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HFE mutations and iron in hemodialysis patients

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Abstract

Introduction: in chronic hemodialysis patients, a disruption in iron metabolism ranging from absolute to functional deficiency, with compartmentalization of this metal into macrophages, is often observed. Chronic inflammation indeed often causes an upregulation of the iron hormone hepcidin, thereby reducing iron absorption and availability to the erythron.

Methods: we systematically reviewed the literature on the role of genetic risk factors on iron metabolism in hemodialysis.

Findings: in this setting, mutations in the HFE gene of hereditary hemochromatosis may confer an adaptive benefit by decreasing hepcidin release, thus improving iron availability to erythropoiesis, anemia control, and the response to erythropoiesis stimulating agents and iron itself, and reducing the side effects of these therapies. The HFE protein together with Transferrin receptor-2 may also have a direct role on erythroid differentiation and iron uptake in erythroid cells. In addition, other genetic determinants of iron status, such as variants in Matriptase-2 (TMPRSS6), have been shown to influence iron metabolism in chronic hemodialysis patients, most likely acting through hepcidin regulation.

Discussion: although data must be confirmed in larger prospective studies, this favorable shift in iron metabolism balance possibly results in reduced mortality, in particular because of cardiovascular and infective diseases. Further genetic studies may offer a valuable tool to test these hypotheses and guide personalized clinical management and the research of new therapies.

Key words: Anemia, clinical nephrology, inflammation, iron overload, HFE

INTRODUCTION: IRON IN CHRONIC HEMODIALYSIS PATIENTS

Chronic hemodialysis treatment (CHD) is typically associated with alterations in iron metabolism, most frequently characterized by decreased circulating iron with reduced transferrin (TF) saturation (TS), resulting in reduced iron availability for the erythropoiesis. This situation is caused by chronic inflammation and/or blood losses, and plays a key role in the pathogenesis of anemia.[1,2] Conversely, hyperferritinemia related to inflammation with altered tissue iron compartmentalization is also frequently

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observed, which is associated with a greater risk of cardiovascular disease and mortality.[3] Indeed, inflammation and oxidative stress determine iron retention in macrophages, with induction of ferritin.[4,5] However, circulating ferritin concentration may also reflect to a certain degree body iron in CHD patients, so that in some patients truly increased body iron stores are observed.[6,7] Chronic intravenous iron supplementation to overcome resistance to erythropoiesis-stimulating agents (ESA) is a key determinant of iron accumulation, but there is a great interindividual variability in the response to therapies, even after accounting for inflammation levels. In fact, individuals who readily incorporate iron into hemoglobin require lower doses of iron and ESA, have less side effects, and may have a better prognosis.[8–10] Therefore, identification of the genetic determinants of iron regulation in CHD may reveal novel biomarkers for risk stratification and personalized clinical management and potential new therapeutic targets.[11]

OVERVIEW OF IRON METABOLISM AND IMPACT OF *HFE* MUTATIONS

Iron is an essential nutrient in human cells, playing a crucial role in vital biochemical activities, as an essential component of enzymes and other molecular complexes.[12–14]

The human body contains approximately 3–5 g of iron, the main part of which is employed to synthesize hemoglobin (Hb) circulating in red blood cells. Other iron-rich organs are the liver and muscles. Approximately 20%–30% of body iron is stored in hepatocytes and in macrophages, to a large extent within polymers of ferritin. A healthy individual absorbs daily 1–2 mg of iron from the diet, which is utilized to compensate nonspecific iron losses by desquamation of enterocytes and epidermis and, in childbearing aged women, by period. Erythropoiesis requires approximately 30 mg of iron per day, mainly provided by the recycling of iron via macrophages, which ingest senescent red blood cells and release iron, which binds to circulating transferrin (TF).

Iron uptake in the duodenum and the jejunum is mediated by a specific set of transporters; the most important for systemic regulation is named Ferroportin-1 (Fp-1), which allows the transport across basolateral membrane of enterocytes, macrophages, and hepatocytes. Ferric iron binds to plasmatic apo-TF to form ferric iron-TF complex, which is the major type of iron present in blood. The TF complex facilitates the transport of iron to cells that express TF receptors (TFR), including erythroid progenitors, and limits the ability of iron to generate toxic radicals.

