PAPER

Haemochromatosis

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Iron is an important component of the Earth's crust, but its own chemistry greatly limits utilisation and also sets the basis for its toxicity. The capacity of readily exchanging electrons in aerobic conditions makes iron essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration. On the other hand, as humans have no means to control iron excretion, excess iron, regardless of the route of entry, accumulates in parenchymal organs and threatens cell viability. In fact, a number of disease states (that is, iron overload diseases) attributable to genetic or acquired factors are pathogenetically linked to excess body iron stores and iron removal therapy is an effective lifesaving strategy in such circumstances.

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DEFINITION

Haemochromatosis (HC)—synonymous with genetic, hereditary, primary iron overload—is the paradigm of a genetic disorder leading to body iron overload and multi-organ failure. HC is attributable to inappropriately increased iron absorption in which iron loading of parenchymal cells in the liver, pancreas, heart, and other organs impairs the function and damages the structure of these organs.[1] This condition is caused by inborn errors of iron metabolism. As shown in figure 1, at least three genes may now be associated with a disease phenotype fulfilling this definition, while a distinction should be made between HC and siderosis (that is, excess iron accumulation in tissues) that indirectly results from other conditions that bring about iron overload (acquired iron overload) (fig 1).

Von Recklinghausen, in 1889, first coined the term HC to indicate an iron storage disease with widespread tissue injury[2]; he was basically referring to the clinical syndrome of portal cirrhosis, diabetes mellitus, and bronze skin pigmentation, described by Trousseau 24 years before.[3] After the identification of the inherited nature of HC by Sheldon,[4] Simon and coauthors defined the incidence and genetic transmission of the disease that seemed to be linked to the HLA complex on the short arm of chromosome 6 and mapped in close proximity to the HLA-A locus.[5 6] A cornerstone of HC genetics had been laid in 1996, when the discovery of a gene candidate for HC was published.[7] This gene, formerly named *HLA-H* (H for haemochromatosis), it was then redefined *HFE* by the WHO Nomenclature Committee for Factors of the HLA system.[8] Recently, an autosomal dominant form of HC has been described[9] and associated to a mutation in the SLC11A3 gene,[10] coding for a main iron export protein in mammals, ferroportin/IREG/MTP-1.[11–13]

In the vast majority of cases around the world, HC is attributable to mutations in the *HFE* gene.[14] However, it must be emphasised that while the presence of mutations in the HFE gene indicates the existence of the genetic form of HC, the clinical diagnosis of HC is made when iron overload is present (see later). Therefore, the identification of any one of the HFE mutations (C282Y homozygosity or C282Y/H63D compound hererozigosity) is, by itself, insufficient for the diagnosis of HC. Instead, the identification of the genetic abnormality provides evidence of *susceptibility* to developing the phenotype. The diagnosis of HC, therefore, must be based on phenotype rather then genotype. As mentioned, mutations in other genes have been associated to HC, including SLC11A3[10 15] and transferrin receptor 2 (TfR2).[16] These mutations may be responsible for hereditary iron overload diseases non-HFE or act as modifiers genetic factors in HFE HC heterozygotes. All the three forms of HC described so far are characterised by tissue iron overload, organ disease and they require iron removal therapy as treatment. However, the form attributable to HFE, and apparently that attributable to TfR2 mutations (although the latter has not been extensively described clinically, yet) seem to share common histopathological (predominant parenchymal iron overload) and clinical features. The form attributable to the SCL11A3 gene mutations (see later) shows peculiar features. In view of these findings the classification of HC has to be redefined (fig 1).

GENETICS, EPIDEMIOLOGY, AND MOLECULAR PATHOGENESIS

HC is the most common inherited autosomal recessive disorder in the white population, affecting about 1 in 200 of northern European ancestry.[14] In the original paper by Feder *et al*[7] most HC patients were homozygous for a single base transition, resulting in the substitution of a tyrosine for cysteine at position 282 (C282Y) of the unprocessed polypeptide. This mutation prevents correct folding of HFE and, hence, assembly with β_2 microglobulin (β_2M) and presentation at the cell surface. The distribution of C282Y mutation coincides with the presence of the disease and is in agreement with the theory of a Celtic or north European origin of the mutation.[14] The

Abbreviations: HC, haemochromatosis; Tf, transferrin; TfR, transferrin receptor; UIBC, unsaturated iron binding capacity; HIC, hepatic iron concentration; HII, hepatic iron index

