Hereditary haemochromatosis and iron metabolism

Joyce Carlson, Sigvard Olsson
Department of Clinical Chemistry, Lunds University Hospital, MAS, S-20502, Malmo, Sweden
Division for Hematology, Sahlgrens University Hospital, S-41345, Gothenburg, Sweden

HEREDITARY HEMOCHROMATOSIS

Hereditary hemochromatosis (HH) is characterized by abnormal iron absorption from the diet resulting in progressive iron overload, causing tissue damage of several organs, particularly the liver (1). Historically HH has been regarded as an extremely rare inborn error of metabolism causing "bronze diabetes", liver cirrhosis and hepatocellular carcinoma due to heavy iron overload in the liver and pancreas. Doctors have therefore rarely suspected that patients presenting with fatigue and abnormal liver tests may in fact may have hemochromatosis. Physicians should now consider HH as "a disorder". To the classical three " A"s , asthenia, arthropathy and ALT elevations (2) may be added "arrhythmia". Abnormal pigmentation may also be seen, especially in cases with concomitant porphyria cutanea tarda (3). Absence of symptoms is nonetheless common, particularly in young subjects, due to variable phenotypic expression of the disease and variations of lifetime accumulation of iron stores. Early detection, in conjunction with routine check-ups or screening procedures, is of utmost importance because an effective therapy is available through phlebotomy (4,5). The diagnosis which previously required extended family studies and HLA-typing has become very simple provided it has been considered. Diagnostic tests using modern DNA technology have become readily available and inexpensive as we have entered into the new millennium.

BASIC ASPECTS OF IRON METABOLISM

The iron content of a healthy adult male is about 4 grams, with 2.5 grams in the red cell mass (1 gram of Hb contains 3.4 mg of iron). The iron content of women is slightly lower because of smaller body size, lower red cell mass and depletion of iron reserves through menstrual iron losses. Iron derived from destruction of erythrocytes is generally recycled through cells of the reticuloendothelial system and exported to re-enter the transferrin bound circulating pool, from which iron is transported into new erythropoetic cells for re-incorporation into heme. Daily absorption of dietary iron is carefully regulated to maintain essentially constant circulating transferrin saturation rates. The main physiological losses of iron from the body occur via desquamation (primarily intestinal epithelial cells) and via menstruation, childbirth and lactation in women. (1)

Ferritin is a polymer of light and heavy ferritin chains which in complex can store a vast molar excess of iron in many cell types. The serum ferritin concentration indirectly reflects the size of the iron stores, and increases rapidly as stores become saturated. Plasma iron content is proportionately low, and saturates the transferrin iron binding capacity (TIBC) to about 30% and consists of iron bound for cellular uptake. Each transferrin (Tf) molecule can bind 2 iron ions. Tf circulates as mono- and diferric Tf as well as "naked" apotransferrin. Receptor mediated endocytosis occurs via transferrin receptors TfR1 and 2 anchored in the plasma or sinusoidal membranes of most cells. The TfRs have much greater affinity for iron saturated Tf (Tf (Fe) 2) than for monoferrous Tf or the iron-free apotransferrin. (6) Further discussion of clinical use of analysis of soluble transferrin receptor lies outside the scope of this article.

Investigation of the genes for ferritin and TfR led to the fascinating discovery of homologous structural "hairpin" or "stem-loop" elements, now called iron responsive elements (IRE) present in the 5' non-coding region of the ferritin mRNA and as repeated structures in the 3' end of the transferrin receptor mRNA (18). IREs are bound with high affinity by two proteins (IRP1 and IRP2) in the absence of iron. Iron ions strongly chelate the IRPs, closing the internal structure which otherwise interacts with IREs. By this ingenious mechanism (see fig. I), reciprocal regulation of ferritin and TfR synthesis is momentarily steered at the translational level. Binding of an IRP to the IRE in ferritin mRNA prevents initiation of translation while similar binding to the TfR mRNA prohibits its degradation, normally occuring from the 3' ->5' direction, thus allowing prolonged translation of multiple protein molecules from a single TfR mRNA. In contrast, introduction of iron to this system initiates ferritin synthesis and accelerates degradation of the transferrin receptor mRNA (6).