Iron uptake in the cells occurs primarily by the endocytic pathway, which involves the interaction between TF and TFR. Two different TFR are known, namely TFR-1, which is found in all cells and shows an elevated affinity for circulating TF, and TFR-2, mainly expressed in the liver and in the hematopoietic cells, which binds the TF complex with a lower affinity.

Not all absorbed iron is utilized in metabolic processes, but it is partly stored as reserve, both for use when iron levels are low, and to prevent toxic effects of free iron in the cell. Under iron overload conditions, ferritin levels increase dramatically, particularly in liver.

Systemic iron homeostasis is achieved by modulation of the amount of iron absorbed in response to iron need and availability and erythropoiesis activity. Conversely, "inflammatory" regulators communicate signals in response to infection or inflammation, resulting in accumulation of iron in macrophages.

A small antimicrobial peptide synthesized by the liver, named hepcidin,[15] is the principal effector of the systemic modulation of iron metabolism, via its ability to bind Fp-1 on cellular surface blocking its iron transport activity, and to increase Fp-1 degradation.[16] In enterocytes, Fp-1 internalization on the basolateral surface causes the retention of absorbed iron with subsequent loss by desquamation, while the same process in macrophages causes the failure to release iron.[17] The final effect is the reduction of plasma iron availability. Hepcidin secretion is reduced in response to signals that cause an increase in iron release from cells, such as iron deprivation, and stimulus to erythropoiesis, whereas it is induced by iron overload or inflammation. Thus, hepcidin represents the common effector of the homeostatic regulation of intercellular iron fluxes in response to the iron stores, erythroid, and inflammatory regulators. It is still not yet entirely clear how systemic iron demand modulates hepcidin release by the liver. The transcription and secretion of hepcidin by the liver is regulated by a mechanism of body iron sensing and is finely regulated by a group of proteins, including the hereditary hemochromatosis protein called HFE, TFR-2, hemojuvelin (HJV), bone morphogenetic protein 6 (BMP6), Matriptase-2 (TMPRSS6), and TF (Fig. 1 A). Mutations in *HFE*, *TFR-2*, *HJV*, and the hepcidin gene (*HAMP*) are responsible for hereditary hemochromatosis (HH), a common iron overload disorder characterized by a deficit of hepcidin release or activity.[17,18]

HFE mutations represent the most frequent cause of HH in Caucasian adults.[19] The most common *HFE* mutation responsible for HH is a single nucleotide substitution that causes the substitution of a cysteine with a tyrosine at

position 282 (C282Y), leading to disruption of a disulfide bridge, protein mysfolding, and lack of expression on the plasma membrane. It is now clear that deletion of HFE specifically in hepatocytes leads to increased iron absorption and systemic iron overload, that is mediated by lack of hepcidin upregulation in response to circulating iron.[20] The homozygous genotype is very frequent in Caucasians, particularly in people from Northern Europe (frequency 1/300–400), whereas the prevalence decreases toward Southern Europe. A second and most frequent mutation is a substitution at position 63 of a histidine with an aspartate (H63D): this is a very common polymorphism in the general population, as 25%–30% of the population carries the H63D variant. The H63D can predispose to a milder form of iron overload in combination with the C282Y variant (C282Y/H63D compound heterozygosity), or more rarely in homozygosity (H63D/H63D), whereas it does not predispose to iron accumulation in the general population.[21] The penetrance of iron overload depends on age, gender, environmental factors, and on the role of the so-called modifier genes,[22] for example, the beta-thalassemia trait.[23,24] Many other molecules have been implicated in the regulation of hepcidin secretion, such as iron-regulated BMP6 ligand, a bone morphogenetic protein of the TGFβ superfamily and its receptors, small mother against decapentaplegic 1/5/8, HJV and TMPRSS6. Notably, a common mutation in *TMPRSS6* encoding for the A736V substitution is associated with iron status and erythropoiesis in the general population, and modulates the phenotypic expression of *HFE* mutations by influencing hepcidin secretion.[25–32]