1 Primary iron overload
- A Haemochromatosis associated to mutations in the *HFE* gene:
  - C282Y homozygosity
  - C282Y/H63D compound heterozygosity
- B Hereditary iron overload associated to mutations in the *SLC11A3* gene (Ferroportin/MTP/IREG-1)
- C Hereditary iron overload associated to mutations in the *Transferrin receptor* 2 gene
- D Aceruloplasminaemia
- E Congenital atransferrinaemia
- F Others (unidentified genes)
  - 1) Juvenile haemochromatosis
  - 2) Neonatal haemochromatosis

2 Secondary iron overload
- A Dietary iron overload
- B Parental iron overload
- C Iron loading anaemias
- D Long term haemodialysis
- E Chronic liver disease
  - Hepatitis C
  - Alcoholic cirrhosis, especially when advanced
  - Non-alcoholic steatohepatitis
- F Porphyria cutanea tarda
- G Post-portacaval shunting
- H Dysmetabolic iron overload syndrome

3 Miscellaneous
- Iron overload in sub-Sahara Africa

Figure 1 Classification of iron overload diseases.

percentages of C282Y homozigosity are >90% in the UK and Brittany with a decreasing gradient northern to southern Europe, with the lowest percentage in Italy (64%) and Greece (30%). Indeed, HC is an example of a founder (the Celtic) effect, combined with a strong positive selection of the C282Y allele, which may confer the ability to absorb more iron and to survive better in ancient times, when iron deficiency was extremely common.

A second mutation resulting in the substitution of an aspartic acid for histidine at position 63 (H63D) was found in a number of patients who carried only one copy of the C282Y mutation.[7] The H63D mutation has a worldwide distribution with the highest frequency among Basques.[14] This mutation may have a clinical significance only in cooperation with other genetic or environmental factors (for example, viral hepatitis, thalassaemia, porphyrias etc). A considerably smaller proportion of patients are compound heterozygotes for the two mutations (genotype HD/CY). A third substitution of cysteine for serine at amino acid position 65 (S65C), has been implicated in a mild form of HC,[17] whereas the role of other rare mutations found in subjects H63D or C282Y heterozygotes, is unclear.[18]

The human HFE protein is closely related to the major histocompatibility complex (MHC) class I molecules. The *C282Y* mutation disrupts a critical disulfide bond in the α_3 domain of the *HFE* protein and abrogates binding of the mutant *HFE* protein to β_2M.[19] This results in reduced transport to and expression on the cell surface. Conversely, mice lacking β_2M show a haemochromatotic phenotype.[20] The HFE knockout and knock-in (carrying only the C282Y mutation) mice recapitulate the human disease.[21 22]

The first indication that HFE can influence iron homeostasis came with the discovery that it binds transferrin receptor 1 (TfR1) with an affinity close to that of Tf, reducing the affinity of the TfR1 for Tf and competing with Tf binding.[23 24] In addition, in an elegant study, it has been shown that binding to TfR is not required for targeting of HFE to the basolateral membrane, but is required for HFE to be transported to Tf positive endosomes and for regulation of intracellular iron homeostasis in cultured cells.[25] This suggests that the biological effect of HFE on TfR may be exerted in the endosomal compartment where iron is released from TfR-Tf complex and not necessarily at the cell surface. At the tissue level, HFE appears to be preferentially expressed in duodenal crypt cells and Kupffer cells of the liver, mainly in the perinuclear compartment.[26 27]

Iron absorption is inappropriate to body iron stores in HC, and steadily increases from birth despite expanding iron deposits[28] (fig2A and B). Typically, in HC subjects there is a positive iron balance of 1 mg to 2 mg of iron daily that during childhood and adolescence in men and in menstruated women does not cause marked iron accumulation because of high growth demands and iron losses, respectively. Afterwards, iron overload in the liver, pancreas, heart, and joints, leads to parenchymal cell damage and organ disease. In view of the available data, any proposed model of HFE function in iron metabolism has to consider (a) the biochemical evidence (that is, HFE interacts with TfR1), (b) the pattern of expression of TfR1 and HFE in vivo, and (c) the phenotype of human HC.

At the biochemical level, data from several tissue culture studies show that over expression of HFE results in significant reduction in the rate of iron uptake, decrease in iron and ferritin and induction in TfR1 expression.[29] The proposed mechanism of inhibition of TfR function by HFE is controversial: HFE does not reduce transferrin uptake at saturating transferrin concentrations or change the cycling of the TfR1.[30–32] However, some caution should be used in extrapolating these in vitro data to the in vivo situation. Most of these studies have been carried out in vitro using concentrations of Fe^{2+}-Tf, which is lower than that found in plasma (8 μM–15 μM), or using bovine serum Tf, which has a low affinity for human TfR1, or expressing inappropriately and