In addition many cells have at least one additional metal ion transport protein. One such protein present on essentially all cells is now named the divalent metal transporter 1 (DMT1), previously known as Nramp 2 and other names. DMT1 is expressed at the apical membrane of intestinal epithelial cells, on erythroid cell membranes and in other cell systems (7). Homologous mutations in this gene have previously been identified in the Belgrade rat

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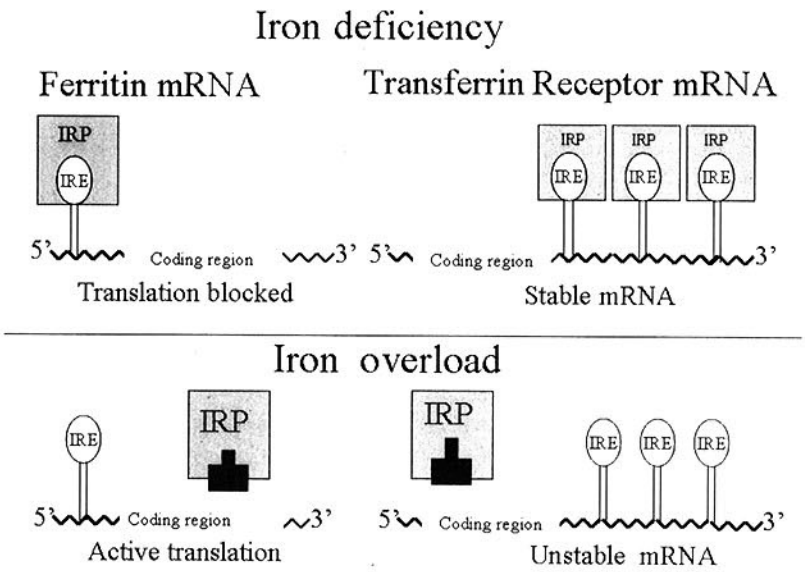

Figure I
Reciprocal regulation of the synthesis of ferritin and transferrin receptor. Freely modified from reference 6

and microcytic anemic, MK, mouse strains, spontaneously develop iron deficiency anemia (8 ,9). It has recently been discovered that the DMT1 gene undergoes alternative splicing to include or exclude 3' IRE sequences, thus enabling or preventing regulation of expression responsive to available iron (7).

Dietary iron exists predominantly in the ferric (Fe(III)) state and is normally reduced in the gastrointestinal tract to ferrous iron, possibly after chelation with mucin at the mucosal surface (10). Ferrous iron can be absorbed in an acid milieu and heme iron is absorbed at neutral or higher pH. Transport across the apical membrane of small intestinal epithelial cells is mediated by specific transport proteins, including DMT1 (fig. II).

GENETIC BASIS OF HH

Sheldon proposed in1935 that hemochromatosis was an inborn error of metabolism (1). In 1975 Marcel Simon and coworkers found that the responsible gene defect should be found on the short arm of chromosome 6 close to the histocompatibility or HLA locus. (11) Siblings who had inherited the same HLA haplotypes (a combination of HLA A and B genes) as a proband with clinical disease had also inherited