IMPLICATIONS OF IRON STATUS IN CHD PATIENTS

In CHD patients, different types of situation related to iron-metabolism are observed: (1) absolute iron deficiency, due to decreased total body iron stores. This situation is common in CHD, due to low-grade but frequent blood losses. (2) Functional iron deficiency, when iron stores are normal or even increased, but the ESA-stimulated bone marrow needs more iron from TF than the iron output from tissue stores, resulting in ESA resistance. Hepcidin can aggravate functional iron deficiency by decreasing the release of stored and macrophage iron and intestinal iron absorption, through Fp-1 downregulation. The most severe form, historically termed 3) "reticuloendothelial blockage," which usually occurs in the setting of acute or chronic inflammation/infection. It can be considered as an extreme form of functional iron deficiency, and is associated with inflammation, low TS, but normal to very high levels of ferritin and tissue iron stores, which are locked by hepcidin and inflammatory cytokines. As a consequence, resistance to ESA and iron easily develops. Thus, the pattern of anemia, hyposideremia, ESA resistance, and high serum ferritin is frequently observed in CHD patients.

Furthermore, excess iron has also been implicated in the pathogenesis of the accelerated atherosclerosis by catalyzing oxidative stress in CHD.[33] In CHD patients, carotid intima media thickness and plaques were correlated with serum ferritin and oxidative stress and reduced plasma antioxidant activity,[6,34] and intima-media thickness was also associated with the dose of iron administered.[35] Furthermore, hepcidin and TNFα levels have also been correlated with vascular stiffness, another reliable predictor of cardiovascular events in CHD.[36] Even after adjustment for malnutrition and inflammatory biomarkers, severe hyperferritinemia, and the intravenous dose of iron were still associated with increased total and cardiovascular mortality.[37,38] Moreover, as iron is an important growth factor for invading pathogens, iron overload has been associated with an increased incidence and severity of infections.[39,40]

IMPACT OF HFE MUTATIONS IN CHD

A few studies have evaluated the impact of the major C282Y and H63D HFE mutations in CHD patients (Table 1).

The two common C282Y and H63D mutations, present in about one third of individuals of European descent, have been shown associated with baseline iron stores, lower requirement of erythropoietin to maintain stable Hb levels, and a trend to a lower requirement of iron supplementation in Italian CHD patients.[8] The relationship between iron parameters and *HFE* mutation in the setting of HD was confirmed by Fernandez et al., who showed increased ferritin levels in patients heterozygous for the C282Y after a 4 months treatment with parenteral iron.[45] Moreover, a possible role of the H63D mutation (when present in homozygosity) as a modulating factor of iron overload in end stage renal disease was also postulated.[46] These data suggest that in the context of CHD, characterized by "stressed" iron metabolism regulation, these genetic variants may not only influence circulating iron parameters and body stores, but also iron handling by macrophages after infusion, and iron availability to the erythron. Importantly, iron stores and the requirement of iron and ESA were comparable between patients carrying only the minor *HFE* H63D mutation and those positive

Table 1 Studies evaluating the impact of HFE mutations in CHD patients

Author, year	N =	Population	Ferritin levels	Outcomes	Main results
Bi et al.[41], 2013	560 CHD patients and 480 healthy controls	560 CHD patients and 480 healthy controls from four centers in North China	H63D homozygous subjects had significantly higher serum ferritin concentrations compared with wild-type individuals	C282Y/H63D mutations of HFE gene, iron status, CVD, and inflammation markers (CRP, IL-6, TNF-alpha) were evaluated	Association between H63D homozygous mutations and CVD in CHD patients. Elevated serum CRP, IL-6, and TNF-alpha levels also related to CVD in HD patients
Bloudickova et al.[42], 2014	1014 CHD patients and 2559 healthy controls	1014 HD patients and 2559 unrelated healthy Caucasians	—	Single-nucleotide polymorphisms (SNPs) in HFE were genotyped to evaluate genetic predisposition to renal disease	Higher frequency of HFE H63D and C282Y mutations in CHD patients than in healthy controls
Brown et al.[43], 2009	172 CHD patients	Patients on CHD for >90 days with no cause of anemia except chronic kidney disease	404 ug/L (275.5)	Erythropoietin and intravenous iron requirements in HD patients with HFE mutations (C282Y and H63D) were evaluated	No significant difference in hemoglobin or serum ferritin comparing patients with > or = 1 HFE mutations to those without mutations. Trend toward lower median weekly erythropoietin dose in patients with > or = 1 HFE mutation. No difference in median weekly intravenous iron dose
Canavese et al.[44], 2002	132 patients	132 patients (34 in peritoneal dialysis, 98 in CHD)	—	C282Y/H63D mutations of HFE gene, iron status, red cells parameters, EPO dosage, major CV events, and C-reactive protein as marker of chronic inflammation were evaluated in patients without iron therapy and after intravenous iron supplementation	No differences in baseline iron parameters in patients bearing mutations alleles nor after iron therapy. Increased prevalence of patients capable of maintaining normal hemoglobin level without EPO therapy in C282Y-mutated patients

