Genetic hemochromatosis: Pathophysiology, diagnostic and therapeutic management

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Summary

The term hemochromatosis (HC) corresponds to several diseases characterized by systemic iron overload of genetic origin and affecting both the quality of life and life expectancy. Major improvement in the knowledge of iron metabolism permits to divide these diseases into two main pathophysiological categories. For most HC forms (types 1, 2, 3 and 4B HC) iron overload is related to cellular hepcidin deprivation which causes an increase of plasma iron concentration and the appearance of plasma non-transferrin bound iron. In contrast, iron excess in type 4A ferroportin disease is related to decreased cellular iron export. Whatever the HC type, the diagnosis rests on a non-invasive strategy, combining clinical, biological and imaging data. The mainstay of the treatment remains venesection therapy with the perspective of hepcidin supplementation for hepcidin deprivation-related HC. Prevention of HC is critical at the family level and, for type 1 HC, remains a major goal, although still debated, at the population level.

The term genetic hemochromatosis (HC) has become a generic one, encompassing a variety of disorders corresponding to systemic iron overload of genetic origin. Therefore, today, one should now think in terms of “hemochromatoses” rather than “hemochromatosis”. Numerous mutations, located on different chromosomes, are involved, leading to varying phenotypes according to clinical expression and severity. The present review will focus on HFE-related (type 1) HC (chromosome 6), by far the most frequent form in Caucasians, and on the non-HFE-related HC, rare diseases involving mutations of the hemojuvelin [1] (HFE2 or HVY) (chromosome 1), hepcidin [2] (HAMP) (chromosome 19), transferrin receptor2 [3] (chromosome 7) (TFR2), ferroportin [4,5] (SLC40A1) (chromosome 2) and ceruloplasmin (CP) (chromosome 3) genes, and corresponding to types 2A, 2B, 3, 4 HC, and to hereditary aceruloplasminemia [6] (HA), respectively [7,8].

Pathophysiology

It will consider four main aspects [9] (figures 1 and 2).
**Hemochromatoses with iron overload due to enhanced cellular iron influx related to deprivation in hepcidin**

**Mechanisms of hepcidin cellular deprivation**

Hepcidin (encoded by the *HAMP* gene) is the iron hormone governing systemic iron homeostasis. Essentially produced by the hepatocytes [10], this 25-aminoacid peptide decreases plasma iron by a double mechanism [11]. On the one hand, it limits digestive iron absorption, on the other hand it decreases iron release from the spleen into the plasma (this splenic iron originates from the normal erythrophagocytic process). Hepcidin modulates the amount of iron release into the plasma by targeting ferroportin, the only known cellular iron exporter [12]. Schematically, after hepcidin binding to ferroportin, the complex is internalized and leads to intracellular ferroportin degradation which, in turn, decreases the iron export capacity mediated by the residual ferroportin at the membrane level [13]. Therefore, every physiological or pathological situation increasing hepcidin synthesis will decrease plasma iron, and conversely. The development of iron overload in hepcidin deprivation-related HC is mediated by plasma iron increase (hypersideremia), through two mechanisms.

The most frequent one is hypohepcidinemia. It is the case for types 1 (*HFE*-related), 2A (*HFE2* or *HJV*-related), and type 3 (*TFR2*-related) HC. In these settings, the causal mutations, through alteration of molecular cascades that are increasingly dissected, and involve especially the BMP-SMAD signaling pathway and/or ERK1/2 pathways [14], lead to abnormally decrease hepatic synthesis of hepcidin with respect to iron status, and subsequently to decrease levels of plasma hepcidin.

The other situation implicated in hepcidin cellular “deprivation” is hepcidin resistance. It occurs during type B ferroportin disease, due to very specific mutations and characterized by an impaired capacity of ferroportin to interact with hepcidin. Heparidin being then unable to decrease ferroportin expression, the cellular consequences are equivalent to those observed during plasma iron overload.