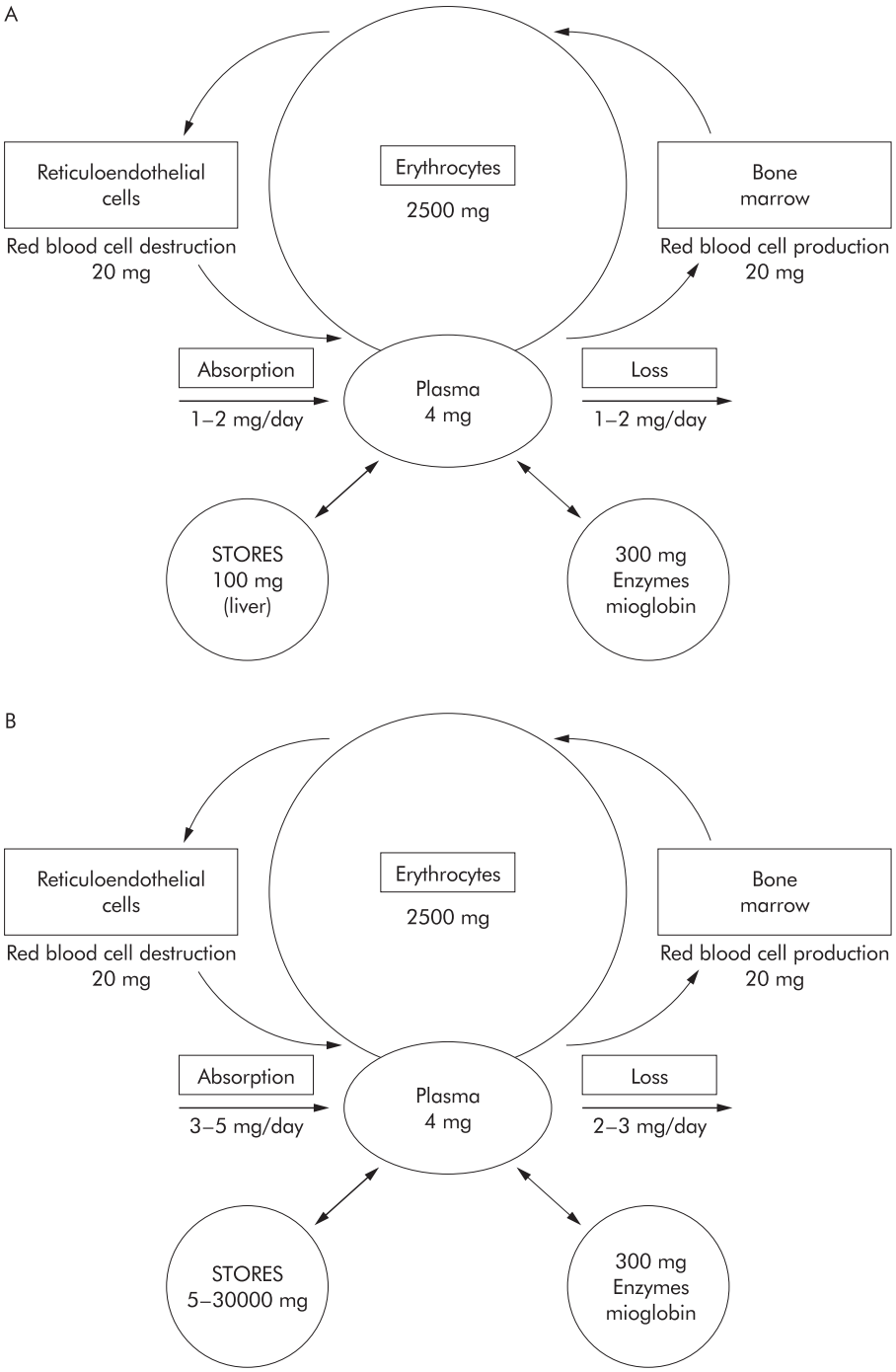

Figure 2 (A) Body iron metabolism. Most iron in the body is present in the red cell comparment and continuously recycles between the bone marrow and reticuloendothelial cells. The only step that is regulated in humans is iron absorption, while iron excretion is mainly a passive phenomenon. (B) In classic haemochromatosis, increased iron absorption leads to dramatic expansion of the iron stores while iron excretion is only slightly modified.

