Genetic hemochromatosis update

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Abstract

Hereditary Hemochromatosis is an autosomal recessive disease, characterized by chronic iron overload. It is mainly due to mutations of the HFE-1 gene. In the large majority of patients, the substitution of tyrosine for cysteine at amino acid 282 (C282Y) is found at the homozygous state. Since the HFE-1 hemochromatosis identification, several other entities of iron overload have been individualized. In the present article, the frequency, penetrance and pathophysiology of HFE-1 hemochromatosis as well as various clinical presentations resulting from different mutations affecting different proteins involved in iron metabolism are described. (Acta gastroenterol. belg., **2005**, 68, **33-37**).

Key words : hemochromatosis, iron overload, genetics, pathophysiology.

Hereditary Hemochromatosis (HH) also called HFE-1 hemochromatosis is a disorder of iron metabolism classically characterized by excessive intestinal absorption of iron. If left untreated, iron may accumulate in vital organs (liver, heart, and pancreas), and lead to life-threatening conditions (1,2)

Eight years ago, the gene coding for one of the key proteins involved in the hyperabsorption of iron was discovered. This gene, located on the short arm of chromosome 6, codes for HFE-1 protein, which regulates the amount of iron absorbed from the intestinal lumen (3). HFE-1 is a membrane bound protein with 3 extra cellular domains (α_1, α_2 and α_3), non-covalently associated with light chain ß$_2$-microglobulin. To date, several point mutations affecting the HFE-1 protein have been identified. In the large majority of HH patients, the substitution of tyrosine for cysteine at amino acid 282 (C282Y) is found at the homozygous state. A minor mutation results from the substitution of aspartate for histidine at amino acid 63 (H63D) and may lead to slight iron excess when associated to C282Y, corresponding to the profile of compound heterozygosity.

Frequency and penetrance

At the time when the HFE-1 gene was discovered, the general belief was that all homozygote C282Y subjects would, at some point in their lives, develop the disease. Since then, however, the phenotypic description of thousands of homozygote C282Y patients has failed to confirm this point of view, emphasizing the incomplete penetrance of C282Y homozygosity. The degree of penetrance is in fact totally dependent on the definition ascribed to this penetrance. Based on the clinical and biochemical abnormalities observed in HH patients (as summarized in Table I), a five stage grading in the course of HH development has been proposed (4). In a recent study published in the Lancet, Beutler *et al.* (5) screened 41,038 subjects. As expected, homozygosity for C282Y was found in 4 out of 1,000 subjects. Amongst these patients, only one subject had severe clinical symptoms corresponding to the historical definition of grade 4 HH (Table I). A year earlier, Asberg *et al.* (6) screened more than 65,000 subjects with both increased ferritin and transferrin saturation. A total of 269 (5 out of 1,000) patients were found to be homozygous C282Y/C282Y, out of whom 14% had their quality of life affected (asthenia and arthralgia). HH grade 4 was observed in 4% of homozygous HH subjects (versus less than 1% in the Beutler study). The interim data from an ongoing study in the USA (called HEIRS study), and for which the inclusion of 100,000 subjects is planned, indicated a ratio of 6/1,000 C282Y homozygotes. Half of the patients already included in this study had an increased ferritin and transferrin saturation (= grade 2), and therefore a potential indication to venesection treatment. Finally, the data collected in 2001 by the French Medical Secretariat for Hereditary Metabolic Diseases revealed that 50% of French HH patients had grade 3 clinical symptoms, compared to 20% who had grade 4 clinical symptoms. On the whole, if the penetrance of grade 4 HH is probably much lower than previously thought, grade 3 and in particular grade 2 have much higher penetrance.