### Glossary

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Hc</td>
<td>haemochromatosis</td>
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<tr>
<td>HAMP</td>
<td>hepcidin antimicrobial peptide</td>
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<tr>
<td>HJV</td>
<td>hemoujuvelin</td>
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<tr>
<td>TFR2</td>
<td>transferrin receptor 2</td>
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<tr>
<td>SLC40A1</td>
<td>human solute carrier family 40 member 1</td>
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<tr>
<td>CP</td>
<td>ceruloplasmin</td>
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<tr>
<td>HA</td>
<td>hereditary aceruloplasminemia</td>
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<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
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<tr>
<td>Smad</td>
<td>Small (phenotype) mothers against decapentaplegic</td>
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<tr>
<td>MAP</td>
<td>mitogen-activated-protein kinase</td>
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<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>TFSat</td>
<td>transferrin saturation</td>
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<tr>
<td>NTBI</td>
<td>non-transferrin bound iron</td>
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<tr>
<td>LPI</td>
<td>labile plasma iron</td>
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<tr>
<td>RPI</td>
<td>reactive plasma iron</td>
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<tr>
<td>GP</td>
<td>general practitioner</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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hepcidin deficiency with a resulting increase efflux of iron from the enterocytes and from the splenic macrophages, and therefore increased plasma iron levels.

**Pathophysiological consequences of hepcidin cellular deprivation**

The key primary biochemical event is increased plasma iron concentration which leads to increased saturation of transferrin, the physiological carrier protein of plasma iron (corresponding to transferrin saturation [TfSat] levels over 45%). As a result, novel forms of circulating iron may appear in the plasma, named non-transferrin bound iron (NTBI). NTBI, in contrast with transferrin-iron that targets essentially the bone marrow, is very avidly taken up by parenchymal cells, first and foremost the hepatocytes [15] but also cardiomyocytes and pancreatic cells. Therefore, NTBI is the major iron species accounting for cellular (and tissue) iron deposition in HC. Moreover, whenever TfSat exceeds 75% [16], a novel NTBI form appears, defined by its capacity to produce reactive oxygen species (ROS), and called labile plasma iron (LPI) [17] or reactive plasma iron (RPI). LPI is considered as the main culprit for cellular iron toxicity in HC, through damaging cellular plasma membranes as well as intracellular organelles. The resulting tissue alterations underlay the clinical organ damage developed in HC, such as hepatic, pancreatic and cardiac lesions.

**Hemochromatoses with iron overload due to decreased cellular iron efflux related to ferroportin deficiency**

**Mechanisms of ferroportin deficiency**

The involved mutations of the ferroportin gene affect the cellular iron export function and not the domain interacting with hepcidin. As a consequence, cellular iron egress is impaired, leading to increased intracellular iron stores. Such a situation is present in type 4A HC, which is the most frequent form of the ferroportin disease [4,5].

**Pathophysiological consequences of ferroportin deficiency**

As a consequence of altered cellular iron egress, plasma iron does not increase and may even decrease (corresponding to normal or decreased TfSat, respectively). Therefore, no plasma NTBI is present, implying that parenchymal cells are only moderately affected by iron deposition, especially as ferroportin activity is particularly pronounced in macrophages. The sites of cellular iron overload are therefore mainly the spleen (particularly rich in macrophages) and, at a lesser degree, the liver (Kupffer cells). The absence of NTBI also means absence of LPI and, therefore, less damaging capacity of excessive stored iron (especially as macrophages are less sensitive to iron-related damage than parenchymal cells). These data likely explains why type 4A HC seems a relatively benign disease as compared to
the hepcidin deprivation-related forms of HC [18]. However, long-term studies remain to be conducted.

**Hemochromatosis of not fully solved pathophysiology**

It is the case for HA [19]. The proposed explanation for iron overload is iron retention due loss of ferroxidase activity normally exerted by ceruloplasmin [20]. Indeed, this ferroxidase property is required for plasma transferrin to take up the iron released, under the ferrous form, from the cells (iron oxidation into its ferric form being needed for transferrin uptake). As an upstream consequence, ferroportin activity for cellular iron export would be altered, leading to cellular iron retention (as in type 4A ferroportin disease). This would fit with the decreased plasma iron levels (and TfSat) observed in HA. However, this mechanism cannot not explain why, in HA, iron overload spares the spleen and affects essentially the hepatocytes (like in hepcidin deprivation-related HC) [9]. Moreover, HA is the sole HC form where iron overload is significantly present in the brain, accounting for neurological manifestations of the disease. Further studies are therefore needed to fully elucidate the mechanisms whereby systemic (including brain) iron overload develop in this disease.

**The issue of penetrance variability**

It has become clear that genetic predisposition does not mean clinical expression. This is particularly clear in type 1 HC where it has been estimated that 1% of women and less than 30% of C282Y/C282Y men would develop the full-blown disease [21]. Many studies are underway to determine the environmental and host factors likely to account for phenotypic variability, which concerns not only the amount of body iron excess, but also, for an equivalent amount of iron overload, the organ targeting of iron excess. Among environmental factors, dietary iron content, physiological iron losses (menstruations [22], pregnancies, breastfeeding), body weight [23] have been identified. Among host factors, the role of male gender (through the hepcidin decreasing effect of testosterone [24,25]) has been proposed for favoring greater stores as compared to females, and genetic factors have been reported for explaining visceral complications, especially PCSK7 polymorphism for favoring hepatic fibrosis [26]) have been reported.

**Diagnostic management**

It is based on a non-invasive strategy, i.e. not requiring in most cases to perform a liver biopsy. Five main diagnostic steps can be individualized [27] (figure 3).

**To suspect iron overload**

*From the clinical viewpoint, many symptoms, more or less associated, can reflect HC*

Chronic fatigue, joint pains, hyperpigmentation (melanoderma), impotence, diabetes, osteoporosis, hepatic features (mild increase of plasma transaminase activities, hepatomegaly,
sometimes cirrhosis or hepatocellular carcinoma), cardiac symptoms (rhythm disturbances, heart failure). Anemic syndrome and neurological symptoms (extrapyramidal syndrome, cognitive dysfunction) can express HA.

When comparing the clinical expression of the various HC types, the following remarks can be proposed:

- type 1 HC is most often a delayed disease, with a long clinically asymptomatic phase until the age of approximately 30–40 years in men and 40–50 in women;
- types 2A and 2B (and sometimes type 3) HC correspond to much rarer but also more severe diseases with clinical expression before the age of 30, and often before 20. They are characterized by severe lesions of the liver (cirrhosis), heart (cardiac failure), and endocrines (hypothalamic-pituitary insufficiency);
- type 4A ferroportin disease is only clinically mildly symptomatic despite strong iron overload.

From the biochemical viewpoint, the most frequent abnormality leading the clinician to suggest iron overload is, by far, hyperferritinemia (usually defined by plasma ferritin levels over 300 µg/L in men, and over 200 µg/L in women)

It is critical, however, to remember that hyperferritinemia may be due to other causes than iron excess [28]. The main differential diagnosis is the metabolic syndrome. Dysmetabolic hyperferritinemia [29] is probably the most frequent cause of hyperferritinemia worldwide. It should be suspected in any patient with an increase of weight (or waist circumference), blood pressure, glyceria, lipidemia, or uricemia. Plasma TfSat is normal and hepatic iron overload (when assessed by magnetic resonance imaging -MRI-) is normal or only moderately increased [30] (less than three times the upper normal limit). Two other possible causes of hyperferritinemia should be ruled out, inflammation and alcoholism [31]. It is only after having excluded these three major causes, that increased plasma ferritin levels can be considered as reliably reflecting body iron excess.

With regard to plasma iron or TfSat, it is important to recall that it can be normal or even low, despite significant body iron excess, in HC forms such as type 4A ferroportin disease and HA.

