Hepcidin, Iron Overload and the Classification of Haemochromatosis

Introduction: The absorption of iron must be carefully regulated as there is no definite pathway for iron secretion by the body. Thus, the small amount of iron that is lost each day, (about 1-2 mg), is matched by dietary absorption of iron. The concentration of iron in the human body is normally about 40-50 mg Fe/Kg body weight, with the majority of iron stored in circulating erythrocytes, and to a lesser extent in the liver.

Iron overload is caused by conditions that:

a) alter (hereditary haemochromatosis, refractory anaemia with ineffective erythropoiesis)

or

b) bypass (transfusional iron overload),

the normal control of body iron content by regulation of intestinal iron absorption. The excess iron is deposited in the liver, endocrine glands, the heart and the skin, and eventually the iron-overloaded organs suffer tissue damage, presumably through the iron-catalysed generation of reactive oxygen species.

A recent classification (Reference. 1) summarises the severity of haemochromatotic iron overload, from grade 0 (neither biochemical nor clinical symptoms) to grade 4 (development of cirrhosis, insulin-dependent diabetes or cardiomyopathy).

Iron Absorption and Transport

Iron is brought into the cell through an active transport process involving the protein DMT-1 (divalent metal transporter-1), which is expressed on the apical surface of enterocytes (Figure.1) in the initial part of the duodenum. DMT-1 is not specific to iron, and can transport other metal ions such as zinc, copper, manganese or lead. The brush border ferric reductase, converts ferric to ferrous iron for use by DMT-1, and Hephaestin, a transmembrane-bound ferroxidase, converts ferrous to ferric iron, creating a concentration gradient of ferrous iron across the cell membrane facilitating iron egress. At low iron conditions the translation of TfR (transferrin receptor), DMT-1 and ferroportin is enhanced, with the opposite occurring at high iron conditions. For the most part, iron bound to ferritin in the enterocyte will remain there. This iron will be lost from the body when the enterocyte dies and is sloughed off from the tip of the villus.

Iron that enters the body from the surface of the enterocyte is rapidly bound to transferrin, an iron-binding protein of the blood. Transferrin delivers iron to red blood cell precursors, which take up iron bound to transferrin via receptor-mediated endocytosis.

Normally, the capacity of transferrin to bind iron in the plasma, greatly exceeds the amount of circulating iron. The transferrin saturation, (percent of transferrin occupied by iron), is measured to determine if an individual has an excessive load of iron in the body. Normal transferrin saturation is in the range of 20-50%. When iron is present in excess, it overwhelsm the capacity of transferrin to bind to it, and therefore becomes bound to smaller, low-molecular weight molecules. Such non-transferrin bound iron (NTBI) more readily participates in oxidative reactions that damage cells.

Hepcidin

Hepcidin is a 25–amino acid disulfide-rich peptide synthesised in the liver. It acts as a systemic iron-regulatory hormone by regulating iron transport from iron-exporting tissues into plasma. Hepcidin inhibits the cellular efflux of iron by binding to, and inducing the degradation of, ferroportin, the sole iron exporter in iron-transporting cells.(see Figure 2.)

More recently, it has been suggested that the synthesis of hepcidin, a hormone of liver origin that slows intestinal iron absorption, may be stimulated by HFE, as well as by infection, iron, TfR2, and haemojuvelin (See Figure 3.).
TfR2 and HFE favour the passage of iron into liver cells, which would be responsible for hepcidin induction. Thus, normal HFE, would slow intestinal iron absorption after inducing the synthesis of hepcidin.

Thus, based on current understanding, the term haemochromatosis encompasses at least four types of genetic iron overload conditions (Reference. 2). Most of these have been recently distinguished from one another as a result of the identification of a series of genes related to iron metabolism, (Summarised in Table 1.). At least three of these entities (HFE haemochromatosis, juvenile haemochromatosis and transferrin receptor 2 (TfR2) haemochromatosis) involve systemic hepcidin deficiency as a key pathogenetic factor.