Table 1 *Continued*

Author, year	N =	Population	Ferritin levels	Outcomes	Main results
Fernandez et al.[45], 2011	290 dialysis patients	290 dialysis patients in Gran Canaria, Spain	C282Y +/− patients (302 ng/mL) C282Y −/− patients (307 ng/mL) C282Y +/− patients and C282Y −/− patients after 4 months of parenteral iron (934 vs. 659 ng/mL)	C282Y mutation, red cells, and iron parameters were studied	Heterozygosity for the C282Y allele associated with differences in iron parameters in dialysis patients
Pelusi et al.[10], 2013	199 CHD patients and 188 healthy controls	199 CHD patients from Northern Italy and 188 healthy controls	265 ng/mL	C282Y/H63D mutations of HFE gene, A736V TMPRSS6 polymorphism, iron status, red cells parameters, EPO, and intravenous. iron dosage, serum hepcidin levels, and C-reactive protein as marker of chronic inflammation were evaluated	The combined HFE (presence or absence of HFE mutations) and TMPRSS6 A736V genotypes influence serum hepcidin levels independently of ferritin and C-reactive protein in CHD patients
Pericole et al.[46], 2005	201 CHD patients	201 CHD patients from Brazil	C282Y negative (744 ng/mL) C282Y heterozygous (207 ng/mL) H63D negative (703 ng/mL) H63D heterozygous (1005 ng/mL) H63D homozygous (2493 ng/mL)	C282Y/H63D mutations of HFE gene, iron, and red cells parameters were evaluated	No differences observed in biochemical parameters between C282Y HFE heterozygous and HFE normal patients with renal failure Possible role of H63D mutation as a modulating factor of iron overload in renal failure when present in homozygosity
Valenti et al.[6], 2007	63 CHD patients	63 CHD patients from Northern Italy	—	HFE and MnSOD genotype were tested. Iron parameters and vascular damage (intima-media thickness, carotid, and femoral arteries plaques) were assessed.	Hyperferritinemia, favored by HFE mutations, represents a risk factor for advanced cardiovascular damage in CHD patients