Pathophysiology (Figs. 1 and 2)

Iron absorption takes place mainly at the duodenal level. In normal conditions, it is currently admitted that the cryptic duodenal cells regulate iron uptake, which occurs at the level of the apical cells. Cryptic cells are involved in the sensing of iron uptake. This signaling process originates at the basolateral border of the cryptic cells, where the non-mutated HFE synthesized within the cytosol binds to β_2-microglobulin and the protein couple migrates towards the basolateral border and

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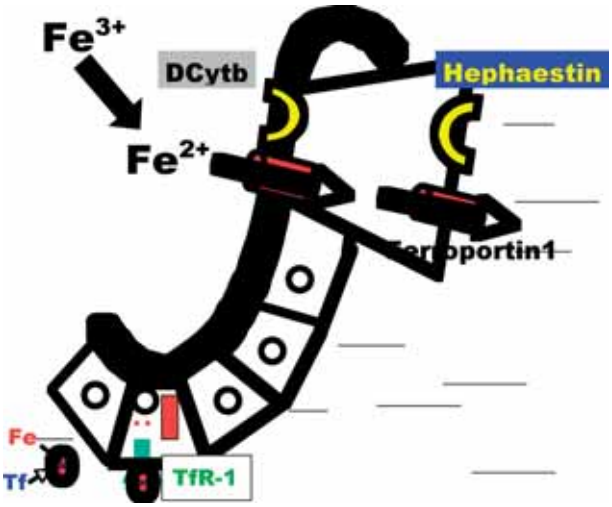
Table I. — **Different grades of HH, based on clinical and biochemical criteria (4)**

0	1	2	3	4
				Potentially lethal symptoms
			Quality of life symtpoms	Quality of life symptoms
		↑ Ferritin	↑ Ferritin	↑ Ferritin
	↑ TfS	↑ TfS	↑ TfS	↑ TfS

TfS = Transferrin Saturation



DMT = Divalent metal transporter
Dcytb = Duodenal cytochrome b
Tf = Transferrin
TfR-1 = Transferrin Receptor-1

Fig. 1. — Schematic representation of the major proteins involved in iron transport at the duodenal level.

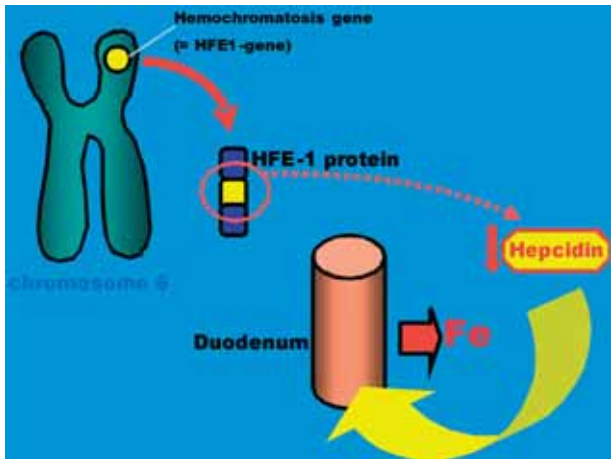


Fig. 2. — Schematic representation of HFE-1 hemochromatosis pathogenesis.

binds to the transferrin receptor1 (TfR-1). The ternary complex modulates the affinity of transferrin iron to TfR-1, and hence the entry of iron into the cells. The amount of iron in the cryptic cells determines the rate of iron absorption at the apical level. In HH subjects, the mutated HFE protein is unable to bind the β_2- microglobulin molecule, which in turn prevents HFE from migrating to the plasma membrane. This results in failure of the apical cells to accurately program the degree of iron uptake. In other words, the crypt cells behave as if they were iron-deficient, despite increased visceral iron stores. In fact, it is more and more admitted that, on one hand, iron hyperabsorption is the result of a systemic (rather than a local i.e. purely duodenal) process and, on the other hand, that excessive iron delivery by the macrophages represents an important component (possibly superior to iron intestinal absorption) of the iron overloading process (7).

In the past few years, a series of transporters and co-transporters, as well as two new important regulatory proteins, have been shown to play a major role in iron metabolism.

Duodenal cytochrome b (Dcytb)

Dcytb is a reductase at the luminal membrane of apical enterocytes, which reduces ferric iron to the ferrous form. This is required for iron uptake by apical duodenal cells. In HH, the expression of Dcytb is increased.