unphysiological levels of HFE. It is in fact clear that the stoichiometry of Tf, HFE, and TfR1 and their relative equilibrium in the cell, in vivo, may be critical in determining a biological effect. Studies of HFE and TfR1 in transfected cells and in solution suggest the stoichiometry is 1:2, or one HFE per transferrin receptor dimer.[33 34] However, the crystal structure indicates that a 2:2 stoichiometry is also possible under very high concentrations of HFE (about 8 mmol/l).[35] The relative concentrations of HFE and TfR1 may be very important in vivo. HFE is mainly expressed in intestinal crypt cells and macrophages of the liver (Kupffer cells) in tissue staining experiments with specific antibodies.[26 27] TfR1 seems to be highly expressed in the crypts.[36] Although macrophages are believed to express low TfR1 and phagocytosis of erythrocytes accounts for most of iron acquired by these cells in vivo, TfR1 mediated uptake may also occur in culture and in vivo in macrophages. Therefore, judging from the results of the available studies, the relative equilibrium of HFE:TfR1 in duodenum and macrophages may be different in vivo, and this may have reflection on the function in the relevant tissue. In one of these studies the expression of HFE in Kupffer cells of the liver was particularly strong,[27] and this might indicate a special role of HFE in these cells. Moreover, the in vitro findings do not explain the HC phenotype: a mutated HFE should lead to iron overload, whereas in HC tissues with strongest HFE (and TfR1) expression are actually iron deficient (that is, crypt cells and macrophages). In fact, in HC patients[37] and in murine models of HC (that is, HFE and β_2M knockout mice)[21 22] little metal is stored in macrophagic cells, and iron regulatory protein activity in HC macrophages

