating systemic iron homeostasis [6]. However, despite recently obtained insights in the molecular basis of iron loading disorders, the prevalence and clinical consequences of increased iron stores in the population are not known [7]. Unfortunately, an increasing number of patients undergo molecular testing just because plasma ferritin and transferrin saturation (TS) are increased.

In this review current guidelines for diagnosis and genetic testing for HH are discussed.

Pathophysiology

The control of iron homeostasis acts at both the cellular and the systemic levels and involves a complex system of different cell types, transporters, and signals. To maintain systemic iron

homeostasis, communication between cells that absorb iron from the diet (duodenal enterocytes), consume iron (mainly erythroid precursors), and store iron (hepatocytes and tissue macrophages) must be tightly regulated. The recently identified ß-defensin-like antimicrobial peptide hepcidin is thought to be the long-anticipated regulator that controls intestinal iron absorption and macrophage iron release [8–11]. Hepcidin is mostly synthesised in the liver when changes occur in body iron needs, such as in anaemia, hypoxia, and inflammation, and is secreted in the circulation. Recent *in vivo* studies have shown that hepcidin is down-regulated by erythropoiesis, anaemia, and hypoxia, which meets the need of increased iron input for erythrocyte production in these conditions [12,13]. Cytokine-induced hepcidin levels in inflammation leads to the iron trapping in the macrophage characteristic of the anaemia of inflammation. Recently, light was also shed on how hepcidin exerts this regulatory function (Fig. 1); it was reported to counteract the function of ferroportin, a major cellular iron exporter protein in the membranes of macrophages and the basolateral site of enterocytes, by inducing its internalisation and degradation [14]. Sequence variations in *HFE* were shown to lead to inappropriately low concentrations of hepcidin, suggesting that *HFE* is involved upstream in the regulation of hepcidin expression [15,16]. Subsequently, this inadequate hepcidin synthesis leads to increased iron absorption in the intestine and subsequent iron overload in *HFE*-related haemochromatosis.

The excessive absorption of iron leads to cellular injury through the Fenton reaction which generate oxyradicals: these, in turn, promote lipid peroxidation of organelle membranes [17,18]. Progressive deposition of iron in the liver can result in fibrosis and ultimately cirrhosis. In early HH, iron remains localised within hepatocytes along the pericanalicular axis of the cell. Increasing levels of hepatic iron increase the risk of cirrhosis, and age and duration of iron loading have been shown to play a significant