Divalent metal transporter1 (DMT-1)

DMT-1 is a ferrous iron carrier at the luminal membrane of the apical enterocyte. In HH patients this protein is over expressed.

Ferroportin-1

Ferroportin-1 is expressed at the basolateral membrane of the apical enterocytes delivering iron to the

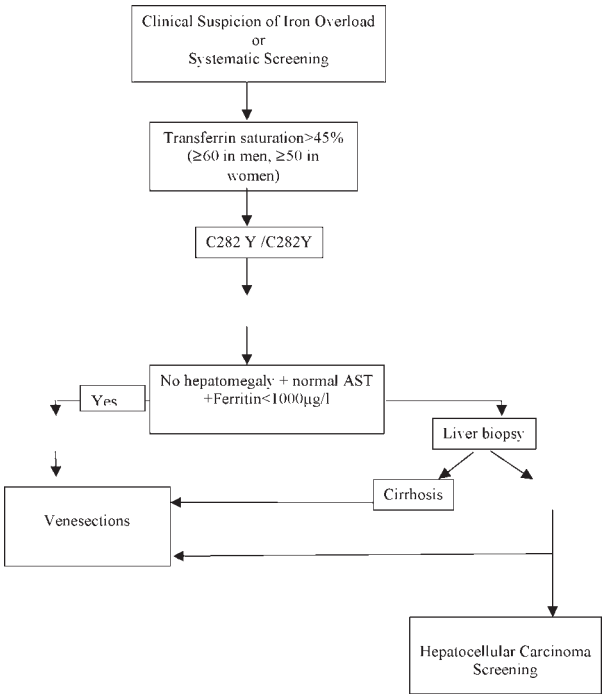


Fig. 3. — Strategy for the diagnosis of HFE-1 hemochromatosis.

plasma. It has also been found in macrophages. The gene coding for ferroportin-1 is located on chromosome 2. In HH, the expression of ferroportin-1 is enhanced.

Hephaestin

Hephaestin, also located at the basolateral membrane of the apical enterocyte, is a ferroxidase involved in iron export, by transforming Fe^{2+} into Fe^{3+}. Ferric iron is then bound by plasma transferrin, and can circulate in the bloodstream. In HH patients, neither mRNA nor protein expression were found to be increased.

Transferrin receptor 2(TFR-2)

TFR-2, encoded by a gene on chromosome 7, is expressed in hepatocytes. When mutated, an iron overload syndrome similar to HFE-1 hemochromatosis is observed (8).

Hepcidin

Hepcidin, whose gene is located on chromosome 19, is a 25 amino acid protein synthesized in the liver and secreted in the blood. Hepcidin's involvement in iron metabolism was first demonstrated by a French team (9). A series of subsequent studies, both in mice and in humans, have documented the key role of hepcidin in iron. In mice lacking hepcidin an hemochromatosis picture was observed whereas mice overexpressing hepcidin were severely anemic (10,11). Hepcidin mutations are responsible for a severe form of human juvenile hemochromatosis (12). Furthermore, in HFE knock out mice and C282Y homozygous subjects (13, 14), the levels of hepcidin were found to be lower than in normal subjects, suggesting the involvement of HFE in the regulation of hepcidin expression. The regulation of hepcidin production by HFE most probably involves macrophagic cells. Thus, in HH macrophages, the iron content, which is lower than normal, normalises following HFE injection (15).

Hemojuvelin

It is the latest "iron protein" identified (16). The corresponding gene is located on chromosome 1. When mutated, this protein is responsible for the most frequent form of human juvenile hemochromatosis.

Diagnosis (Fig. 3)

As previously described, HFE1 is not the sole protein involved in the molecular management of iron. In fact, as mentioned above, mutations affecting one or several other key proteins may complicate the diagnosis of HH. The following individual cases were reported in order to illustrate clinical presentations resulting from different mutations affecting various proteins involved in iron metabolism.