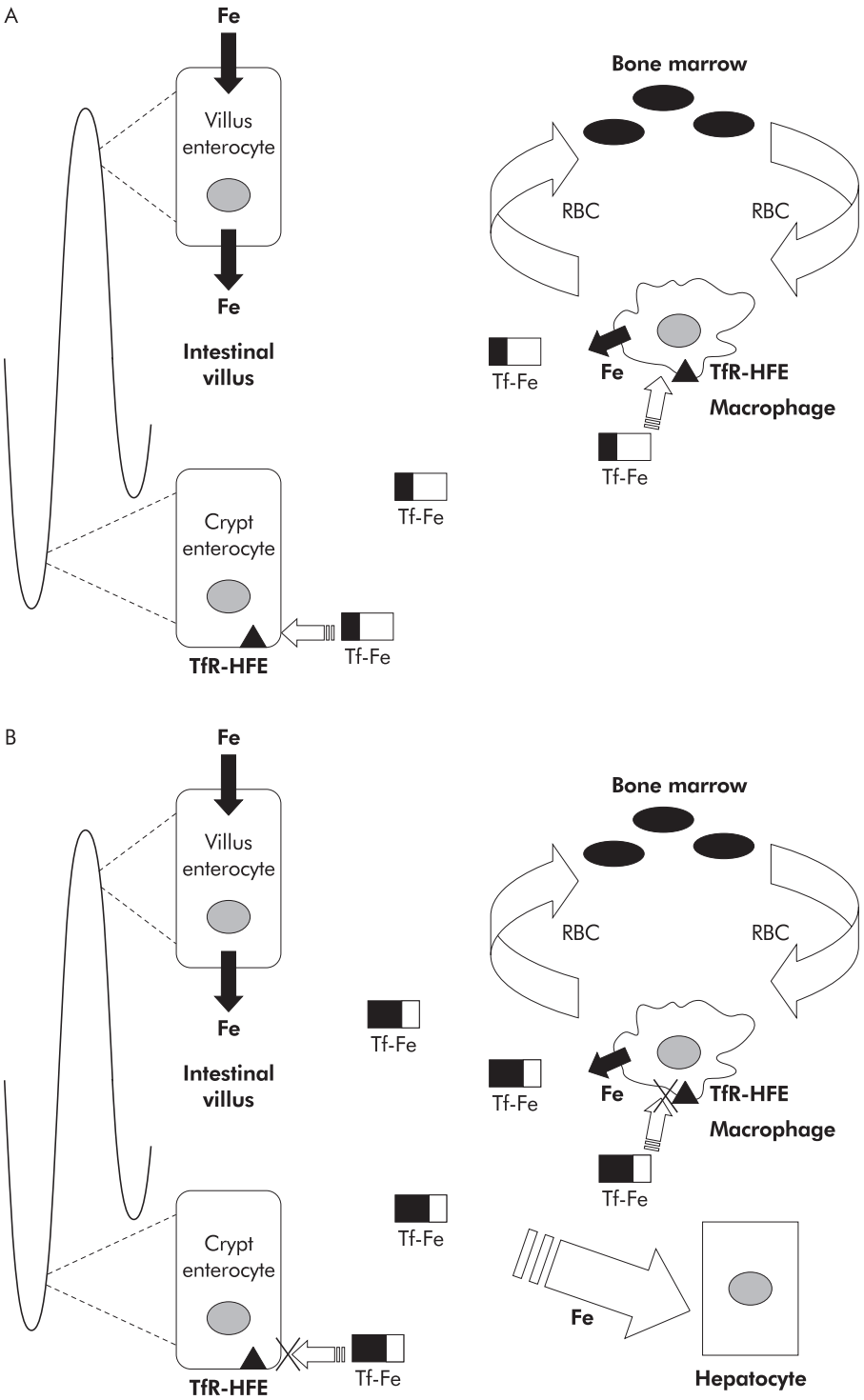

Figure 3 A model for the regulation of body iron homeostasis in normal subjects and in haemochromatosis. (A) In normal subjects iron is continuously recycled between the bone marrow and reticuloendothelial (RE) cells, with serum transferrin acting as a shuttle to deliver iron to the erythron from RE cells and enterocytes. The "information" on the erythron and body iron status is transferred to the crypt cells of the distal duodenum by transferrin: the extent of transferrin saturation with iron acts as the "signal" that is transferred through the transferrin receptor/HFE pathway to the stem cells of the crypts. This sets the level of "free iron pool" in the crypt cell that will be also reflected in the mature enterocytes upon differentiation and migration to the villus. The "free iron pool" will dictate the level of expression of apical and basolateral iron transporters in the mature enterocytes of the villus, and, in turn, of iron absorption. (B) In haemochromatosis, because of the defective HFE and the faulty HFE/TfR pathway, both RE cells and crypt cells receive an incorrect signal of "iron deficiency" despite increasing saturation of circulating transferrin with iron. This, paradoxically, will lead both macrophages and duodenal enterocytes, as in a "true" iron deficiency state, to "release" more iron. Duodenal cells will accomplish this by activating the iron uptake-transfer machinery. Excess circulating iron will be directed mainly through non-Tf/TfR pathways to parenchymal cells of various organs such as the liver.

is inappropriately high indicating a paradoxically low iron pool.[37] The accumulation of iron in parenchymal cells of the liver is most probably a passive phenomenon following increased circulating iron, as hepatocytes express very low TfR1 and no HFE, and may be mediated by TfR2 and other carriers delivering the non-transferrin bound iron fraction (fig 3B). In a recent study by Montosi *et al*[38] it was shown that a lower Fe^{2+}-Tf accumulation is a primary defect of HC macrophages, persisting in vitro, and is corrected by expression of normal HFE. In agreement with this conclusion, in the *HFE* knockout mice, spleens are comparatively resistant to dietary iron loading, possibly reflecting decreased accumulation of transferrin bound iron by the $HFE^{-/-}$ splenic macrophages. Interestingly, the same phenomenon occurs in the intestine of HC patients. Some 30 years ago Astaldi *et al*[39] reported the absence of ferritin bodies in intestinal cells in HC. Then, it was found that high activity of IRP was responsible for inappropriately low ferritin in duodenal cells of HC patients.[40 41] This again implies that these cells are actually iron poor. In agreement with the data by Montosi *et al*[38] and with a role of wild type HFE in favouring intracellular transferrin iron accumulation. Waheed *et al* have recently shown that when in vitro adequate amount of β_2M are expressed along with HFE in transfected cells, increased transferrin iron uptake results.[42]

In summary, a mutated HFE may lead to a defective Tf-iron accumulation particularly in crypt enterocytes and reticuloendothelial cells. How does this translate into increased iron absorption in HC?

As in iron deficiency, the intestinal iron transporters, DMT1 and ferroportin1/IREG1/MTP1, are activated in HC.[43 44] It is probable that these transporters are upregulated by an intracellular iron deficient state. The rate of intestinal iron absorption is usually inversely related to the serum Tf saturation. The main supply of iron to the intestinal crypt cell is from serum transferrin (fig 3A). In anaemic states, increased red cell production induces an increase in plasma iron turnover (with uploading of iron onto circulating transferrin) that leads to a diversion of iron from the intestinal and reticuloendothelial cells to the bone marrow, with increased iron absorption.[29] In conditions of low Tf saturation (for example, anaemia), the crypt becomes starved of iron leading to activation of the genetic iron import-export machinery. When the plasma iron turnover decreases (and transferrin saturation rises) (for example, acquired iron overload) more iron is diverted to the intestinal crypt cells where the expansion of the "free iron pool", once the cells differentiate to iron absorbing enterocytes, down regulates the expression of iron carriers, and decreases iron absorption. In this model, macrophages have a central role. It has been estimated that macrophages in vivo deliver 10 million atoms of iron each minute to circulating transferrin.[45] The uptake of Fe^{2+}-Tf through TfR1 by macrophages may be the means for macrophages to be informed on the body iron status and erythroid demands. In the presence of low Tf saturation, as in iron deficiency, macrophages are prompted to release iron to upload circulating Tf and support the erythron. In HC, they may be prompted to release iron (despite high Tf saturation) because of the faulty TfR1 cycle due to a mutated HFE. The same phenomen occurs in the intestinal crypt cells. The faulty TfR-HFE system will lead to iron deficiency in crypt cells and, consequently, in villus absorptive cells (fig 3B). Intracellular iron deficiency will lead to activation of the intestinal iron carriers.[43 44] This reinforces the concept emphasised in this article that the defect in HC is shared by both macrophages and intestinal cells, but intestinal cells are the final effectors of an increased iron absorption because of their inability to sense the saturated levels of iron in the circulation and to modify accordingly the absorption machinery.

CLINICAL, DIAGNOSTIC, AND SCREENING ISSUES

The advent of HFE testing has profoundly modified our approach to diagnosis and screening of HC.

Diagnostic settings

Clinical diagnosis of HC is difficult. The full clinical expression of HC includes cirrhosis of the liver, diabetes and other endocrine failure, joint inflammation, heart disease. However, the actual symptoms at presentation—fatigue, malaise, arthralgia—are rather non-specific and common in the aging population. Early diagnosis in HC is especially important as treatment by venesection before irreversible end organ damage has occurred can restore a normal life expectancy. Therefore, there is a need for a test that would identify people at risk of developing iron overload before the occurrence of significant organ damage. Within a diagnostic setting, when HC is clinically suspected, a genetic HFE testing should be performed. However, some caution should be used in interpreting the results. First of all, the HC genotype shows incomplete penetrance.[46] There are cases of a known mutation where there is no iron overload and there is a wide range of variation in the expression of symptoms in individuals with disease. The detection of a genotype such as C282Y/C282Y or C282Y/H63D identifies a high risk for iron accumulation but is not tantamount to HC. There is a wide gap between both the frequency of iron overload and of homozygous C282Y, and the frequency with which HC is diagnosed (about 1 in 10 000). The anomaly in regard to HC, in fact, is the discrepancy between the prevalence of the genotype and the number of cases found either by clinical observation or death records. This presents the possibility that the HFE gene may require the interaction of other genes and/or environmental factors to produce life threatening conditions. On the other hand, it is also very possible that the genotype contributes to risk of developing many chronic diseases (for example, liver diseases, diabetes, heart diseases, arthritis and arthralgia, impotence, and infertility) perhaps producing cases of these conditions that are, for example, more serious and/or of earlier onset.

In view of the above considerations, people suspected to have HC and found homozygote for the C282Y mutation should be always checked for iron overload. Iron overload can be evaluated through various methods. The biochemical methods includes: fasting transferrin saturation or unsaturated iron binding capacity (UIBC) and serum ferritin. Transferrin saturation may provide information on higher tissue iron turnover and on increasing body iron levels. Therefore, TS above 50% in men or 35%–40% in pre-menopausal women, may suggest early iron accumulation. Serum ferritin provides a good correlation with the degree of iron excess in tissues (provided confounding factors susceptible to interfere with its level, such as inflammation, cytolysis, or a dysmetabolic iron overload syndrome, has been ruled out). Therefore, serum ferritin is accurate for detection of tissue iron overload, but has low specificity. On the other hand, a normal serum ferritin in an HFE HC homozygote may simply indicate that liver iron overload is not established yet. HC is highly unlikely if the transferrin saturation is normal, the ferritin is normal, and the HFE genotype wild type. An alternative method for assessing the extent of iron overload is by measuring the total number of phlebotomies required to obtain a normal serum iron and ferritin. If a substantial amount of iron can be removed by phlebotomy without inducing iron deficiency anaemia, physicians can be confident that iron overload was present. A substantial amount of iron (and thus the number of units) differs in men and women, being 5 g and 3 g of iron, respectively (assuming 225 mg/iron per unit).

Magnetic resonance imaging can be used to detect increased tissue iron overload, particularly liver iron overload. The present limitation of low sensitivity could be overcome by new technologically more advanced devices. The paramagnetic properties of hemosiderin and ferritin permit the use of a superconducting quantum interference device (SQUID) to measure the hepatic magnetic susceptibility and correlates with hepatic iron concentration.