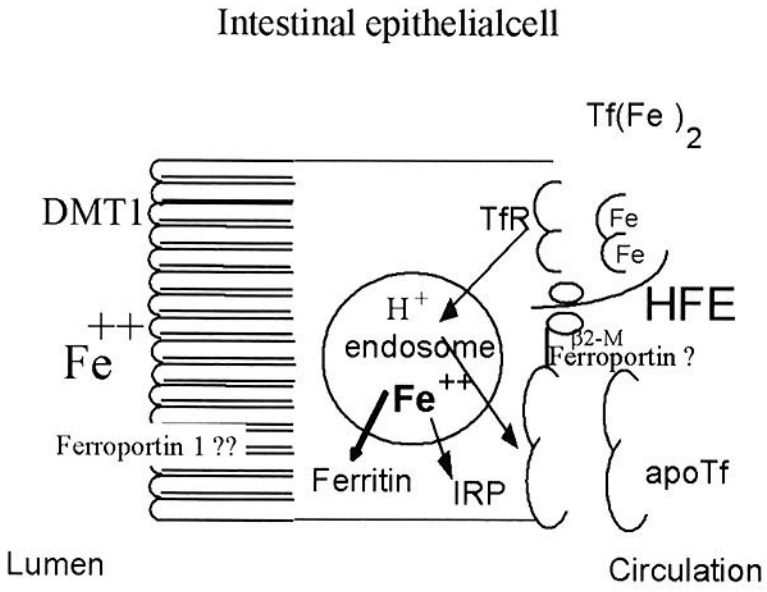

Figure II
Schematic diagram of transport mechanisms for iron across intestinal epithelial cells

hemochromatosis. Simon suggested that the original mutation had taken place in a person of celtic origin living in northwestern Europe and carrying HLA A3B7 or A3B14 haplotype (12). The finding that some families carried HLA haplotype markers different from the ancestral A3 was believed to be due to genetic recombination.

During the past ten years microsatellite DNA markers became available and an intense search for the mutation was started using positional cloning. In 1996 Feder et al. found a candidate gene originally called HLA-H, and later renamed HFE, coding for a major histocompatibility complex type 1 protein and localized at a physical distance of 4.5 mB telomeric from HLA-A (13). A single mutation 845G->A, giving rise to the amino acid substitution C282Y was found in 85% of HFE alleles from patients with verified HH and slightly less than 10% of alleles from normal controls. Another mutation 187C->G gving rise to H63D amino acid substitution was rarely present in homozygous form in patients lacking the C282Y mutation, but was present in about 7.3% of patients who were compound heterozygotes for the two mutations. Both mutations were present with increased frequency in patients with porphyria cutanea tarda (PCT).

The C282Y mutation in HFE removes an essential cysteine which normally participates in a disulfide bond, forming a structural conformation capable of interaction with β 2-microglobulin (14). Association of β 2-microglobulin (β 2-M) to HFE is necessary for intracellular traffic and incorporation of the HFE molecule in the cell membrane. These observations were further strengthened by the fact that β 2-M knock-out mice had been shown to develop iron storage disease (15).

PATHOGENETIC MECHANISM

Lebron and Feder soon demonstrated association of normal or wild type (wt) HFE with the transferrin receptor (TfR) molecule at the cell surface (16), and recent studies have further shown that the intact wt HFE molecule induces phosphorylation and consequent inactivation of TfR (17). This not only reduces affinity for iron saturated transferrin (Tf), but also impairs endocytosis of the TfR, with decreased cellular iron uptake as a result.

In B-lymphoid cell lines derived from normal (wt HFE) and C282Y HFE individuals, the C282Y cells expressed less HFE protein at the cell membrane and 1/2 to 1/3 as much TfR , with lower affinity for Tf than that found in wt cells (18). Considering the number of TfRs in the two cell lines, the relative Tf internalization rate was nonetheless greater in C282Y cells. In addition the Tf independent iron uptake was also significantly greater in C282Y than in wt cells. Despite this, ferritin content was lower in C282Y cells, which were also more sensitive to oxidative stress. Similarly, macrophages isolated from iron overloaded C282Y patients incorporated less iron than macrophages from healthy controls (19). Overexpression of wt HFE in these macrophages resulted in increased uptake of diferric Tf with a 30-45% increase in intracellular ferritin and a slight decrease in surface TfR density. It is uncertain if this increase in iron accumulation depends on increased TfR mediated uptake, increased receptor independent uptake, or decreased egress of iron from the cells. These authors speculate that Ferroportin 1, a ferrous ion transporter identified on the basolateral surface of entrocytes and in Kupffer cell membranes may.