Table 1 *Continued*

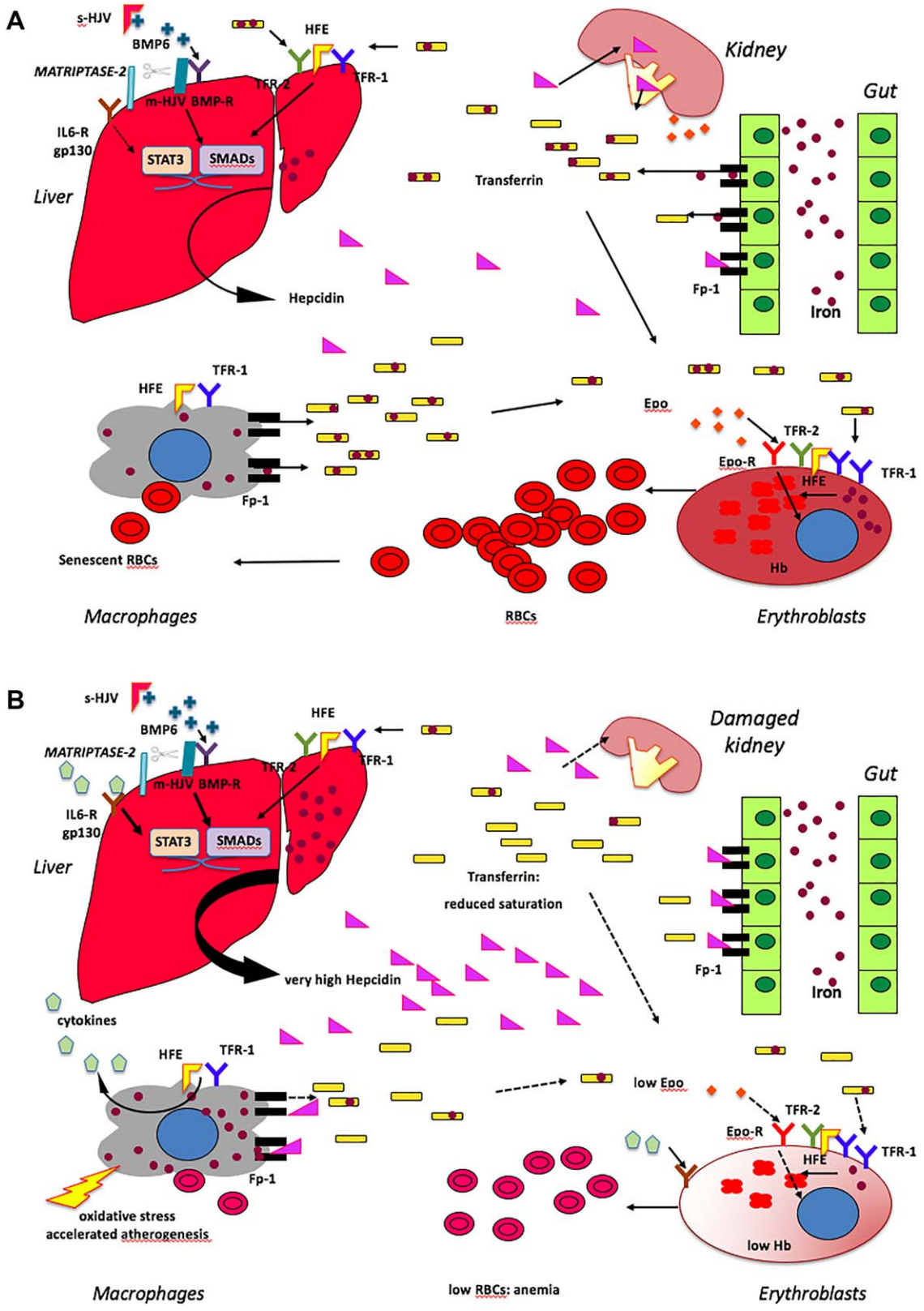
Author, year	N =	Population	Ferritin levels	Outcomes	Main results
Valenti et al.[8], 2008	96 CHD patients	96 CHD patients from Northern Italy evaluated at enrolment and followed prospectively for 3 years	Patients positive for HFE mutations (617 ng/mL) Patients negative for HFE mutations (423 ng/mL)	HFE genotype was tested, iron, and red cells parameters were monitored. Epo and iron requirements were evaluated	HFE mutations reduce the amount of Epo and iron necessary to support erythropoiesis in CHD patients
Valenti et al.[9], 2009	65 CHD patients and 57 healthy controls	65 CHD patients from Northern Italy and 57 healthy controls	236 ng/mL (128–410)	HFE genotype was tested. Serum hepcidin levels, iron, and red cells parameters were measured	Serum hepcidin levels (regulated by iron stores and inflammation) are increased in CHD patients and relatively reduced in subjects carrying HFE mutations

CHD = chronic hemodialysis; CVD = cardiovascular disease; CRP = C reactive protein; IL-6 = interleukin 6; TNF-alpha = tumor necrosis factor alpha; MTHFR = methylenetrahydrofolate reductase; Epo = erythropoietin; MnSOD = manganese-dependent superoxide dismutase.

for the more severe variant C282Y or homozygous for the H63D mutation. Therefore, it can be speculated that chronic inflammation and blood losses typical of CHD provide enough environmental pressure to magnify subtle alterations in cellular iron handling also in carriers of the milder and more common H63D mutation, which thus reach clinical significance, unlike to what it is observed in general population.[21] In keeping, Brown et al. confirmed a trend toward lower median weekly erythropoietin, but not in intravenous iron dose in patients carrying at least one mutated allele in the *HFE* gene.[43] