11. Introduction to

"Diagnosis of juvenile hemochromatosis in an 11-year-old child combining genetic analysis and non-invasive liver iron quantitation"

by

De Gobbi M, Caruso R, Daraio F, Chianale F, Pinto RM, Longo F, Piga A, and Camaschella C.

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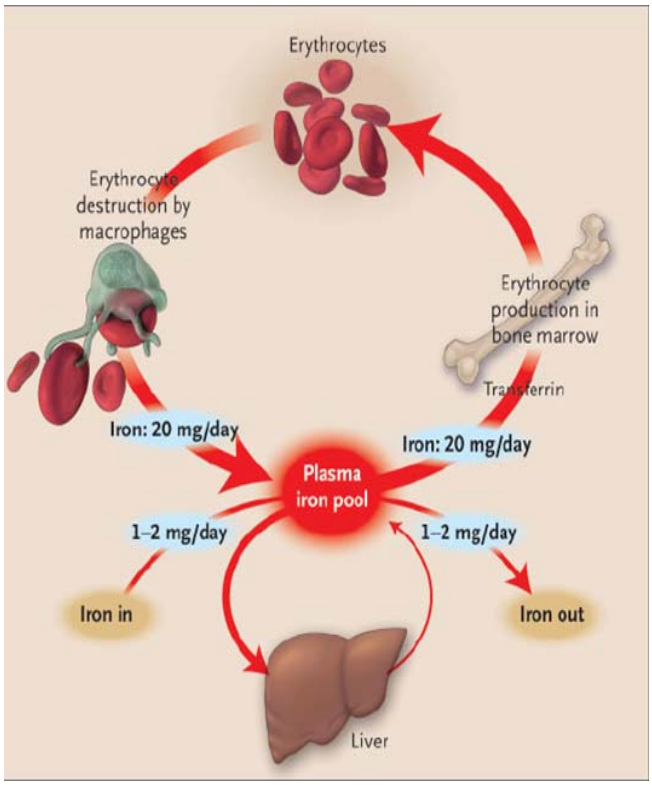
11.1. Human iron homeostasis [108,109]

Human iron homeostasis depends on the coordinated functions of numerous genes. Plasma levels of iron are closely regulated to ensure a daily supply of approximately 20 mg to the bone marrow, for incorporation into hemoglobin in erythroid precursors and mature red cells. Most of the iron found in the plasma derives from the continuous breakdown of hemoglobin in senescent red cells by reticuloendothelial macrophages. Approximately 1 to 2 mg per day is also taken up by duodenal enterocytes and transferred to the plasma compartment or, depending on body needs, stored in the enterocytes as ferritin. These stores are eliminated when enterocytes are sloughed at the end of their life cycle; apart from menstrual blood loss, this is the only significant means by which excess body iron is excreted. Iron recycled by macrophages (as well as that absorbed from the gut) is loaded onto serum transferrin and delivered primarily to the bone marrow for reincorporation into new red-cell precursors. The remaining body iron (approximately 1000 mg) is stored, primarily in hepatocytes.

In physiological conditions, the normal iron content of the body is approximately 3–4 g. To be absorbed, the dietary iron, which is mainly in the poorly soluble and absorbable ferric state, must be reduced from the ferric to the ferrous state by ferric reductase, expressed on the luminal surface of the duodenum. The ferrous iron is then taken up by a specialized luminal iron transporter, called divalent metal transporter-1 (DMT1), and it may be stored within the mucosal cell as ferritin or transported across to the plasma via ferroportin.



<u>Normal iron homeostasis</u>. From Pietrangelo, New Engl. J. Med 2004 (Ref. 108).

11.2. Hereditary Haemochromatosis [108,109]

Hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in Caucasians, affecting 1 in every 200–400 individuals and leads to a progressive accumulation of iron in the body due to increased intestinal iron absorption. Excess iron causes tissue damage and fibrosis with irreversible damage to various organs. Symptomatic organ involvement generally begins in midlife, often with non-specific symptoms such as unexplained fatigue or joint pain. Liver disease (ranging from slightly elevated aminotransferase levels, with or without hepatomegaly, to cirrhosis and even hepatocellular carcinoma) usually predominates, but endocrine disorders (diabetes, hypogonadotropic hypogonadism, impotence, and hypothyroidism), cardiac problems (arrhythmias and heart failure), and joint disease (destructive arthritis) are also found. Although iron metabolism is abnormal, erythropoiesis is not jeopardized, and hematologic anomalies are not usually seen.

Hereditary hemochromatosis type 2, or juvenile hemochromatosis (JH), is a rare autosomal recessive disorder that affects males and females equally and is characterized by an early onset of iron overload that leads to severe organ impairment, usually before the age of 30. JH is characterized by rapid iron loading, which may result in a combination of hypogonadotropic hypogonadism, cardiac

disease, liver cirrhosis, diabetes, arthropathies, and skin pigmentation as seen in classical HH, but with an earlier onset and an increased severity. Cardiac symptoms dominate the course of the untreated disease, and heart failure and/or major arrhythmias are recorded as the most common causes of premature death. Thus, for the more advanced cases, the only therapeutic choice is heart transplantation.

11.3. Genetic background [108,110,111]

Classic hereditary hemochromatosis with adult onset is associated with mutation of the *HFE* gene, located on chromosome 6 (hemochromatosis type 1); in most cases the mutation is a single-base change that results in the substitution of tyrosine for cysteine at position 282 of the HFE protein (C282Y), the major-histocompatibility-complex class I–like protein, HFE. Newly synthesized HFE binds to beta2-microglobulin, an event necessary for its expression on the cell surface and endosomal membranes, where it interacts with transferrin receptor 1 (TfR1), the major receptor for transferrin. By disrupting a disulfide bond in HFE that is critical for its binding to beta2-microglobulin, the C282Y mutation impairs cell-surface expression of HFE and the interaction of HFE with TfR1. According to the first *in vitro* observations, HFE would function as negative regulator of iron uptake by negatively regulating TfR1, while more recent findings suggest that HFE normally facilitates, rather than hinders, TfR1-mediated cellular uptake of transferrin-bound iron. However, the presence of HFE with the C282Y mutation, which is unable to interact with TfR1, leads to iron-deficient duodenal crypt cells, which give rise to iron-deficient daughter cells. These cells are programmed to react to iron starvation by hyperactively and persistently absorbing iron from the intestinal lumen and transferring virtually all of it into the bloodstream, regardless of actual erythropoietic needs.

Although relatively few cases have been described to date, the iron-overload phenotype associated with mutations in the gene encoding transferring receptor 2 (*TfR2)* appears to be very similar to that of classic, *HFE*-related hemochromatosis (hemochromatosis type 3). Few is known about the function of TfR2. TfR2 shows 66% homology with TfR1, and in transfected cells it mediates the uptake of transferring-bound iron, possibly through a mechanism similar to that described for TfR1. TfR2 differs from TfR1 in its affinity *in vitro* for transferrin (1/25 to 1/30 as

strong), its high level of expression on hepatocytes, and the fact that its expression is not downregulated by hepatic iron overload.

Two subtypes of juvenile hemochromatosis (hemochromatosis type 2) have been recognized. The first subtype is caused by a mutation in a recently identified gene (*HJV*) located on chromosome 1q21, whose protein product has been called hemojuvelin (HJV). The second subtype of juvenile hereditary hemochromatosis is characterized by a particularly severe iron overloading, more severe than in first described one, and is caused by mutations in the *HAMP* gene, coding for hepcidin.

The discovery of hepcidin greatly contributed to the understanding of iron metabolism and its regulation. Hepcidin is a small cysteine-rich peptide (20–25 amino acids), which has been proposed to be a hormone produced by hepatocytes, targeting duodenal enterocytes and macrophages. In its target tissues, hepcidin affects the transport of iron through the membrane. The secretion of hepcidin is induced by interleukin 6. The redistribution of iron makes it less available for microorganisms that need iron as a growth factor. Moreover, the hepcidin molecule directly is a weapon against bacteria: acting similarly to defensins, hepcidin disrupts the bacterial membrane. In the hepcidin model, the rate of iron influx into the plasma depends primarily on the activity of hepcidin: when plasma iron levels are high, the synthesis of hepcidin increases, diminishing the release of iron from enterocytes and macrophages, possibly through interaction with iron-export proteins, such as ferroportin. When plasma iron levels drop, the synthesis of hepcidin is down-regulated, allowing these cells to release increased amounts of iron.