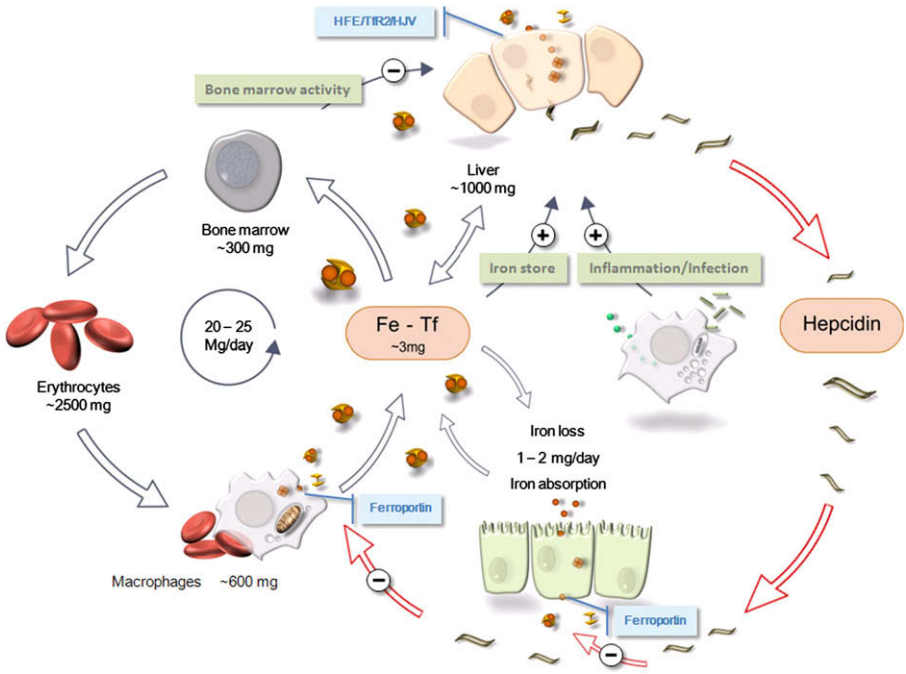

Fig. 1. Iron uptake and recycling. Most of the utilised body iron is recycled from senescent erythrocytes by macrophages, and returned to the bone marrow for incorporation in erythroid precursors. The liver and reticuloendothelial macrophages function as major iron stores. 1–2 mg of iron is absorbed and lost every day. The liver-produced peptide hepcidin controls the plasma iron concentration by inhibiting iron export by ferroportin from enterocytes and macrophages. This means that an increased hepcidin production leads to a decrease in plasma iron concentrations. Hepcidin expression is regulated by body iron stores, inflammation, erythroid iron demand, and hypoxia via regulation pathways involving expression of *HFE*, *TfR2*, *TfR1* and *HJV* genes. Hb: haemoglobin; Tf: transferrin. *Adapted and reproduced with permission from Swinkels et al, Neth J Med* [28].

role in the development of advanced fibrosis. There is a threshold of hepatic iron concentration associated with the development of cirrhosis. Substantial hepatocyte and Kupffer cell iron loading is required before fibrosis becomes evident. Hepatic injury in *HFE*–HH develops in the absence of significant necrosis or marked inflammation. At extreme levels of hepatic iron loading sideronecrosis occurs, this is thought to be responsible for macrophage activation, which may lead to both the development of fibrosis and redistribution of iron towards non-parenchymal cells. Although a potential role for inflammatory mediators in the pathogenesis of iron-induced fibrosis has been proposed, the sources of these mediators remain to be elucidated.

When present, excessive alcohol consumption, chronic infection with hepatitis C virus, and obesity-related steatosis have been shown to act as cofactors in the development of fibrosis and cirrhosis [19,20]. In non-HH liver disease, iron may be a cofactor in exacerbating liver injury.

Clinical picture

The traditional bronze diabetes phenotype of HH commonly found in early reports of the disorder is now rare. Iron accumulation can result in a number of nonspecific symptoms Fig. 2. Furthermore clinical symptoms can differ substantially between individuals with HH and between different forms of HH. Early clinical symptoms are described to encompass weakness, fatigue, joint pain, abdominal symptoms, and skin pigmentation, whereas massive iron overload will ultimately lead to arthritis, severe fatigue, chronic abdominal pain, liver enzyme elevation, liver cirrhosis, primary liver cancer, diabetes mellitus, hypopituitarism, hypogonadism, congestive heart failure, cardiac dysrhythmias and increased risk of certain bacterial infections [21–26]. However, none of the early clinical symptoms have been proved to occur more frequently among subjects with the genetic condition of HH than among control subjects. The occurrence of any of these symptoms, therefore, does not justify the performance of diagnostic tests for HH in first-line care [27,28]. However, according to international guidelines, serum iron status should be considered in patients of Northern European descent who have been referred to a specialist after at least six months of unexplained symptoms as described above [29–31].

Diagnosis

Detection of iron overload

During the first diagnostic phase, the combined measurement of serum iron, transferrin (and the calculation of TS) and ferritin, offers a simple and reliable first approach for determining the amount of iron in the body. TS is the most sensitive parameter for identification of susceptibility for haemochromatosis. Serum ferritin reflects body storage iron, and increased iron stores are often suspected when the serum ferritin concentration is elevated. Universal reference values, however, are absent, due to different populations examined and the shortcomings in standardisation of ferritin assays.

