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POINT OF VIEW

Non-HFE hemochromatosis

J. A. Solís Herruzo and P. Solís Muñoz

Service of Digestive Diseases. Hospital Universitario 12 de Octubre. Madrid, Spain

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INTRODUCTION

Hereditary hemochromatosis (HH), a designation first used by v. Recklinghausen in 1888 (1), is an inherited, autosomal recessive disease characterized by excessive iron deposition in the liver, pancreas, heart, and other organs as a result of excessive intestinal iron absorption. In organs where iron excess accumulates, lesions develop that are responsible for clinical manifestations (liver cirrhosis, joint disease, heart failure, arrhythmias, endocrine disease, skin pigmentation, etc.). The search for a genetic cause led first to acknowledge an association with the short arm of chromosome 6, the area coding for the HLA-A3 molecule (2), and then to recognize the hemochromatosis gene-HFE (3). Two mutations were initially identified in the HFE gene–845G→A (C282Y), and the substitution 187G→C (H63D). The search for the C282Y mutation in the HFE gene of Northern European patients with HH has revealed that more than 80% of such individuals are homozygous for this mutation (4). In Northern European countries some C282Y non-homozygous patients are C282Y/H63D double heterozygotes. In these same countries the frequency of the C282Y mutation amongst the general population is 1 in 100 inhabitants in Ireland, and 1 in 400 inhabitants in the United States (5,6). In Southern European countries the frequency of this mutation is lower. In Greece, for instance, it is to be found not even in 1 in 100,000 population (7). This mutation is presumed to have developed in a Celt individual during the Bronze Age, later to spread in countries where Celtic penetration was greatest (8).

Protein HFE belongs in the family of HLA class 1 histocompatibility molecules. Similar to these molecules, it is 343-aminoacid glycoprotein located at the plasma membrane in some cells. It is made up of three extracellular loops (α_1, α_2, α_3), a transmembrane portion, and an intracytoplasmic portion. It interacts with transferrin receptors at a gap between loops α_1 and α_2 (9). A thiol bridge between two cysteine molecules gives rise to loop α_3. This loop is crucial for its non-covalent binding of β_2-microglobulin (β_2MG), and its expression in the surface of cells (10). It is currently accepted that this protein plays a relevant role in iron metabolism. However, its precise mechanism of action is unknown. Experiments performed in cultures of cells transfected with an HFE-expression plasmid revealed that iron passage into cells, and thus cell iron and ferritin content, decreased, while the number of transferrin receptors 1 (TfR1) increased. Hence, HFE seemed to behave as a negative regulator of TfR1 function (11-13) and a blocker of iron passage into cells. HFE was suggested to compete with transferrin (Tf) in binding TfR1 (14). However, these experiments have been criticized – overexpression of HFE alone is ineffective, as the presence of β_2MG is required for its expression on the surface of cells. When an overexpression of both proteins – HFE and β_2MG – occurs, cell iron and ferritin content dramatically increases (15). That is, HFE behaves as a facilitator of iron passage into cells. In fact, HFE mutations involving the region through which the binding of TfR1 occurs block internalization of the Tf/TfR1/HFE complex (16).

Two mechanisms have been suggested through which HFE may regulate intestinal iron absorption. One is related to the role ascribed to duodenal crypt cells as sensors

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Correspondencia: J. A. Solís-Herruzo. Servicio de Medicina de Aparato Digestivo. Hospital Universitario 12 de Octubre. Avda. de Córdoba, s/n. 28041 Madrid. e-mail: jsolis.hdoc@salud.madrid.org

of total body iron stores. HFE is found at the base membrane of these cells together with β_2MG and TfRs. Upon arrival, iron-saturated Tf incorporates itself into the HFE/β_2MG/TfR complex, which is then wholly internalized within an endosome (17). Tf releases iron within duodenal crypt cells. These cells maturate into enterocytes the way iron-rich cells should, that is, by inhibiting the synthesis of all those proteins favoring iron passage (Dcytb, DMT, ferroportin, hephaestin). Under such conditions intestinal iron absorption decreases (Fig. 1). In contrast, when little iron is transported by Tf the amount of iron Tf may deliver to duodenal crypt cells is small. These cells then maturate into enterocytes as iron-poor cells. That is, the synthesis of proteins favoring iron passage increases (Fig. 2) (reviewed in 18). 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 hemojuvelin (reviewed in 19). TfR2 and HFE favor the passage of iron into liver cells, which would be

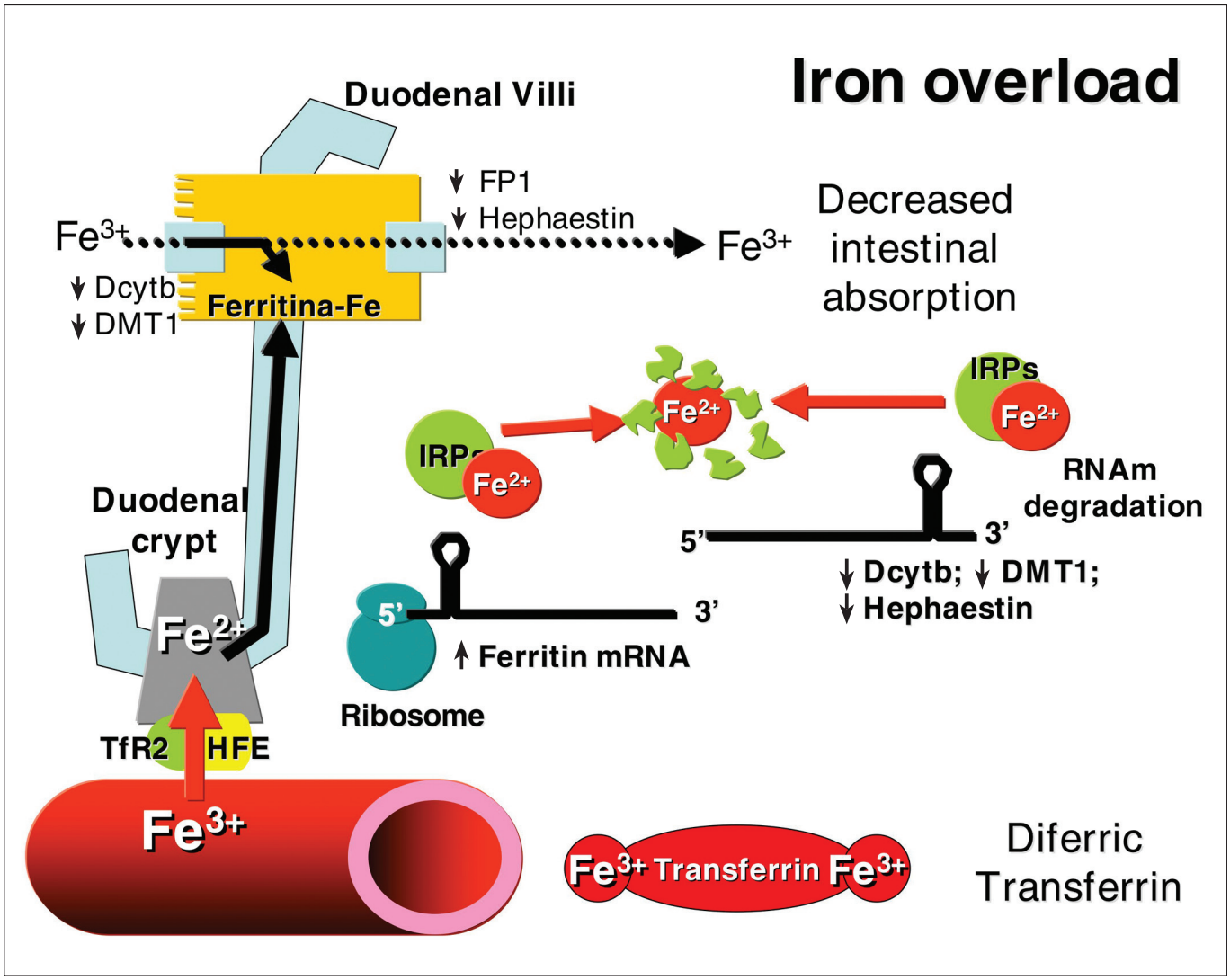

Fig. 1-. *Iron overload.* Transferrin-bound iron is captured by duodenal crypt cells due to the presence of transferrine receptors (TfR1 y TfR2) together with protein HFE at the base membrane. These cells become rich with iron, which binds IRPs (iron-regulatory proteins) and helps in their degradation. A consequence of absent IRPs is that mRNAs with IREs (iron responsive elements) at their 5'-end (ferritin) may be expressed and thus increase this protein in cells upon their maturation into enterocytes. In contrast, mRNAs with IREs at their 3'-end (Dcytb, DMT-1; hephaestin) are cleaved, since they lack protection from IRPs. As a consequence their amount is reduced in mature enterocytes, and iron transportation through the latter decreases. That is, intestinal absorption of iron decreases.

Sobrecarga de hierro. *El hierro transportado por la transferrina es captado por las células de las criptas duodenales gracias a la presencia de receptores de transferrina (TfR1 y TfR2) junto a la proteína HFE en su membrana basal. Estas células se enriquecen de hierro, por lo que este se une a las IRP* (iron-regulatory protein) *y contribuye a su degradación. Consecuencia de la ausencia de IRP es que los ARNm que poseen el IRE (iron responsive element) en su extremo 5' (ferritina) pueden expresarse y aumentar el contenido de la proteína en las células cuando maduran a enterocitos. Por el contrario, los ARNm que tienen el elemento IRE en su extremo 3' (Dcytb, DMT-1; hephaestin) se degradan al no estar protegidos por las IRP. En consecuencia, su cuantía disminuye en los enterocitos maduros y el transporte de hierro a su través disminuye. Es decir, la absorción intestinal de hierro desciende.*

responsible for hepcidin induction. Thus, normal HFE would slow intestinal iron absorption after inducing the synthesis of hepcidin.

The C282Y mutation results in the substitution of tyrosine for cysteine 282. This brings about a severe structural distortion in the HFE molecule. Specifically, it blocks the formation of the α_3 loop upon the disappearance of its constituent thiol bond. This molecular malformation prevents HFE – following its synthesis in the endoplasmic reticule – from binding β_2MG and therefore reaching the base membrane of cells. The absence of this protein may increase intestinal iron absorption through either of the above-mentioned mechanisms: a) its absence at the base membrane of duodenal crypt cells blocks the passage of iron into these cells. Iron deficiency in these cells determines their maturation through increasing iron-binding proteins and favoring intestinal iron absorption; and b) similarly, a lack of HFE determines a decrease of hepcidin formation in the liver, and hence an increase in intestinal iron absorption. In fact, patients with HH have been found to have greatly reduced hepcidin concentrations in their urine (20).

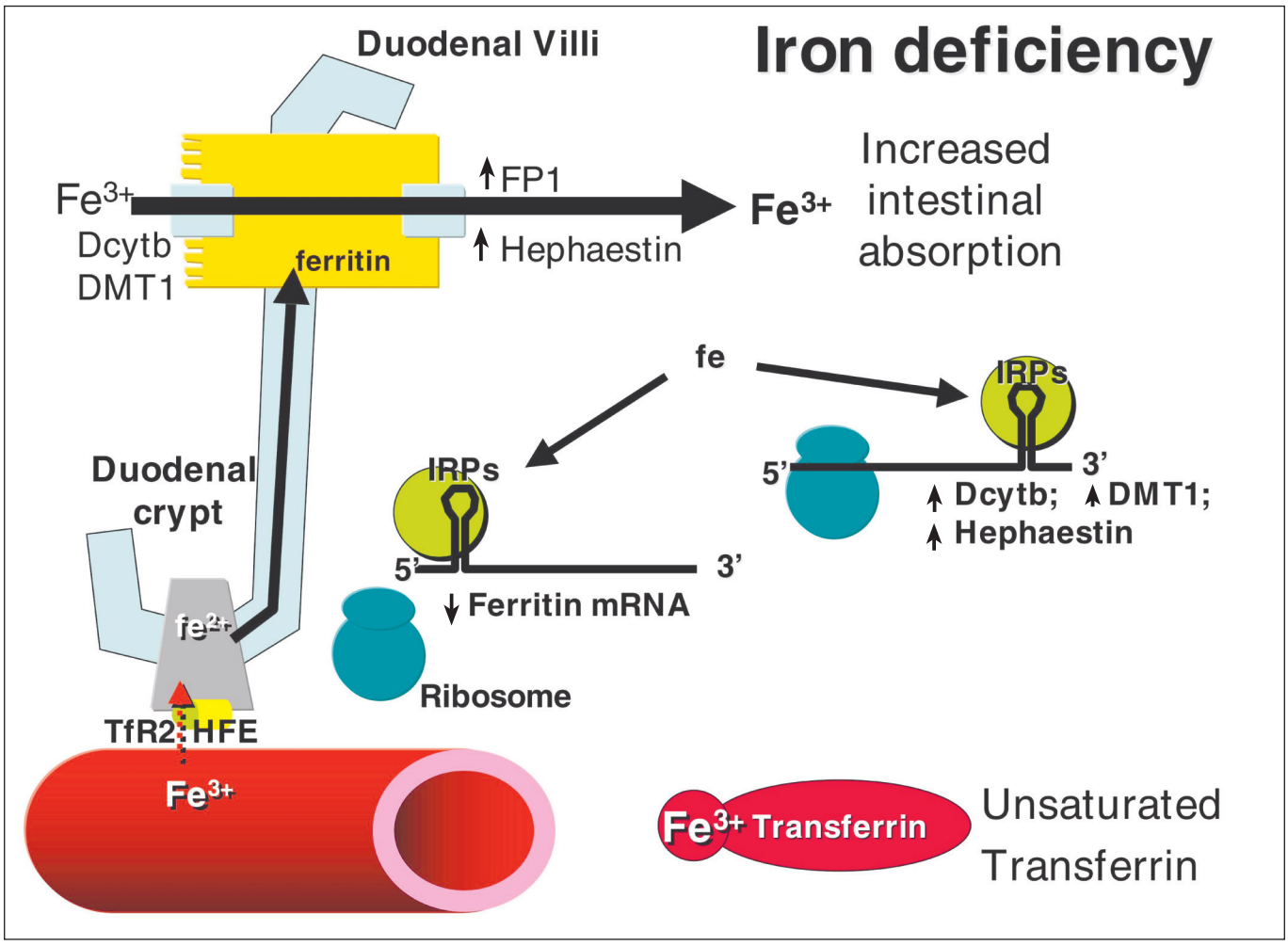

Fig. 2-. *Iron deficiency.* In this situation little iron may pass from blood transferrin into glandular crypt cells. Iron paucity in these cells allows the binding of IRPs to mRNA IREs. If IREs are at the 5'-end (ferritin), the presence of IRPs prevents mRNA from entering ribosomes and its expression. That is, ferritin diminishes in these cells. In contrast, mRNAs with IREs at their 3'-end (Dcytb, DMT-1, hephaestin) are more stable in the presence of IRPs, and their expression increases. Thus, the amount of such transporters increases inside enterocytes, and hence the intestinal absorption of iron. In hereditary hemochromatosis type I or III, the passage of iron into crypt cells is made difficult by the absence either HFE or TfR2, respectively. The maturation of these cells into enterocytes occurs in a way similar to that seen in iron deficiency conditions.

Deficiencia de hierro. *En esta situación, es poco el hierro que puede pasar desde la transferrina de la sangre a las células de las criptas glandulares. La escasez de hierro en esas células permite la fijación de las IRP a los IRE de los ARNm. Si estos poseen el IRE en su extremo 5' (ferritina), la presencia de la IRP impide la entrada del ARNm en los ribosomas y su expresión. Es decir, la ferritina en esas células disminuye. Por el contrario, los ARNm que poseen su IRE en el extremo 3' (Dcytb, DMT-1, hephaestin) son más estables en presencia de las IRP y su expresión aumenta. En consecuencia, aumenta la cantidad de estos transportadores en los enterocitos y, por ello, la absorción intestinal de hierro. En la hemocromatosis hereditaria tipo I o III, el paso de hierro a las células de las criptas se encuentra dificultado por la ausencia, bien de la HFE o bien del TfR2, respectivamente. La maduración de esas células a enterocitos se produce de la misma forma que lo hacen las células en situaciones de carencia de hierro.*

Mutation H63D is very common in Europe, particularly in Spain. It has been estimated that 22% of the European population are heterozygous for this mutation, 2% are homozygous for this mutation, and 2% are compound heterozygous in combination with C282Y (21). Even with homozygous status this mutation has little impact on iron homeostasis. Only patients with C282Y/H63D may exhibit morbid iron overload. Overall these are moderate, low-penetrance overloads (22).

While many other mutations in the HFE gene have been described of late, their clinical significance is uncertain. S65C is the most common of these mutations, and may result in some siderosis when in association with C282Y, particularly when alcohol abuse or other factors favoring iron overload are also present (23). Other mutations include G93R, I105T (24), Q127H (25), V272L (26), Q283P (27), E168X, W169X (28), V68δT, P160δC (29), IV53, and IG-T (30).

Despite our better understanding of HFE nowadays, many patients remain with unexplained iron overload. This is particularly common in Southern Italy, where up to 40% of patients with HH lack these mutations (4,27,31). In Spain the frequency of HFE-associated HH is similar to that of Nordic countries (32). In countries with non-European-origin populations (Asia, Africa, etc.), mutation C282Y is exceptional in frequency. It is for this reason that other genetic factors –in addition to those related to HFE– are thought to exist, which would influence the development of HH in countries where populations are not of Celtic descent. From studies in HFE knock-out mice it was also concluded that other genetic factors leading to iron overload must exist (33).

Once the secondary causes of iron overload (thalassemia, hemolytic anemia, chronic viral hepatitis, alcoholism, oral or parenteral iron administration, transfusion, porphyria cutanea tarda, portosystemic shunts, etc.) had been ruled out, the genetic study of patients with iron overload and absence of the C282Y mutation led to the identification of mutations in the genes coding for a number of proteins involved in the regulation of body iron stores. These new HH forms have been designated HH types II through IV, with type I being reserved for HFE-associated HH.

HEREDITARY TYPE II HEMOCHROMATOSIS OR HEREDITARY JUVENILE HEMOCHROMATOSIS

In 1932 Bezançon et al. (34) described in France the case of a 20-year-old male with liver cirrhosis, infantilism, and multiple endocrine insufficiencies who died from heart failure. More recently Goossens, in 1975 (35), and Lamon et al., in 1979 (36), described similar cases.

The presence of a rare, serious form of hereditary hemochromatosis involving children or adults of both genders younger than 30 years of age is currently admitted. It develops in Caucasian individuals of European descent (35,36), and is clinically characterized by patients having hypogonadotropic hypogonadism, miocardial disease, hepatomegaly, liver cirrhosis, and melanic skin pigmentation (36,37). These patients may also present with hypothyroidism with reduced response to TRH (35), adrenal insufficiency (38,39), and decreased prolactin and growth hormone (40). This form of hemochromatosis has been designated juvenile hemochromatosis (JH) or hereditary type II hemochromatosis. It results from iron overload secondary to excessive intestinal iron absorption (1-4 mg/day) (41). The disorder is inherited in an autosomic recessive fashion (36,37), and bears no relationship to the HFE gene, transferrin receptor 2, or ferroportin. It is currently acknowledged that differing genetic defects hide behind a single phenotype. In most cases JH is linked to chromosome 1q, which has been designated as type IIa hemochromatosis. In a minority of patients the disease is linked to chromosome 19, this being type IIb hemochromatosis.

The first mutations identified included those related to chromosome 19, where the HAMP gene coding for *hepcidin* (*hep*atic bacteri*cid*al prote*in*) is located. It is a small, 25-aminoacid peptide with antimicrobial properties that is synthesized by liver cells in response to inflammation, iron overload, and interleukin 6, and is decreased during hypoxia or when the need for erythropoiesis increases (42-45). The gene of *hepcidin*, which has three exons and two introns, codes for a polypeptide with 83 aminoacids that is a precursor to mature hepcidin. The latter results from the separation of 25 aminoacids at the carboxylic end (43). Its genetic expression is regulated by transcription factors C/EBPα and HNF4 (*Hepatocyte Nuclear Factor* 4) (46), but not by iron regulatory proteins (IRPs). Hepcidin messenger RNA (mRNA) lacks the iron responsive element (IRE) (47). Its function is to block iron passage through bowel cells (43,48), and iron release from macrophage stores (49). The mechanism by which these effects occur is unknown. Two distinct mechanisms have been suggested. Nicolas et al. (50) suggested that *hepcidin* interacts with the transferrin-transferrin receptor-HFE protein complex in duodenal crypt cells, thus favoring iron entry through these cells' base wall. As a consequence these iron-rich cells maturate into enterocytes while inhibiting the expression of proteins involved in intestinal iron absorption. In contrast, Frazer and Anderson (19) suggested a direct effect on proteins involved in the passage of iron through enterocytes as an alternative mechanism. To this effect hepcidin has been seen to bind, internalize and inactivate ferroportin 1 at duodenal mature enterocytes (51). Intestinal iron absorption is thus blocked (Fig. 3). Whatever the mechanism of action of hepcidin, its absence favors intestinal iron absorption and the release of iron stored in the reticuloendothelial system (RES). This is seen in all situations where this hepatic hormone is low (iron-deficient diet, bleeding, hypoxia, types I, II, III

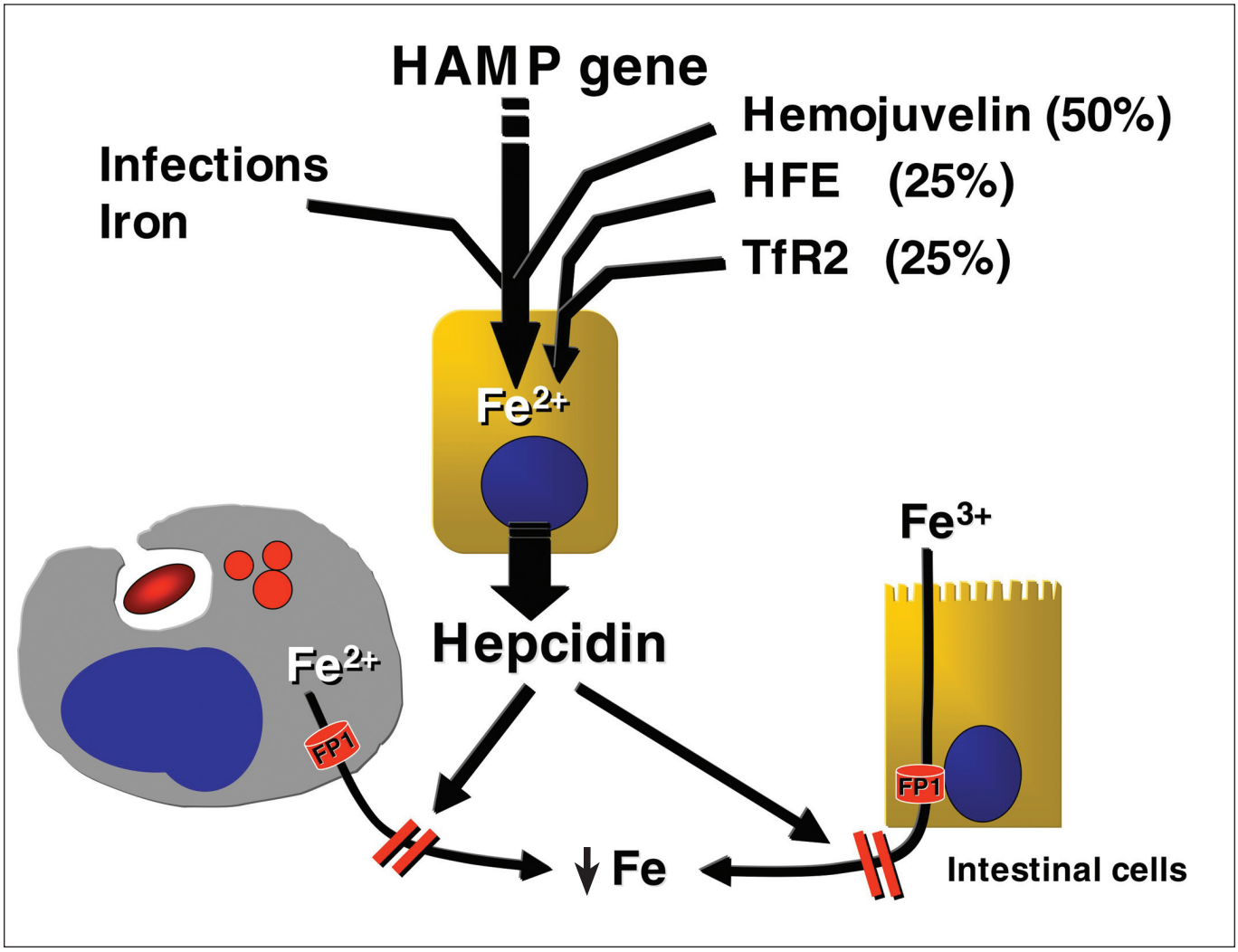

Fig. 3.- *Hepcidin and regulation of iron metabolism.* Hepcidin is a peptide expressed by gene HAMP in liver cells in response to infection and iron overload. Hemojuvelin, protein HFE, and transferrin receptor 2 (TfR2) also contribute to increase hepcidin production. This slows the passage of iron through enterocytes (intestinal absorption) and the release of iron from macrophages. In these cells iron stems from the degradation of phagocyted old red blood cells. Hepcidin has been suggested to exert these effects by internalizing ferroportin 1 (FP-1) within cells.
Hepcidina y regulación del metabolismo del hierro. *La hepcidina es un péptido, producto del gen HAMP, producido por la células hepáticas en respuesta a la infección y a la sobrecarga de hierro. La hemojuvelina, la proteína HFE y el receptor 2 de la transferrina (TfR2) también contribuyen a aumentar la producción de hepcidina. Esta frena el paso del hierro a través de los enterocitos (absorción intestinal) y la salida del hierro presente en los macrófagos. El hierro de estas células procede de la degradación de los hematíes viejos fagocitados por esas células. Se ha sugerido, que estos efectos los realiza la hepcidina por internalizar en las células la ferroportina-1 (FP-1).*

hemochromatosis, etc). On the contrary, increased hepcidin (inflammation, infection, exogenic iron overload, liver adenomatosis, etc.) (52) results in decreased intestinal iron absorption and iron retention in RES cells. During inflammation and infection hepatic hepcidin synthesis increases (52), which translates into decreased intestinal iron absorption (53,54), iron retention within macrophages (55), and anemia (45,53,56).

A number of mutations in the HAMP gene have been found in some patients with JH (57,58). A change G→A in the sequence +14 at the 5'-untranslated end (5'-UTR) has been reported in a Portuguese family, which creates a new AUG sequence that inhibits the translation of normal hepcidin mRNA, and probably results in the formation of a new, abnormal, unstable and degradable peptide (59). Other mutations reported include R56X, which creates a *"stop codon"*, the deletion of guanine 93, 175G→C (R59G), which precludes prohepcidin activation into hepcidin by convertases (60), particularly by furin, and 212G→A (G71D), which alters this peptide's structure and function (61).

In most patients with JH the disorder is linked to chromosome 1q (57), but the gene involved has remained unknown until very recently. In 2004, Papanikolaou et al. (62) published the results of a thorough study of chromosome 1q, where they unveiled a locus of previously un-

known function, *LOC148738,* which was associated with JH. The gene involved was initially designated HFE2, and more recently HJV. In this gene, which was made up of four exons separated by three introns, they found numerous mutations, and one of them, G320V, was present in all patients of Greek, Canadian, and French descent with JH (62). This gene codes for a 426-aminoacid protein that has been called *hemojuvelin*. Various regions have been identified in its molecule, including a short transmembrane region, a signal peptide, a v-Willebrand-like region, another RGD region through which it relates to extracellular matrix proteins, and a receptor-binding region (62). The mechanism of action of *hemojuvelin* is unknown, but seems to be closely linked to that of hepcidin. It is known not to be a hepcidin receptor (62), as it is not expressed in organs where hepcidin acts (intestine, spleen) (62). When mutations exist in the HJV gene, urine hepcidin decreases (62). In JH urine hepcidin is deeply reduced despite the fact that body iron is strongly elevated. Hemojuvelin is therefore thought to be a hepcidin-modulating protein, so that the former's decreased levels or inactivity results in the latter's reduced presence. Such decreases would be responsible for the increased intestinal iron absorption and iron overload found in patients with JH (62).

Since first described by Papanikolaou et al. (62), many other authors have confirmed the presence of mutation 959G→T (G320V) in patients with type IIa HH, in addition to their finding a few more. Huang et al. (63) described mutations 962G→A, 963C→A (C321X), 18G→C (Q6H), and 842T→C (I281T). The former two determine early transcription termination, and the latter involves the signal peptide region. Lee et al. added 238T→C (C80R), 302T→C (L101P), 665T→A (I222N) (64) and C321W (65). Lanzara et al. also confirmed mutation G320V, and then identified 17 new mutations. Most of them were located in exons 3 and 4, particularly within the molecular region corresponding to the von Willebrand-like domain (66), and many were determinant of transcription termination. These mutations included a deletion of 13 base-pairs (CGGGGCCCCGCCC), which may be expected to result in a nil phenotype. They found two mutations in another patient – 220delG, which creates a transcription end signal at 113, and 806-807insA, which leads to molecule truncation at position 331 and the formation of a 310-aminoacid molecule. Mutation 1153C→T (R385X) originates a protein devoid of 42 aminoacids at its carboxylic end, precisely those corresponding to the transmembrane portion. Mutation 295G→A (G99R) affects the RGD region, as does mutation G99V, already reported by Papanikolaou et al. (62). Mutations 253T→C (S85P) and 302T→C (L101P) also occur around this region.

Patients must be diagnosed and treated during the early stages of disease. As in HFE-linked HH, treatment primarily relies on an intensive program of periodic bleeding (67). Even in the presence of heart disease, heart failure, and arrhythmia patients may recover with this therapy (36,68,69). Combining bloodletting and deferroxamine may be useful in the presence of severe, life-threatening heart disease (68). Cases have been reported where heart transplantation was life-saving (40,68,70). As expected, liver iron contents clearly diminishes with bleedings, and fibrosis has also been shown to decrease or disappear with this therapy (36,39). There is no evidence that these patients may have a higher risk for liver cancer, but it is only logical to think so. In fact, foci of iron-depleted hepatocytes have also been reported in these patients (70).

HEREDITARY TYPE III, TRANSFERRIN RECEPTOR 2-LINKED HEMOCHROMATOSIS

In a number of patients with iron overload in whom the presence of HFE gene mutations or its being secondary to other conditions was excluded genetic research demonstrated some homozygous mutation in the gene coding for transferrin receptor 2 (TfR2). A number of the families where these mutations were unveiled originated in Southern Italy (71-74), but they have also been detected Japan (75), Portugal (76), and Northern France (77).

Patient characteristics are varied, but may be similar to those of patients with classic, type I hemochromatosis. However, they commonly experience symptoms at younger ages, even before turning 30 (74,76,77), and cardiac manifestations (74), hypogonadotropic hypogonadism (74,76), joint pain, skin hyperpigmentation, and liver cirrhosis are common. Manifestations may resemble those of type II or juvenile hemochromatosis.

TfR2 is a transmembrane glycoprotein exhibiting a large extracellular part. Sixty percent of aminoacids in this molecular region are similar to those in the extracellular domain of transferrin receptor 1 (TfR1). This is an area through which it contacts iron-carrying transferrin (Tf) (78), albeit with a lower affinity as compared to TfR1 (79,80). A potential binding of the HFE protein is currently debated (81,82). It is preferentially expressed by liver cells (82), but has also been identified in duodenal crypt cells (83). The binding of iron-carrying Tf to TfRs represents the primary entry of iron into cells. This bond is modified by HFE. Diferric Tf, TfR, HFE, and β_2MG all make up a complex that is internalized by endocytosis. Tf-bound Fe^{3+} is released in the endosome's acid pH, and is then transported into the cytoplasm by means of a reductase ($Fe^{3+} \rightarrow Fe^{2+}$) and of divalent metal transporter 1 (DMT1) (reviewed in 84). Both Tf and TfR return to the cell's surface for reuse.

In 1999 Kawabata et al. (78) cloned the TfR2 gene and showed that two transcripts resulted from it – TfR2-α and TfR2-β. The former is highly homologous to TfR1; its product is expressed on the surface of some cells, and plays a role in the regulation of iron metabolism. TfR2-β gives rise to a smaller protein that stays within cells. Iron

plays no part in the regulation of TfR2 expression, since its mRNA lacks an IRE (82).

Genetic studies performed thus far allowed to identify the following mutations: 84-88insC (E60X) (72), R105X (77), 515T→A (M172K) (72), Y250X (71,85), Q317X (74), 1780-1791del (AVAQ 594-597) (75,73), and 2069A→C (Q690P) (76,86). Some of these mutations (E60X, R105X, Q317X) result in a transcription termination sequence, which blocks protein expression.

From what we know so far about this receptor's function, how its depletion or loss of activity may lead to increased intestinal iron absorption and iron overload is difficult to understand. However, its causal role in this condition has been demonstrated in *tfr2*-deleted mice, which also develop iron overload (87).

Two hypothetical mechanisms may explain the fact that TfR2 or HFE defects, both of them involved in iron entry into cells, may lead to iron overload through increased intestinal iron absorption. One of them is based on the role duodenal crypt cells play in the regulation of body iron (83). The other one relies on the role of HFE, hemojuvelin, and TfR2 in the regulation of hepcidin synthesis or function (74). One within the body, the passage of iron into cells is facilitated by TfR1s, which remain present in this disease (87).

HEREDITARY TYPE IV HEMOCHROMATOSIS. FERROPORTIN DISEASE

It has been known for years that people from the Solomon Islands commonly have abnormal iron overload (88). During the study of 81 living members of an extensive family of Melanesian descent 31 people were found to show evidence of iron overload. The fact that their disorder was inherited in an autosomal dominant fashion, and that HFE involvement could be ruled out stood out among these subjects' characteristics (88).

In 1999 Pietrangelo et al. (89) studied 53 members of an Italian family, some of which had iron overload in the absence of HFE gene mutations. Other authors have described similar families in various countries (The Netherlands, Canada, Italy).

These patients share common characteristics, which differ from those of patients with HFE-linked hemochromatosis. The disease is transmitted in an autosomal dominant pattern. Considerably high blood ferritin levels is a common feature in these patients, this increase being not paralleled by transferrin saturation. The latter is even normal or slightly high in a number of cases (89,90-92). Hemoglobin may be diminished in young women. This disproportion between serum ferritin rates and transferrin saturation is particularly noticeable in early disease. Liver biopsy confirms a big amount of iron within the liver, both inside hepatocytes and reticuloendothelial system cells, specifically Kupffer cells and macrophages located in portal spaces (90,92,93). During early disease iron preferentially settles in Kupffer cells, but siderosis increases in hepatocytes as the disorder progresses. The predominantly periportal location of hepatocyte siderosis that is common in classic hemochromatosis is not seen in such cases. Iron rather distributes itself evenly throughout the lobule. Anyway, this siderosis is well tolerated, and liver fibrosis is mild or nonexistant (90,91,93,94). Such iron overload may be treated using periodic bleedings, and tolerance may be normal. However, a number of patients do not tolerate bleedings and develop anemia even in the presence of high serum ferritin levels (90,92).

HFE gene mutations and iron overload secondary to other diseases were all excluded in patients with this conditions.

The study of the ferroportin 1 (FP-1) gene, also designated SLC11A3, IREG1, and MTP1, has revealed a number of mutations. Njajou et al. (95) found mutation 430A→C in exon 5 (N144H) in a Dutch family. Arden et al. (88), in members of a family of Melanesian descent, identified a similar mutation – 431A→C (N144T). In the extensive Italian family studied by Pietrangelo et al. (89), Montosi et al. (96) unveiled mutation A77D in exon 3. In turn, Wallace et al. (93), Devalia et al. (90), Cazzola et al. (97), and Reotto et al. (91) found a deletion of three base pairs (485_487delTTG) in a region of exon 5 containing three repeat TTGs in the populations of various countries. This mutation results in the loss of 1 of the 3 valines in positions 160 to 162 (V162del) at FP1. This valine triplet is maintained by most species (98), and is thus supposed to play a relevant role in iron binding or transportation. Other mutations reported include 190T→C (Y64N) (99), 774A→G (D177G), 850G→T (E182H), and 1272G→T (G323V) (100). Jouanolle et al. (92) found a mutation in exon 8 that results in a G490D change between FP1 helices 8 and 9. This change must disrupt the molecule's packing and structure. While Njajou et al. (95) suggested that these mutations would increase the functional capabilities of FP1, most other authors agree that they result in loss of function (88,96).

The gene coding for FP-1, located in chromosome 2q32, responds to inflammation, hypoxia, and iron demands by the bone marrow (101-103). Its mRNA has an IRE in region 5'-UTR (104). This element binds an IRP *(Iron-Regulatory Protein)*. The expression of this mRNA in the presence of iron is contrary to expectations for an mRNA having an IRE near the 5'-UTR end. In all mRNAs having an IRE in this area, the presence of iron determines increased expression (105). Such is the case of ferritin (18). In contrast, FP1 mRNA decreases its expression in the presence of iron, only to increase it under iron deficiency conditions (106). The cause of this abnormal behavior in FP-1 mRNA is unknown. FP-1 is located in cell membranes, piercing them in up to 9 different sites (90). While the above-mentioned mutations occur throughout the FP-1 molecule, most of them develop between the first and fourth transmembrane domains, in extracellular helices 1 and 3. FP1 has been presumed to es-

tablish functional relations with apotransferrin, ceruloplasmin or hephaestin via these helices (107). Their function is not accurately understood, but is thought to be essential in allowing iron release from RES cells. These cells play a primary role in the reuse of iron from old RBC destruction. The loss of its function determines iron retention in these cells, and thus potential iron scarcity regarding erythropoiesis. Secondary to this deficiency intestinal iron absorption would increase (108). Why this protein's malfunction impacts iron release from RES cells while not preventing iron passage through duodenal enterocytes remains unclear (109). It may possibly result from the fact that iron flow through RES is much more intensive than iron flow through enterocytes (110). Thus, a failure in FP1 would have a greater impact on RES *versus* the bowel.

While these patients have been usually treated with periodic bleedings, some authors have questioned their need. Although the extent of iron overload may be very significant, iron excess is generally well tolerated, and relevant medical injuries are usually few. Conversely, some patients do not tolerate bleedings (90,92) and develop anemia despite still elevated levels of serum ferritin. If bleedings are eventually used, caution and frequent hemoglobin and hematocrit monitoring are recommended.

OTHER HEREDITARY HEMOCHROMATOSES

Congenital atransferrinemia

In 1961 Heilmeyer et al. (111) described the case of a young woman with serious hypochromic anemia associated with generalized iron overload. Eight additional patients have been subsequently described in Slovakia (112), Japan (113), Mexico (114,115), France (116), Samoa Islands (117), and the USA (118). All these patients shared similar clinical features. They had severe hypochromic, sideropenic, refractory anemia since childhood in association with severe siderosis in the liver and other organs. Some of them were prone to infection (111), and two died from pneumonia (111,115). Blood iron is usually very low, but serum ferritin rates are highly elevated (2000-8000 μg/L). Blood transferrin (Tf) is very low or undetectable (118). In patients having undergone liver biopsy extensive hepatic siderosis was seen to compromise both hepatocytes and Kupffer cells (118). Varying degrees of liver fibrosis were found in some patients (117). Other organs may also be damaged by siderosis (myocardium, pancreas, thyroid, kidneys); in contrast, iron is absent from the bone marrow.

A similar condition was found in mice with atransferrinemia. A Tf gene mutation has been identified in these mice (119,120). Genetic studies available on this disease are few in human beings. The condition is transmitted with an autosomal recessive pattern. Beutler et al. (118) found two Tf gene mutations in a female patient – one in exon 5 and one in exon 12 (double heterozygote). The former was a 562_571 deletion followed by a 572_580 duplication, which represented a transcription interruption point. The second mutation was a 1429G→C (A477P) substitution, which probably determined the synthesis of an unstable Tf. A mutation 1180G→A (E394K) (121) was identified in other patient, and a homozygous mutation 229G→A in exon 3 (D77N) was identified in the Slovakian patient (122).

Tf functions to carry iron in the plasma and then deliver it to the erythron and other tissues. Tf absence determines the development of severe anemia from iron deficiency (113). Intestinal iron absorption and deposition in tissues increases as a result of anemia (118).

Treatment for this condition includes periodic i.v. infusion of apoTf (113) or standard fresh plasma (118). Bleedings prior to plasma infusion have been used to control iron overload (118), and deferrioxamine has also been administered (112).

Hereditary aceruloplasminemia

In 1987, Miyajima et al. (123) reported on a Japanese 52-year-old patient who has diabetes, retinal degeneration, extrapyramidal symptoms, and total lack of serum ceruloplasmin. Shortly afterwards similar cases were reported in Ireland and Japan (124,125), where blood ceruloplasmin (CP) was undetectable. The ceruloplasmin gene, located in chromosome 3q23-q24 (126), has 20 exons and codes for 1046-aminoacid protein (127). Various mutations associated with CP loss of function or plasma CP absence have been identified in this gene (128,129). Many other cases have been reported with this disorder ever since, most of them in Japan, but also in Caucasian populations (124,130-132). The disease has an autosomal recessive pattern of inheritance.

Genetic studies have uncovered numerous mutations, homozygous on occasion, double heterozygous in other instances. Mutations commonly result in transcription termination points, and thus the synthesized CP is abnormal, inactive, and degradation-bound (128,129,131-133). Harris et al. (128) found in their patient a 5-base pair insert that gave rise to a truncated protein. So was also the result of change G→A in the sequence corresponding to amino acid 991 as found by Yoshida et al. (129). In the case reported by Bosio et al. (131) two mutations were encountered. The first was a 436C→G (Q146E) change, and the other was an adenine insertion at position 2917. The latter induced transcription termination, and hence the production of a truncated protein in amino acid 983. The missing area just includes the sites where copper binds apoceruloplasmin, which then acquires ferroxidase activity. Okamoto et al. (133) also described an adenine insertion in exon 3, the area corresponding to amino acid 184, which translates into early transcription termination.

In the patient reported by Loréal et al. (132), these authors found a 2-base pair deletion in one allele's exon 11, which brought about a transcription termination signal (TGA) in codon 632. In the other allele, they found change 694T→A (TAT→TAA), which also results in transcription termination.

When symptomatic, these patients are usually in the fourth of fifth decade of life, and show neurologic changes including dementia, dysarthria, and dystonia (123-125). Retinal degeneration and insulin-dependent diabetes mellitus are common findings (125). All these manifestations result from iron deposition in the central nervous system, primarily in the base nuclei, retina, and pancreas β-cells. Liver biopsy commonly demonstrates the presence of intense siderosis (hepatic iron > 1.500 μg/g) compromising both hepatocytes and RES cells (134). Despite such iron overload and elevated serum transferrin levels, fibrosis or hepatocellular necrosis is uncommon for most patients. In contrast, hyposideremia, and normocytic, normochromic anemia are common, since iron is not bound by transferrin and cannot reach the bone marrow (123,134). Intestinal iron absorption increases secondary to anemia, as *hephaestin* –a molecule with a structure similar to ceruloplasmin and sharing the latter's iron oxidizing role– is found in enterocytes (135).

CP is essential for Fe^{2+} oxidation into Fe^{3+} (136,137), which allows iron to leave cells and then be transported by Tf to organs in need of it (138). In the absence of CP Tf cannot bind iron, and iron cannot leave RES cells (136,139,140), thus remaining confined in them. In addition, plasma unbound Fe^{2+} deposits itself in tissues (liver, pancreas, etc.), as occurs in atransferrinemia and classic hemochromatosis when Tf saturation reaches 100%. The expression of TfR1 and DMT1 diminishes in iron-laden hepatocytes, since their mRNAs have an IRE at their 3'-end whose expression is inhibited by iron through IRP (141). Neurologic lesions result from iron preferential deposition in astrocytes. CP cannot go across the blood-brain barrier, but is usually synthesized by said cells. In aceruloplasminemia astrocytes synthesize no CP, and iron is retained within these cells (142).

Aceruloplasminemia may be mistaken for Wilson's disease, but differs from the latter in that serum ferritin levels are very high –resulting from insulin-dependent diabetes– and magnetic resonance imaging shows excessive iron in base nuclei of the brain. It differs from hemochromatosis in that Tf saturation is low, as are serum iron rates. This differentiation is relevant to avoid bleedings. Some patients have received subcutaneous deferroxamin (2 g/day; 5 days/week) (132). This managed to decrease serum ferritin and liver iron deposition, as well as to stabilize diabetes; however, it had to be discontinued on occasion because of aggravated anemia. Iron deposits in the nervous system remained unaltered.

Iron overload associated with H-ferritin mutation (type V HH)

Only one Japanese family with iron overload resulting from a mutation of ferritin's H subunit has been reported so far (143). Subunit L mutations result in the hereditary hyperferritinemia-cataract syndrome, where no iron overload is present (144-146).

In 2001, Kato et al. (143) described a Japanese family with 4 out of 8 members suffering from hyperferritinemia. Moreover, hypersideremia increased transferrin saturation, and iron deposition in tissues were all demonstrated in some of them. Liver biopsy showed siderosis to be distributed around hepatocytes in lobular areas 1 and 2. Other causes of iron overload were ruled out, including HFE and TfR2 mutations, as well as aceruloplasminemia. The study of ferritin subunits L and H mRNA revealed the former to be normal, but the latter had a heterozygous point mutation at position 49, corresponding to the 5'-IRE loop, with an U replacing A (A49U). The study of genomic DNA showed a mutation 49A→T. This change was only found in family members with hyperferritinemia, and the mutation is thus seemingly transmitted in an autosomal dominant fashion. Such mutation in this area confers IRPs a higher affinity for IRE, and hence a suppression of H-ferritin mRNA translation. Conversely, IRP binds L-ferritin mRNA no as firmly as H-ferritin mRNA, which translates into an increased expression of the former subunit (143).

H-ferritin has ferroxidase activity, which is necessary for Fe^{3+} to be able to incorporate L-ferritin capsules (147). Mutation A49U conditions a decrease in the binding of iron to L-ferritin (143), and this metal's deposition in the cytoplasm of cells. H-ferritin-knockout mice die during embryo stages from excessive body iron accumulation (148). In the hyperferritinemia-cataract syndrome there is no iron overload despite severely increased L-ferritin levels (144). This likely due to the fact that H-ferritin is normal and retains its iron-oxidizing activity.

Iron overload in Subsaharian Africa (Bantu siderosis)

The fact that iron overload is very common among Subsaharian Africans has been known for over 70 years now (149,150). In some rural areas the frequency of this condition exceeds 10% of the total population (151). Iron overload has also been seen to be common in urban areas and among Afro-Americans (152,153). Historically this iron overload was attributed to the consumption of iron-rich food, specifically beer fermented in non-galvanized iron drums (154). However, it has been shown in recent years that besides this diet-related factor –currently nonexistant in African cities– a genetic non-HFE-related factor must be involved in the pathogenesis of this iron overload (151,154,155). Whereas heterozygotes for this

factor need an iron-rich diet for iron overload to develop, homozygotes may have this condition even in the absence of such foods. Studies performed in the Afro-American population reached this same conclusion (153). To this day no gene suspected of being involved in the pathogenesis of this disease has been identified.

Iron overload initially compromises RES cells, both in the liver and the marrow or spleen (155,156). Thus, during early disease liver biopsy shows that iron deposition preferentially involves Kupffer cells, with the typical iron gradient present in other hemochromatosis forms remaining unrecognized in this one (151). In later stages of disease iron also deposits itself inside hepatocytes, fibrosis is encountered in varying degrees, and cirrhosis or even hepatocellular carcinoma may develop (157). Furthermore, there is excess iron in the heart, lungs, spleen, and other organs (153,157). As in other iron overloads preferentially compromising the RES, transferrin saturation may be normal or slightly high. The characteristics of this iron overload resemble those of FP1-induced disease. A mutation in the FP1 gene has been recently identified in the Subsaharian and Afro-American populations, which may well explain this disease (158).

To conclude, new mutations in the genes coding for various proteins involved in iron metabolism and accounting for some non-HFE hereditary hemochromatosis have been identified in recent years. Besides HFE, these proteins include hepcidin, hemojuvelin, transferrin receptor 2, transferrin, ceruloplasmin, and ferritin H subunit. In upcoming years we shall no doubt witness the finding of new changes in other proteins to account for all hereditary hemochromatosis types. Also in upcoming years will insight be gained into the mechanisms of action of all these proteins.

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