To confirm iron overload

It is valuable to get a direct visualization of tissue iron overload. For this purpose, hepatic MRI has replaced liver biopsy. Some techniques correspond to relaxometry approaches [32,33], defining indices such as $T_2^*$ or $R_2^*$. A simple and reliable method is based on the signal intensity ratio [34]. The decreased $T_2$ hepatic signal (as compared the spinale muscle signal which serves as a reference) is inversely correlated with the increase in hepatic iron concentration (the darker the liver, the higher the hepatic iron concentration). "Iron-MRI" also allows to assess the iron status of the spleen and pancreas (and, with relaxometry techniques, of the heart). A further important MRI information is provided by comparing the liver and spleen signals [9]. Schematically a "black" liver together with a "white" spleen orientates toward a type of HC with hepcidin deprivation, whereas a "black" spleen together with a "gray" liver favours the usual (type A) form of ferroportin disease. Therefore, iron-MRI not only ascertains and quantifies iron overload but, by showing the iron balance between liver and spleen, provides a valuable indication on the pathophysiology of iron overload development, an important clue for approaching the HC type.

To suspect the genetic nature of iron overload

An acquired form of iron overload is usually easily ruled out. Transfusional iron overload is obvious in the context of chronic anaemia such as haemoglobinopathies (thalassaemias [35,36], sickle cell disease [37]), myelodysplastic syndromes [38] or aplastic anaemia related to bone marrow transplantation procedure [39]. Similarly, iron overload due to excessive parenteral iron supplementation [40] is diagnosed by the detailed patient’s history.

Family data indicating problems of iron excess is another important clue in favor of a genetic disease.

To orientate toward the pathophysiological category of HC

Combining plasma TfSat and imaging data is here essential. TfSat is a pivotal diagnostic parameter, since increased TfSat favours hepcidin deprivation-related HC, whereas normal or low values are observed in the usual form of ferroportin disease and in HA.

MRI is also, as previously mentioned, an interesting indicator by establishing an iron balance between liver and spleen, thus suggesting hepcidin deficiency or decreased macrophage iron release.

To definitely identify the genetic HC type

Guided by the combination of clinical, biological, and imaging data, the final diagnostic step is appropriate genetic testing.

HFE-related HC

It corresponds, in the vast majority of cases, to C282Y (new nomenclature p.Cys.282Tyr) homozygosity (C282Y/C282Y). As to the other HFE mutations, the following statements can be proposed:

- the H63D (His63Asp) mutation is a simple polymorphism;
- compound heterozygosity (C282Y/H63D) or H63D homozygosity are not susceptible to cause significant body iron overload unless they are associated with factors such as alcoholism, the metabolic syndrome, or other mutations impacting iron metabolism gene (digenerism) [41];
- the S65C mutation has no diagnostic interest;
- exceptional profiles of compound heterozygosity can be responsible for clinically overt forms of HC.
**Non-HFE HC** [42]

They are rare diseases, with a reserve for type 4A HC (type A ferroportin disease) that may be more frequent than initially thought (probably related to its dominant mode of transmission) [43]. The corresponding specific genetic testing requires duly accredited laboratories [41]. It should be noticed that, for Ha, it is good clinical practice to check that plasma ceruloplasmin levels are very low or not detectable and/or ferroxidase activity is decreased before performing specific genetic testing. The new technical approach resorting to high throughput sequencing (NGS: next generation sequencing) offers the advantage of its power but should not lead to forget the need for a preliminary clinical orientation, at best managed by clinical reference centers. Moreover, it presents the drawback to identify an increasing number of new mutations whose deleterious nature is often difficult to establish and requiring additional family and/or functional studies [44].

**Therapeutic management**

It will be confined here to the management of iron removal [27] (figure 4).

**Treatment of HFE (type 1) HC**

*Venesections (phlebotomies) remain the key procedure*

By removing total blood, they remove red blood cells which contain half the total quantity of body iron (2 g), and lead the body to pump iron into its reserves in order to produce new erythrocytes. The induction phase usually consists of weekly venesections (7 mL/kg body weight without exceeding 550 mL) until plasma ferritin reaches approximately 50 μg/L [45], provided haemoglobin levels remain superior to 11 g/dl (or do not fall more than 2 g from the baseline levels). Thereafter starts the maintenance treatment, theoretically for life, whose goal is to prevent recurrence of iron overload by maintaining ferritin levels around 50 μg/L. It usually requires one venesection every one to 3 months. Checking plasma TfSat has no interest during the major part of the induction phase since this parameter, in contrast with plasma ferritin levels, does not fall until the very end of this phase. During maintenance therapy, it may be advised to monitor TfSat for instance twice a year in order to ensure that the patient does not exhibit a biological profile permanently marked by the contrast between satisfactory ferritin levels and a strong rise in transferrin saturation (especially over 75%, a threshold that may correspond to some risk of iron toxicity due to the presence of LPI). In terms of global results, venesection therapy is simple, cheap, efficient, and well tolerated. Some limitations, however, should be pointed out:

- efficiency may only be partial when organ lesions were too severe when starting the treatment (arthropathy, liver cirrhosis with the persistent risk of liver cancer despite appropriate iron removal);
- tolerance is not devoid of side effects affecting the quality of life (discomfort of the needle puncture especially) [46].

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**Figure 4**

Present and future therapeutic approaches for HFE and non-HFE haemochromatosis
Erythocyteapheresis
This method is to remove only red blood cells. Rarely used, it is more complex and expensive than venesections, but more efficient, and globally well accepted by the patients [47]. It can be particularly suitable for people whose professional activity (frequent travellers) is not conducive to frequent repeated bleedings.

Iron chelation
It can be discussed in the rare situations where phlebotomies are not possible either for psychological or technical (poor venous access) reasons. Although desferrioxamine is the only approved drug in this indication, its modalities of administration (prolonged subcutaneous infusions by a portable pump, twelve hours a day and 5 days a week) are rather dissuasive and explain why an oral chelator (such as deferasirox) may be preferred despite its status of off label medication and some possible side effects (leading to a prescription under the clinician responsibility and with an informed written consent by the patient).

Therapeutic perspective
The improved mechanistic knowledge of iron overload development in HC opens the road for applying an innovative approach consisting in hepcidin supplementation. Two main ways are theoretically possible, exogenous administration of hepcidin (minihepcids [48], full hepcidin, or hepcidin agonists) or endogenous stimulation of hepcidin synthesis by targeting one of the molecular steps involved in the hepcidin synthetic pathway. Normalizing plasma hepcidin levels would restore normal iron homeostasis, and could be indicated as an adjunct to venesections during the induction phase (in order to shorten this phase) or for totally replacing maintenance venesections.

Treatment of non-HFE HC
Types 2, 3 ad 4B HC
Venesections treatment is fully indicated given the phenotype of hepcidin deprivation. Chelation therapy may be associated in case of massive iron overload such as in juvenile HC (Type 2A [49,50] and 2B). Hepcidin supplementation is also a logical perspective (except for type 4B HC characterized by hepcidin resistance).

Type A 4 HC
Venesections remain indicated although their tolerance is less satisfactory than in the group of hepcidin deprivation-related HC. Indeed, given the impairment of cellular iron export, the iron recycling process induced by the venesection procedure is less efficient and exposes to the risk of anemia if the phlebotomy schedule is too strong. Therefore, it is advised to alleviate this schedule and, usually, one venesection every two weeks is feasible and efficient [18]. It should be noticed that the plasma ferritin levels reflecting “de-ironing” may be significantly higher than in type 1 HC (the correlation between plasma ferritin concentrations and tissue iron overload being different) so that iron-MRI can be helpful to get an objective assessment of residual body iron stores. Restoring ferroportin activity in its specific iron export property would represent the future therapeutic approach but still seems a distant prospect.

In conclusion, hemochromatoses are potentially severe diseases, especially because their diagnosis can be ignored during a long asymptomatic period or misdiagnosed due to frequently aspecific clinical expression. Diagnosing HC is non-invasive, based on combined, clinical, biological, and imaging data. HC treatment, mostly based on venesection therapy, is remarkably simple and efficient when considering the global field of genetic diseases. Moreover, for most HC entities, this symptomatic treatment should, in the future, be completed or replaced by hepcidin supplementation. Prevention at the family level, resorting mainly to genetic testing, is essential, and, for HFE-HC, population systematic screening [51] (likely based on combined plasma TfSat and ferritin), although still debated, should remain a major objective.

References
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