Traditionally, the gold standard for diagnosing HC has been the quantitation of hepatic iron and observing the histological distribution of iron. Obviously, this gold standard entails a liver biopsy—with the attendent morbidity and mortality risks. The emergence of biochemical and genetic testing may permit the avoidance of liver biopsy in the vast majority of cases. However, when serum ferritin >1000 ng/ml and/or in the presence of hypertransaminasaemia or hepatomegaly, histological examination is required to asses the extent of liver damage and disease. In equivocal cases, liver biopsy may permit hepatic iron assessment through semiquantitative staining or by measurement of dry weight liver iron (hepatic iron concentration, HIC). The hepatic iron index (HII) (dry weight liver iron concentration/age) is one method for differentiating HC from other iron overload states (particularly that associated with alcohol misuse). An HII >1.9 is indicative of HC. The presence of iron in hepatocytes with a decreasing gradient from periportal zone to centrilobular area is typical but not specific of HC. Relative sparing of Kupffer cells is typical of HC but is not seen in association with causes of secondary iron overload.

In conclusion, a positive HFE testing requires confirmation of iron overload. Raised transferrin saturation and decreased

Box 1

Haemochromatosis

- Early increase of transferrin saturation before increase of serum ferritin
- No haematological abnormalities
- Early parenchymal cell iron load; at later stages, also reticuloendothelial cell iron load
- During phlebotomy, serum ferritin declines in parallel with decrease of transferrin saturation and haemoglobin

Hereditary iron overload due to mutations of the *SLC11A3* gene

- Early increase of ferritin before increase of transferrin saturation
- Hypocromic anaemia with normal or high serum ferritin in young individuals (mainly female)
- Early and predominant reticuloendothelial cell iron load; at later stages, also parenchymal cell iron load
- During phlebotomy, low transferrin saturation and low haemoglobin may be reached rapidly despite high-normal serum ferritin

UIBC provide early information of iron overload. Ferritin and liver biopsy provide evidence of iron accumulation in tissues and tissue damage. In equivocal cases, HC may be diagnosed in the presence of HII >1.9 or on the basis of the histological pattern of iron distribution in the liver; or quantitative phlebotomy if liver biopsy is not feasible. In the case of convincing biochemical signs or iron overload but negative HFE testing, a liver biopsy may still represent the gold standard for diagnosis. In these cases, sequencing for the SLC11A3 and TfR2 gene is required (see below).

Screening settings

Screening for HC is a very attractive concept because HC is very common, is fatal if untreated, treatment is safe and effective, there is a long asymptomatic phase, and simple and inexpensive screening tests are available. Moreover, the benefit of identifying a homozygote avoids medical expenses, increased years of productivity, but also identification of additional diseased subjects through family studies. The central ethical and policy questions are whether the benefits of screening outweigh the costs to a sufficient degree as to justify general screening on public health grounds, and whether and how to allow people to make informed choices about participating in screening, either for research or therapeutic reasons. Adverse social effects of screening are: (a) increasing social pressure; this may diminish solidarity and obligate people to do screening although they are not ready for it. Insurance companies and employers will increasingly be interested in people being screened and will put restrictions on people refusing testing and on people found being homozygous for the mutations and exclude them from insurances based on false perception of risks based on genetic testing. These concerns increase if the screening test used generates a high rate of false positive results; (b) confusion, as patients who participate in genetic screening programmes are commonly confused about the meaning of results. This confusion can result in decisions (for example, reproductive decisions) made on the basis of inaccurate information; (c) stigmatisation, a quality of being perceived by others, or by oneself, as being marked in a negative way. In the case of HC, such negative self regard may occur without any actual disease condition, being based solely on genotype not phenotype.

Transferrin saturation is the most sensitive method to detect homozygosity, and a fasting transferrin saturation of greater than or equal to 45% may identify affected homozygotes without necessitating further investigation, but will also identify a large number of patients that do not have HC.[47] Raising the threshold to 55% will increase the specificity but more HC cases will be missed. UIBC has been a component of the transferrin saturation in many laboratories and independently has been demonstrated to have a similar sensitivity and

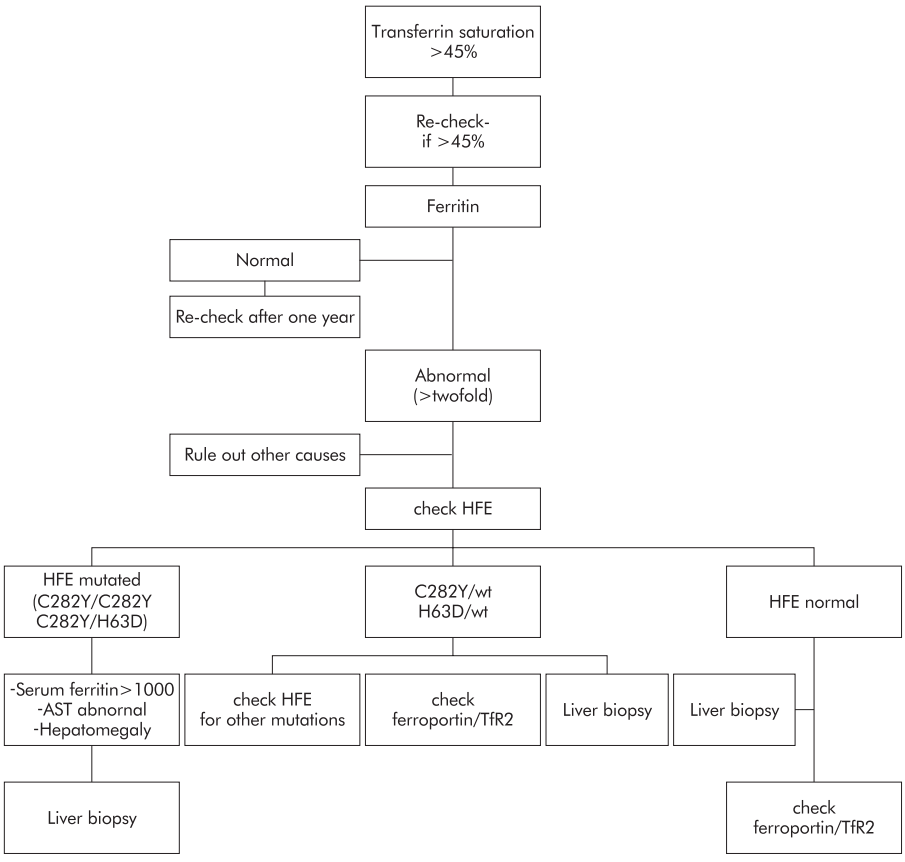

Figure 4 A possible algorithm to screening for haemochromatosis (see text for details).

specificity to transferrin saturation but at a lower cost because it is a one step automated test. A possible algorithm for screening HC is reported in figure 4.

The lack of conclusive data on the penetrance of the genetic defect in HC, on natural history of the disease in unselected populations, on the outcome of venesection treatment in people identified through population screening programmes, are the main questions to be answered by ongoing screening studies. In selected countries with high prevalence of HFE HC, wide population screening for HC should be implemented. Cost effectiveness of genotyping in newborns or biochemical testing in young adults should be carefully evaluated. In any country, targeted screening of selected patient populations (subjects with liver disease, diabetes, endocrinopathies, etc) through HFE testing should be considered. The simple use of a measurement of biochemical iron such as transferrin saturation to detect an iron overload condition should be universally encouraged.

NON-HFE HEREDITARY IRON OVERLOAD DISEASES

It has become apparent that mutations in *HFE* do not account for all cases of HC, particularly in southern Europe.[48] At least two genes have been associated to non-*HFE* linked HC, *SLC11A3* (coding for ferroportin1/IREG1/MTP1) and transferrin receptor 2, *TfR2*. Patients with an autosomal recessive iron loading disorder similar to HC carry mutations in *TfR2* gene.[16] Despite the intriguing homology with TfR1, the function of TfR2 has not been established, and it remains unclear how mutations in TfR2 lead to HC as available studies indicate that TfR2 does not bind HFE and is not expressed in intestinal cells.

Recently, a new clinical entity has been characterised where the clinical phenotype may be confused with classic HC, but the disorder shows autosomal dominant inheritance and the patients do not have mutations in *HFE*.[9] Additional distinguishing features are anaemia early in life despite increased serum ferritin concentrations and early iron accumulation in reticuloendothelial cells. The disorder is attributable to mutations in ferroportin1/IREG1/MTP1.[10 15] It has been suggested that the mutation results in a loss of function causing a mild but significant impairment of iron recycling by reticuloendothelial macrophages[10]; iron retention by macrophages would lead to decreased availability of iron for circulating transferrin (in fact transferrin saturation is inappropriately low as compared with serum ferritin concentrations in this disease) and for the haematopoietic system. This, in turn, would activate feedback mechanisms to increase intestinal absorption and compensate the underlying anemia, contributing to iron overload. Distinguishing features of this new hereditary iron overload disease are reported in box 1.

For many years fundamental questions on the regulation of iron metabolism and trafficking in humans and the genetic basis of hereditary iron overload diseases have remained unanswered. The discovery of the *HFE* and non-HFE haemochromatosis genes and the advent of molecular genetics have dramatically changed our concepts of the complex processes governing body iron metabolism also greatly modified our approach to the diagnosis and treat HC patients.

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