1. The first case report describes the clinical features of a classical presentation of HFE-1 hemochromatosis :

A 45 year-old male patient was hospitalised with asthenia and arthralgia. His ferritin level and transferrin saturation were 700 µg/l and 100%, respectively. The results of mutation analysis were positive for C282Y homozygosity. The patient was diagnosed with HH. Having normal transaminases, no hepatomegaly, and a ferritin level of less than 1000 µg/l, no liver biopsy was indicated since this patient was not at risk of presenting cirrhosis.

2. *A case of TFR-2 mutation*

A 42 year-old male patient was found to have asthenia, arthralgia and a transferrin saturation of 100%. His ferritin level (1,900 µg/l) was much higher than that of the first patient. Based on the patient's clinical and biochemical presentation, classical HH was suspected, and a mutation analysis for C282Y was requested. However, no C282Y mutation was detected. A liver biopsy was performed and showed massive iron overload whose distribution (mainly hepatocytic and periportal) was similar to that of HFE-1 hemochromatosis. In this case, the patient was suffering from an exceptional mutation affecting the TfR2 gene, present on chromosome 7.

3. *A case of Juvenile Hemochromatosis*.

A 23 year-old woman with melanodermia, heart failure, a serum ferritin level of 7,215 µg/l and a transferrin saturation of 100%, was reported. The results of C282Y mutation analysis were negative. Liver biopsy revealed massive iron overload. The development of heart failure

at 23 years of age, with concomitant iron overload, led to the diagnosis of juvenile hemochromatosis. Two mutations are known to induce juvenile hemochromatosis. One affects hepcidin, the other one hemojuvelin. The latter mutation was here responsible for the disease.

4. *A case of dysmetabolic hyperferritinemia.*

The case report discussed next is a good example of clinical features accounting for approximately 30% of erroneous diagnosis of HH.

A 38 year-old male patient with gout and an elevated serum cholesterol was admitted to hospital. Serum ferritin was 1,100 µg/l. Liver ultrasound revealed "overload". No Tf saturation test was performed. The mutation analysis showed heterozygosity for H63D. The diagnosis of HH was therefore done. In fact, this was a misdiagnosis for the following reasons : it should be emphasized that H63D heterozygosity has no clinical significance. Moreover, the so-called "overload" was in fact steatosis and not iron (ultrasounds cannot detect iron) and the results of transferrin saturation performed later showed a normal saturation rate (40%), which ruled out HH. The increase in ferritin in this case was due to the dysmetabolic hyperferritinemia syndrome (also called dysmetabolic hepatosiderosis, 17).

5. *A case of Ferroportin mutation*

A 54 year old female with a normal clinical examination, with the exception of asthenia, is described. Her ferritin level was extremely high (8,943 µg/l), even for a patient with the most severe form of HH. Surprisingly, her transferrin saturation was almost normal (53%). The patient underwent a liver biopsy which showed iron overload, mainly in Kupffer cells. Family investigations showed that her two daughters (34 and 23 years of age), born from different fathers, had low transferrin saturation values (19% and 28%, respectively). For both sisters, the level of ferritin was higher than 1,000 µg/l (1,290 and 1,400, respectively). The fact that the children were born by two different fathers indicated that the mutation was dominant. A ferroportin mutation was detected (18). This new cause of iron overload (19, 20) is now considered to be more frequent than previously thought.

6. *A case of aceruloplasminemia*

The last case report described is that of a 62 year female patient with mild anemia (11.2 g/dl). Biochemical tests revealed a low iron level (5.6 µmol/l) and a transferrin saturation of 10%. The ferritin concentration was 1,450 µg/l. No bleeding or hemorrhage was detected. Iron overload was first detected on MRI, and confirmed by liver biopsy. Because of a few neurological symptoms (extrapyramidal), aceruloplasminemia was suspected, which was confirmed by the fact that no plasma ceruloplasmin could be detected (21).

Acknowledgments

European Community Grant QLK1-CT-2001-00444 and Association Fer et Foie.

Authors would like to thank Orphan-Europe for sponsoring this workshop on rare disease.

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