In the further diagnostic workup hyperferritinemia unrelated to iron overload should be excluded. Main confounding factors include:

- Alcohol: alcohol induces ferritin synthesis. They revert to normal usually after three months of abstinence [32].
- Metabolic syndrome: it is one of the most frequent causes of hyperferritinemia today, that is often accompanied to normal TS values. This condition is also named insulin resistance-associated iron overload or dysmetabolic hepatosiderosis. Ferritin levels may be elevated up to 1200 μg/L [33].
- Inflammatory conditions: in this case hyperferritinemia is associated with low TS levels. Anaemia can be present, corresponding to the classical anaemia of chronic disease. Therefore, plasma CRP should be checked.
- Acute or chronic hepatitis: when hepatocyte damage is present, intracellular ferritin can be released into the blood stream due to either real membrane damage or altered membrane permeability. In order to lead to significant hyperferritinemia, cytolysis must be severe, corresponding to highly elevated plasma ALT.

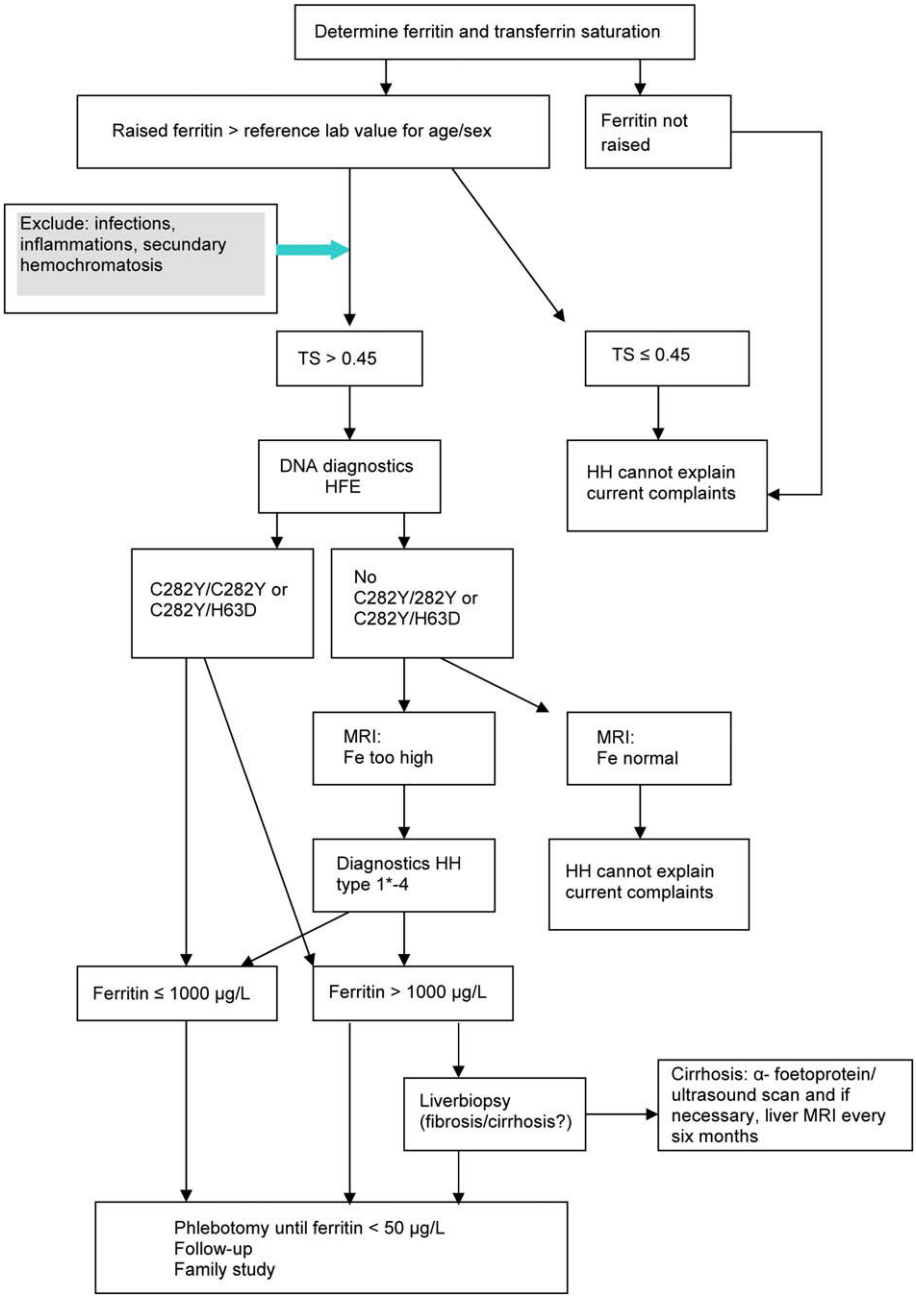

Fig. 2. Diagnostic diagram for suspected iron accumulation. TS = transferrin saturation, HH = hereditary haemochromatosis, MRI = magnetic resonance imaging, *Type 1 diagnostics consists of testing the gene for rare mutations (i.e., not the frequent C282Y and H63D mutations). In addition to the information in the diagram the diagnostic route taken may depend on: clinical presentation, haemoglobin (low in secondary types of iron accumulation and in some forms of ferroportin disease), family history (hereditary disease), concomitant clinical pictures (hepatitis, alcohol abuse), age upon presentation (young in the case of juvenile haemochromatosis). *Adapted and reproduced with permission from Swinkels et al, Neth J Med* [28].

- Hereditary hyperferritinemia cataract syndrome, an autosomal dominant genetic disorder associated with elevated serum ferritin level without iron overload and cataract [34,35].
- Excessive oral or parental iron supplementation [36]. Although this is rare, asking the patient about iron use is mandatory.
- Ferritin is often significantly elevated in the context of infectious (Epstein–Barr virus), inflammatory (Still's syndrome) or iron loading anaemias.

For the reason stated above, among primary-care patients the proportion of patients with elevated ferritin levels that actually reflects clinically relevant increased iron stores is unknown.

If TS is slightly increased, normal or low, it is mandatory to answer the question whether hyperferritinemia reflects iron excess. This can be done by either (1) liver biopsy, (2) MRI, or (3) retrospectively by phlebotomy. This is discussed in the section 'detection of visceral iron overload'. In this setting the most common diagnosis is that of the metabolic syndrome and its features. In the absence of metabolic abnormalities and/or confronted with a significant iron overload (LIC (Liver Iron Concentration) >150 μmol/g and/or removed iron >2.5 g), rare genetic causes of iron excess may be sought for.

Genetic investigation

When TS is $>45\%$ and ferritin levels exceed the upper reference laboratory limits, *HFE* mutations should be investigated.

- The finding of C282Y homozygosity allows to establish the diagnosis of *HFE* haemochromatosis. Then, disease stage has to be assessed and, according to stage, follow-up or phlebotomy should be proposed.
- The finding of any other *HFE* genotype must be interpreted with caution since (a) compound C282Y/H63D heterozygosity does not result in clinically relevant iron overload, i.e., only 20–25% of compound heterozygotes present with mild increase in TS and 10% slight hepatic iron excess, and (b) H63D homozygosity, C282Y heterozygosity and H63D heterozygosity may never explain abnormal serum iron and ferritin levels in the absence of an associated cause of impaired iron metabolism [37].

Non-*HFE* haemochromatosis becomes an option once (i) liver disease and haematological disorders have been ruled out and (ii) iron excess has been proven by direct assessment, i.e., by MRI or liver biopsy. Juvenile forms of haemochromatosis will be considered first in young patients with severe iron excess resulting in heart and endocrine symptoms whilst other adult forms of HH (discussed later) will be first sought for in case of mild and late symptoms, mainly related to joints and liver.

Detection of visceral iron excess and damage

Liver biopsy

The gold standard for the diagnosis and prognosis of liver iron overload is still a liver biopsy [38]. Indications for a liver biopsy are [28]:

- to assess the prognosis by evaluating iron overload consequences in terms of fibrosis and cirrhosis, especially in case serum ferritin levels are above 1000 μg/L [39].
- to assess the diagnosis and/or prognosis in the presence of concomitant clinical pictures such as hepatitis and alcoholic or non-alcoholic steatohepatitis.
- to diagnose iron overload in the absence of C282Y homozygosity and C282Y/H63D compound heterozygosity and after exclusion of secondary causes of iron overload.