Intestinal epithelial cells not only regulate uptake of dietary iron but also represent one of the body' s few options to reduce an iron overload by desquamation. Recent studies have demonstrated up-regulation of the DMT1 transporter in hemochromatosis and HFE knock-out mice (20), with a doubling of the rate of uptake for ferrous iron (and increased rate for ferric iron after reduction), which could be blocked by antibodies to DMT1. DMT1 and ferroportin 1 (FP1) mRNA levels were significantly increased in duodenal biopsies from patients with iron deficiency and hemochromatosis but not in cases of secondary iron overload (21). Immunhistochemical studies have similarly shown increased expression of a putative stimulator of Fe transport (presumably DMT1) in iron deficiency and hemochromatosis with decreased expression in secondary iron overload (22). TfR expression was uniformly increased across the crypt-to tip gradient in iron deficiency, intermediate in hemochromatosis patients and similar to controls in secondary iron overload. A conflicting observation was made in in vitro studies with overexpression of the wt HFE gene in a human intestinal cell line (Caco-2). Excess wt HFE created a marked reduction in apical iron uptake despite a functioning IRE-IRP system and an eightfold mass increase of the apical DMT1 transporter (23). These and other investigations have been summarized in a recent review (24). The balance of these regulatory systems may vary with cell type. It seems reasonable that the TfR-wtHFE complex functions as a type of thermostat, registering circulating levels of transferrin saturation. With good availability of iron, intracellular iron increases, saturating IRPs, which upregulates the synthesis of ferritin and downregulates the synthesis of transferrin receptors and DMT1. Conversely, iron deficiency increases intestinal uptake of dietary iron via upregulation of DMT1, and simultaneous increase in TfR synthesis. The exact intracellular steps of this regulation in different cell systems are not yet fully elucidated.

POPULATION GENETICS

According to a recent pooled analysis of the prevalence of HFE mutations in HH, about 73% of cases can be attributed to homozygosity for the C282Y mutation, about 6% are compound heterozygotes for the two common HFE mutations, and only about 1% are homozygotes for the H63D mutation (25). Numerous other mutations in the HFE gene have been reported including S65C, with much lower frequency and apparently lower penetrance for HH (26). The prevalence of homozygosity for C282Y HFE is currently estimated at about 2.5 per 1000 in northern European based populations, and proportionately fewer cases of

clinical HH are attributable to mutations in this gene in southern European, African and Asian populations.

In Italy a number of cases have been attributed to at least 3 different missense or truncation mutations in the gene for the transferrin receptor 2 (27 ,28). Polymorphisms in this gene have been identified in other populations but there appear to lack association with HH (29 ,30).

At least one DMT1 mutation has been identified in a non homozygous C282Y HH patient (7).

Mutations potentially disrupting the stability of ferritins IRE could theoretically increase intracellular ferritin synthesis and thus potentiate iron stores, and indeed, a relatively rare hyperferritinemia - cataract syndrome is caused by such a mutation in the L- ferritin gene. In this condition it is thought that excessive serum L- ferritin results from leakage of intracellular ferritin. Intracellular iron stores are not increased and phlebotomy results in anemia. Cataracts presumably result from increased levels of circulating L- ferritin bound iron, but the mechanism is not clear (31). IREs are present in other genes including the erythroid specific 5-aminolevulinic acid synthase (ALA-S2) gene whose expression in hemoglobin synthesizing cells is dependent on access to iron (32), and the 3' region of DMT1 (7).

PREVALENCE

Screening studies using phenotypic iron tests have shown a prevalence of 2÷8/1000 in populations of northern European origin. Genotype screenings have shown higher figures and are continurously updated in the OMIM database (26). One estimate of penetrance based on genotyping data is that about 50% of C282Y homozygotes will develop disease. The variability of phenotypic expression means that the benefit of an early diagnosis is uncertain. Therefore a general screening of the population has thus far not been recommended (32).

LABORATORY DIAGNOSIS OF HH

An early laboratory finding seen in HH is an abnormal saturation of transferrin (TS) to a level > 45% (33). This elevation is absent in rapidly growing adolescent males and in menstruating and reproducing females (34). Transferrin saturation increases successively with age in adults with HH. People with TS > 45% in repeat test should be studied for iron overload using serum ferritin concentration (33). If iron overload is suspected, HFE genotyping should be performed. HFE genotyping is now available at public and private laboratories, at essentially all university hospitals and at many regional centers. The costs of such testing are rapidly decreasing and currently range from approximately 25 to 250 USD. The PCR-based methods used incorporate all currently available forms of technology, and are too numerous to list. The C282Y and H63D mutations are easily detected by restriction fragment length polymorphisms (RFLP), and by all variations of these techniques. One example for a duplex analysis for these two mutations with fluorescent detection following capillary electrophoresis is illustrated in figure III.

CLINICAL EFFECTS OF HFE GENOTYPING

The advent of the HFE genotype test has revolutionized the diagnosis and management of patients and families with HH. A simple blood test taken by the local doctor and submitted for genotyping has replaced the inconvenience and cost of hospitalization for a diagnostic liver biopsy for the patient with iron overload and for family members. Liver biopsy can now be reserved for patients with heavy iron overload for prognostic information. We now know that female family members can present a

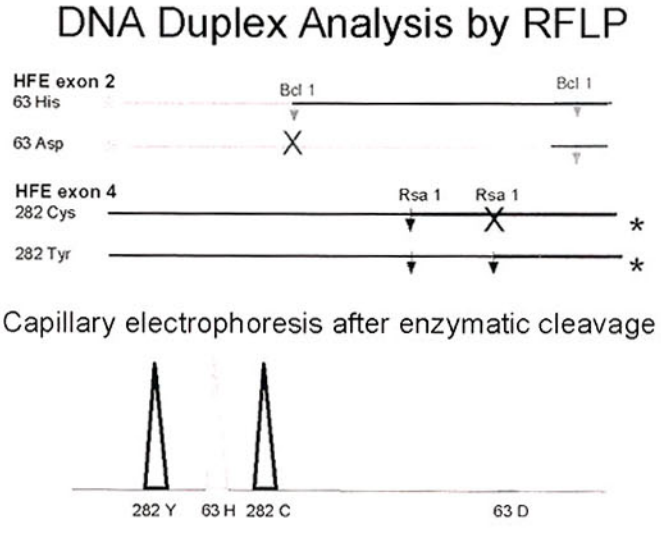

Figure III
Principle of one method for duplex PCR analysis of the two common HFE mutations causing hemochromatosis

phenotypic expression of iron deficiency despite being homozygotes for the C282Y mutation. Availability of genotyping allows identification of relatives at risk, who may be followed using transferrin saturation to detect the development of iron overload, at which time treatment may be initiated.

Awareness of the unexpectedly high prevalence of HFE mutations should alter medical practice, such that all newly detected abnormalities in liver function tests in geographic areas of significant prevalence for HH should include measurement of transferrin saturation and ferritin to detect potential cases. Additional knowledge gained concerning iron metabolism will hopefully stimulate further genetic investigations, e.g. search for mutations in DMT1, ferroportin1 and other genes, in cases of dysfunctional iron metabolism. Furthermore, preliminary studies investigating the relationship between iron overload and oxidative stress as a risk for cancer in general and for cardiovascular disease suggests that treatment may reduce general morbidity and mortality in HH patients and that additional surveillance of patients identified with a long duration of iron overload may be warranted (36, 37).

TREATMENT

Iron is easily removed from tissues through regular phlebotomy once a week until depleted iron stores are evident by S- ferritin < 30 µg/l. Maintenance phlebotomy is then continued 3÷5 times yearly. The prognosis is excellent provided the diagnosis is made early and therapy started before the development of cirrhosis (4, 5).

BLOOD BANKS

Blood banks should be encouraged to screen new applicants for iron overload especially in those countries in which iron supplements are given after each donation. This supplement is potentially dangerous for people with HH. Screening may also detect "superdonors" and several countries are accepting blood for transfusion from healthy HH donors (38).

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