Most juvenile-onset cases of hemochromatosis have been mapped to chromosome 1q, where the *HJV* has been identified. The putative full-length protein is a 426 amino acids GPI-anchored-protein. The function of hemojuvelin was elusive until recently, when it was reported that hepcidin levels are depressed in individuals with *HJV* mutations and in *HJV* knock-out mice, and that *HJV* is a transcriptional regulator of hepcidin.

Bone morphogenic proteins (BMPs) represent a large subfamily of the (TGF-β) superfamily of ligands that upon triggering of their cell surface receptors lead to the activation of Smads transcriptional factors, which in their turn translocate to the nucleus where they modulate gene transcription. Hemojuvelin is indeed a co-receptor that enhances BMP signaling in the hepatocyte via the classical BMP pathway,

involving BMP ligands, BMP receptors and BMP receptor-activated Smads. Loss of hemojuvelin function leads to decreased BMP signaling in liver cells, which then decreases hepcidin expression. Impaired regulation by hepcidin leads to ferroportin overactivity, thereby resulting in increased intestinal iron absorption, increased macrophage iron release, elevated serum iron and abnormal tissue iron deposition.

11.4. Treatment of patients with hemochromatosis [108]

Thanks to improvements in early diagnosis, the classic triad of cirrhosis, bronze skin and diabetes is now rare in adult-onset hereditary hemochromatosis. For young adults with signs of juvenile-onset disease (hypogonadotropic hypogonadism or unexplained heart failure), the workup foresees first-line genetic testing for mutations in the *HAMP* and *HJV* genes, and measurement of biochemical parameters, namely of ferritin levels and hepatic iron index (which is calculated as the hepatic iron concentration [in micromoles per gram of liver, dry weight] divided by the patient's age [in years]; it will invariably be higher than 1.9, whereas normal values are less than 1.0). Once a diagnosis of hereditary hemochromatosis (adult- or juvenile-onset) has been established, further clinical workup is necessary to quantify the iron overload, define its possible visceral or metabolic consequences, and identify risk factors for progression. Family members, particularly siblings, should undergo biochemical testing.

Patients who have symptoms generally require phlebotomy. Phlebotomy may be deferred in some patients with adult-onset disease who have fairly normal liver function, but it is mandatory when the serum ferritin level is more than 1000 ng per milliliter, because of the risk of underlying hepatic fibrosis. Phlebotomy is the safest, most effective, and most economical therapeutic approach. Initially, 1 or 2 units of blood (each containing approximately 200 to 250 mg of iron) are removed weekly until the serum ferritin level is less than 50 ng per millilitre and the transferrin saturation drops to a value below 30 percent. Initiated early, this regimen can prevent organ damage and improve survival. Established cirrhosis, hypogonadism, destructive arthritis, and insulin-dependent diabetes cannot be reversed, but their progression can be slowed.

11.5. Presentation of the article by De Gobbi *et al.* (Eur. J. Ped., 2003)

Despite the recent advances in understanding of the genetic basis of juvenile haemochromatosis, and consequently in its diagnosis, it remains critical to detect the disease as early as possible, as treatment in presymptomatic stages prevents organ damage. The article by De Gobbi *et al.* reports the successful case of early diagnosis and treatment of juvenile haemochromatosis in a 11-year-old child. In this case-report, the early symptoms of iron overload, such as weakness, dark skin pigmentation, or abdominal pain and the classic clinical manifestations were absent. However, in the absence of secondary iron overload, juvenile hemochromatosis was suspected on the basis of strikingly elevated serum iron and serum ferritin levels. Liver iron content was evaluated with a non-invasive superconducting (SQUID) susceptometer, that values the magnetic susceptibility of liver non-heme iron and represent an alternative choice to the invasive method of liver biopsy.

After the exclusion of classical mutation causative of hemochromatosis type 1 or 3 (HFE- or TfR2-linked), the diagnosis of juvenile hemochromatosis was established by linkage analysis. Linkage analysis to chromosome 1q was investigated by intrafamilial segregation of microsatellites mapping within the critical region, in a period where the causative *HJV* gene was still unknown.

Based on these results, phlebotomy was immediately started, and the follow-up demonstrated significant improvement of hematic iron parameters without adverse effects.