The most common form of hereditary haemochromatosis in populations of European origin is due to mutations in the HFE gene on chromosome 6. This results in an autosomal recessive disorder of low penetrance that clinically, predominantly, affects older men. Homozygosity for the major gene mutation C282Y, homozygosity and/or compound heterozygosity involving the minor mutation H63D(p.His63Asp) and much rarely S65C (p.Ser65Cys) are collectively responsible for the majority of iron overload in haemochromatosis.

Of the others, mutations in transferrin receptor 2, (TfR2), are uncommon but cause a similar phenotype. The autosomal recessive diseases, due to mutations in the hepcidin gene, HAMP or the haemojuvelin gene, (HJV), most often cause a much more severe phenotype (“juvenile haemochromatosis”), affecting young men and women equally.

The autosomal dominant haemochromatosis, due to mutations in the ferroportin gene, differs from other haemochromatoses by causing early iron overload in the Kupffer cells (liver macrophages) rather than hepatocytes, but more recent evidence suggests that some ferroportin mutations cause the classical pattern of parenchymal iron overload.

Lastly other rare forms of genetic iron overload – due to mutations in the caeruloplasmin gene (with haematological and/or neurological presentation), the transferrin gene (atransferrinemia, expressed by severe iron deficiency anaemia and parenchymal iron overload) or, recently,
in the DMT-1 gene, also responsible for iron deficiency anaemia and hepatic iron excess – have been reported.

**Unexplained Iron Overload, (with normal or low transferrin saturation and negative HFE testing).**

Hyperferritinemia, (which may exceed 1000 µg/L) with normal transferrin saturation (i.e. < 45%), and a negative HFE test, is not an uncommon presentation seen in clinical practice. Often it is as a result of dysmetabolic hyperferritinemia, also termed insulin resistance-associated iron overload. There are well recognised associated features such as obesity, hypertension, diabetes and hyperlipidemia, and a possible consequence of these metabolic effects is the development of a condition known as Non-alcoholic Fatty Liver Disease (NAFLD). A concomitant increase in the hepatic iron (usually non-parenchymal), may be observed and non-invasive techniques such as the R2 MRI (Ferriscan) can be used to document the hepatic iron concentration.

Ferroportin disease, in its “A” form, presents with:

- Low transferrin saturation (TS), and sometimes mild anaemia,
- Predominant macrophage iron excess and
- Absent or mild iron related complications.

Hereditary aceruloplasminaemia is a very rare disease that is due to mutations in the caeruloplasmin gene located on chromosome 3. It mimics HFE haemochromatosis in that it is familial, and can be associated with major hepatocyte iron overload and diabetes mellitus. Two main features suggest this disease:

- The finding of low TS (and often anaemia) in the face of marked hyperferritinemia without inflammation and
- The presence of a neurological syndrome (extrapyramidal signs, cerebellar ataxia, retinal degeneration and dementia).

Note that caeruloplasmin, (CP), is essential for Fe2+ oxidation into Fe3+, which allows iron to leave cells and then be transported by Tf, thus, in the absence of CP, Tf cannot bind iron, iron cannot leave RES cells and plasma unbound Fe2+, deposits itself in tissues (liver, pancreas, etc.), as occurs in atransferrinemia and classic haemochromatosis when Tf saturation reaches 100%. Neurologic lesions result from iron preferential deposition in astrocytes.

**References**
1. www.has-sante.fr

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**Table 1**

The Characteristics and Classification of Haemochromatosis.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>HFE related Hereditary Haemochromatosis</th>
<th>Juvenile Hereditary Haemochromatosis</th>
<th>TIR2 related Hereditary Haemochromatosis</th>
<th>Ferroportin related Iron Overload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene product</td>
<td>HFE</td>
<td>HJV &amp; HAMP Haemojuvelin &amp; Hepcidin</td>
<td>TIR2 Transferrin receptor 2</td>
<td>Ferroportin</td>
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<td>Inheritance</td>
<td>AR</td>
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<td>Variable.</td>
<td>High</td>
<td>High</td>
<td>Phlebotomy or menstruation induced anaemia</td>
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<td>2nd or 3rd</td>
<td>4th or 5th</td>
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<td>Hepcidin</td>
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<td>Decreased</td>
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