MRI

The presence and severity of iron overload can also be determined by MRI [40–43]. Quantitative MRI is increasingly used as an alternative to liver biopsy to assess the iron liver content but needs

additional expertise and validation. An advantage of MRI is that it can evaluate hepatic versus splenic iron load since a dominant splenic iron excess will mean preferential macrophagic iron deposition, therefore, orientating the diagnosis towards transfusional iron excess or ferroportin disease [44].

Next to non-invasive measures of iron overload, it is also currently investigated whether liver stiffness measurement using transient elastography (Fibroscan, Echosense) is correlated to liver fibrosis in various chronic liver diseases [45,46].

Treatment

Therapy of HH is relatively simple, reducing iron accumulation by phlebotomy, which can prevent and possibly reverse tissue damage [3,47]. Life expectancy is reduced in patients with HH and cirrhosis, but not in those without cirrhosis, compared to an age matched and sex-matched normal population.

During the depletion phase, weekly 500 ml bloodlettings are performed based on haemoglobin, MCV and serum ferritin, until ferritin levels are less than 50 μg/L [28,48]. It has to be noted that in ferroportin disease haematological tolerance may be somewhat problematic due to the risk of anaemia, so that the scheme should be monitored by careful follow-up of Hb levels.

During the maintenance phase, most guidelines recommend to check ferritin levels at least half yearly and to keep levels below 50 μg/L, which may involve several phlebotomies per year. A paradoxical observation is that phlebotomies that target low ferritin levels may increase iron absorption in C282Y homozygotes [11,16,49]. Recent data point out that this can be attributed to very low hepcidin levels that result from low body iron levels and/or increased erythropoiesis, which may favour iron absorption [49]. This might implicate that the reference level for ferritin currently targeted in most venesection protocols should be increased. The exact value, however, has to be determined, probably by using serum hepcidin levels.

Frequency of phlebotomies should not be based on TS levels, since as in C282Y homozygotes elevated TS generally appear to be rather resistant to lowering by phlebotomy, this might result in iron deficiency anaemia.

Therapeutic perspectives

Erythrocyte apheresis

The removed amount of iron may be increased two or threefold for each procedure by using erythrocyte apheresis, a modern equipment for selective withdrawal of red blood cells [50,51]. Possible drawbacks of this technique may be higher costs, prolonged time for each therapeutic procedure, and certain requirements to the patients. The possible advantages are the reduced number of therapeutic procedures and less strain for the patient. At the moment randomised studies are being performed comparing cost-effectivity apheresis to phlebotomy.

Use of oral iron chelators

Until recently, and despite its potential efficacy, the only available oral iron chelator, deferiprone (Ferriprox®), was not found to be suitable for genetic iron overload due to its rare but unpredictable risk of agranulocytosis. The new oral iron chelator Exjade® is now available for posttransfusional iron overload with good efficacy and, so far, acceptable tolerance [52]. It is not registered for HH, and should only be used in exceptional cases, since conventional phlebotomies have much less side effects [53,54].

Other experimental treatments

Recently it was suggested that administration of proton pump inhibitors to patients with HH can inhibit the absorption of non-haem iron from a test meal and the habitual diet. During long-term treatment of patients with HH it was observed that proton pump inhibitors reduced the requirement for maintenance phlebotomy [55,56].

Furthermore $Ca2+$ channel blockers may reverse iron overload by a new mechanism via divalent metal transporter-1 [57].

Role of genetic testing in (differential) diagnosis and/or treatment

The discovery of the *HFE* gene has been a crucial step for identifying the major form of haemochromatosis. The mutation prevalence is high in Caucasian populations since it is found, at the heterozygous state, in approximately 10% of the subjects and in the homozygous state in 3–5 subjects per thousand. In contrast, the C282Y mutation is almost absent in the non-Caucasian populations. Hereafter, the way was opened for the identification of other forms of genetic iron overload which turned out to be non-*HFE* related.

Non-*HFE* haemochromatosis becomes an option once liver disease, haematological disorders and other causes of hyperferritinemia have been ruled out and iron excess has been proven by direct assessment, i.e., by liver biopsy or MRI. These, other well defined but rare genetic disorders of iron metabolism result from mutations in additional recently discovered genes and comprise the various types [58].

The most common of the inherited forms of haemochromatosis is type 1 or *HFE*-related HH. Approximately 90% of subjects with HH are homozygous for the missense mutation that results in the substitution of tyrosine for cysteine at amino acid 282 (C282Y) [4]. A more common mutation is the substitution of aspartate for histidine at amino acid 63 (H63D). This mutation may contribute to minor increases in iron level but rarely causes iron overload in the absence of C282Y. Approximately 1–5% of subjects with HH may be compound heterozygous for both the C282Y and the H63D mutations. Other rare *HFE* mutations have also been described [59,60].

Type 2 or juvenile haemochromatosis (*JH*) differs from *HFE*–HH in that it has an earlier age of onset of manifestations of iron overload, usually by the second or third decade of life [61]. *JH* is related to mutations on either the hemojuvelin (*HJV*) gene (type 2A) or the hepcidin (*HAMP*) gene (type 2B) and usually results in a severe autosomal recessive disorder of early onset affecting both genders equally and is relatively rare. Clinical manifestations are severe and include early hepatic iron loading, cardiac failure and arrhythmias, diabetes, hypogonadism resulting from pituitary involvement and occasionally joint disease. Most *JH* patients who are not treated, die due to cardiomyopathy.

Type 3 or transferrin receptor-2 (*TFR2*)-related HH is an extremely rare iron overload disorder with autosomal recessive inheritance that manifests in the 3rd–4th decade of life with a phenotype similar to that of *HFE*-related HH [6].

Type 4 or ferroportin (*FPN*)-related HH is inherited in an autosomal dominant fashion. Age of clinical onset for this disease is in the 4th–5th decade of life [62]. There are two categories of *FPN* mutations. The first category includes loss-of-function mutations that reduce the cell surface localisation of *FPN*, reducing its ability to export iron. This results in iron deposition primarily in macrophages, and this disorder is sometimes termed '*ferroportin disease*'. Treatment of iron overload in subjects with this type of HH might be problematic because the nature of the underlying disorder limits the ability of phlebotomy therapy to mobilise iron stores. The second category includes 'gain of function' mutations that do not alter cell surface expression but rather abolish hepcidin-induced *FPN* internalisations and degradation. Distribution of iron is similar to *HFE*–HH, being primarily parenchymal. Treatment of individuals with this disorder is similar to that for *HFE*–HH [63].

In all these types of HH, iron overload results from the impairment of the hepcidin–FPN regulatory pathway. Mutations or the absence of *HFE*, *HJV*, *HAMP*, and *TFR2* genes, which cause HH types 1, 2A, 2B, and 3, respectively, reduce hepcidin expression. Furthermore, 'gain of function' mutations in *FPN*, which cause HH type 4B, prevent the hepcidin-mediated degradation of FPN.

Other iron overload disorders such as aceruloplasminemia, atransferrinemia, neonatal haemochromatosis and H-ferritinemia [64] are extremely rare conditions.

Before the above described investigations are being performed, we recommend to send/or consult atypical patients to specialised centres that can perform investigations with an up-to-date, targeted approach since unfortunately, an increasing number of patients undergo molecular testing just because plasma ferritin and TS are increased. In our experience in a tertiary haemochromatosis centre for the Netherlands and Belgium, the latter often leads to an unnecessary search for hereditary conditions that are characterised by similar abnormalities in serum ferritin and/or TS, such as hepatitis, excessive alcohol consumption, several conditions co-occurring in the metabolic syndrome and iron loading anaemias.

Genotype/phenotype correlation

The genotype of HH does identify those subjects at risk for the development of phenotypic disease. It should be noted, however, that clinical expression of *HFE*–HH is not as high as previously thought [65]. A considerable inter-subject variability in both serum iron indices and symptomatology exists, even among patients with identical genotypes [37,66,67].

Although large, systematic, population-based cross-sectional studies have shown that the majority of C282Y homozygotes show a biochemical penetrance, i.e., have increased TS and serum ferritin levels, approximately 25–35% of homozygotes have normal ferritin levels and may not develop tissue iron overload. Also ferritin levels may vary in time in individuals with HH [68].

Some confusion in the literature is related to an imprecise definition of haemochromatosis penetrance. It has become clear that the full-blown form of the disease (especially with cirrhosis) is rare. Cross-sectional studies indicate that cirrhosis occurs in 1–10% of C282Y homozygotes. To estimate clinical penetrance strictly objective criteria should be used, e.g., on the basis of serum liver enzyme levels, clinical examination, liver biopsy, MRI and physicians diagnosis in the context of documented iron overload.

It is suggested in published studies that other genetic and environmental factors are involved in modifying the clinical and biochemical penetrance of C282Y homozygosity [69]. Acquired factors may be diet, tea consumption, blood donation, inflammation, hepatic dysfunction, the metabolic syndrome and drugs. Genetic factors may be related to gender and genes involved in either iron homeostasis or tissue damage, but no factor is found yet that is likely to account for the penetrance of a significant proportion of C282Y homozygous patients [70].

Recently, the clinical penetrance of *HFE*–HH has been characterised in a large Australian longitudinal study of 31,000 adults for a 12-year period. A total of 28% of men and 1% of women developed definite iron overload related disease. Men with a serum ferritin level above 1000 μg/L had an increased risk of fatigue and liver disease compared with men who had serum ferritin levels less than 1000 μg/L. Among the male C282Y homozygotes, the prevalence of hepatic fibrosis was 13.5% and that of cirrhosis 2.7%. These values are conservative since biopsy specimens were obtained from only 43% of C282Y homozygotes with serum ferrtin above 1000 μg/L. The presence of arthritis was not related to the severity of iron overload [71]. This finding confirms and extends the observation of Guyader et al that a serum ferritin level of 1000 μg/L or more is associated not only with cirrhosis but also with symptomatic HH in C282Y homozygotes. Arthropathy, as defined by clinically abnormal metacarpophalangeal joints, was unrelated to serum ferritin levels in homozygotes, a finding that confirmed results reported previously [3,72–74].

Two other longitudinal studies have attempted to characterise the penetrance with the use of population-based data. Andersen retrospectively assessed 23 homozygotes during a 25-year period and found no evidence of liver disease associated with HH [67]. Olynynk et al retrospectively assessed 10 homozygous patients (including six women) during a 17-year period, reported that of six patients that underwent liver biopsy, three had cirrhosis or fibrosis [75,76].

In a recent systematic review, Whitlock et al estimated, after accounting for patients who were lost to follow-up, that disease would eventually develop in 25–60% of homozygotes [27].

On the basis of a cross-sectional population study of subjects between the ages of 20 and 80 years, Beutler et al suggested that disease attributable to HH occurred in less than 1% of all C282Y homozygotes, regardless of gender. However, they did not perform clinical examinations or liver biopsies, and a quarter of the homozygotes were excluded on the basis that they had received a previous diagnosis of HH. They found no association between C282Y homozygosity and the presence of fatigue or arthritis [77].

A particular group of *HFE* genotypes consists of persons who are compound heterozygous for C282Y and H63D. These individuals have been described as being at higher risk to develop iron overload, but in a generally much milder form than in C282Y homozygotes [37,78–80]. However, given the fact that the clinical penetrance of C282Y homozygosity is already low, compound heterozygotes with clinical disease will be scarce. A third sequence variant, S65C, with an allele frequency as low as 1.6%–2.0%, was also found to exert a consistent but small effect on serum iron indices, particularly when present in combination with other *HFE* genotypes, such as C282Y and H63D [81].

Family members of individuals with clinically detected HH are noted to be at higher risk for being homozygous and are more likely to have an increased biochemical and clinical expression compared to their non-homozygotes counterparts. In fact, we performed the Haemochromatosis Family study (HEFAS) in the Netherlands, that was focussed on first degree relatives of C282Y homozygous patients with clinically overt HH and designed to analyse the biochemical and the clinical penetrance. We found that iron overload was more severe in C282Y homozygous siblings of probands with higher ferritin levels relative to the upper reference limit.

Furthermore, in the search for determinants in clinical expression the HEFAS study revealed that genotype, age and gender were predictive for the development of iron dependent organ disease. Surprisingly, with genotype in the model, there was neither an additive predictive value of the serum iron parameters, nor of BMI or alcohol intake.

Family screening

Screening for HH either by phenotypic tests (TS and ferritin) or by genetic testing (*HFE* mutation analysis) has been considered in mainly three groups of individuals: (1) patients with symptoms suggestive for HH (case detection); (2) family members of an affected proband; and (3) the general population.

In the years after the discovery of the *HFE* gene in 1996 population screening of C282Y homozygosity seemed an attractive strategy encouraged by the high prevalence of homozygosity for the C282Y mutation in the *HFE* gene among both clinically overt HH patients (around 80%), and in individuals of the Northern European descent (around 0.5%) [82]. It was especially after the publication of Beutler et al in 2002 on an incomplete penetrance of the C282Y sequence variant that questions arised on the cost-effectiveness of population screening [77]. Several groups have analysed various aspects of costs and benefits. The most comprehensive cost analysis was conducted by Gagne and colleagues. They evaluated the cost-effectiveness of 165 haemochromatosis population screening algorithms involving biochemical or genetic screening tests by developing a computer program that simulates all possible scenarios [83]. They found that population screening is only cost-effective with a prevalence of three cases per 1000 individuals and a penetrance of the biochemical phenotype greater than 70%. These data, combined with a growing concern regarding the potential for genetic discrimination and psychological harm due to identification of a genetic defect, steered many experts away from recommending a population-based screening strategy.

Instead, early case detection by family (cascade) screening and increased awareness for the disease in the presence of symptoms that are consistent for HH seems more likely to have a significant effect [82]. Theoretically, an index patient's siblings and his children/parents have ca 25 and 5% chance, respectively, of being predisposed to HH. Moreover, family members that share environmental and genetic modifiers with the symptomatic proband, are likely to have a similar clinical penetrance or the mutated *HFE* gene, making family screening promising and cost-effective, especially if contributing factors are elucidated. This implicates that relatives to the first degree should be evaluated on the basis of iron parameters and, in the event of an *HFE*-related form of HH, on the basis of *HFE* genotyping as well.

Discussion/conclusion

We have described a systematic diagnostic strategy for patients suspected of HH and their relatives. HH should not be defined by genotype in isolation, but in combination with biochemical evidence of raised serum iron indices. We recommend that after initial clinical and laboratory investigations and exclusion of acquired causes of hyperferritinemia, atypical patients are send to specialised centres that can perform investigations with an up-to-date, targeted approach, including rational targeting of the gene variations to be screened. Diagnostic screening of suspected non-*HFE* HH by assessment of hepcidin values might reduce the workload and costs of the cumbersome procedures of screening for sequence variations in the multiple genes responsible for haemochromatosis.

Finally, screening family members of known affected individuals should be considered the standard of care. With the advent of *HFE* mutation testing in family members, it is possible to diagnose

'predisposition for haemochromatosis', that is, individuals at increased risk of clinically significant iron loading based on the finding of C282Y homozygosity. Assessment of hepcidin levels or hepcidin/ferritin ratio's might be potentially useful in the prediction of biochemical or clinical penetrance of HFE-related HH as well [16].

Phlebotomy treatment is safe and effective to remove excess iron and can reverse preexisting hepatic fibrosis [39]. Technology or industry driven new treatment options such as erythrocyte apheresis and iron chelation should be carefully evaluated before introduced in clinical practise. We furthermore suggest that in the absence of solid evidence to target at ferritin levels below 50 μg/L, to aim at ferritin levels below the upper limit of the reference range (generally 300 μg/L for men and 200 for women) instead of 50 μg/L. Future well controlled studies should be designed to collect evidence that these novel recommendations result in a decreased frequency of maintenance phlebotomies without affecting the prognosis of the patient. In monitoring of phlebotomy treatment, hepcidin levels below a certain threshold might become an indication to change the phlebotomy interval [49].

The diagnostic and therapeutic strategies proposed above, however, may change in time with advances in noninvasive techniques for the assessment of hepatic iron and tissue damage, the availability of serum hepcidin measurements, the identification of new key players in iron homeostasis and the advent of novel therapeutic approaches.

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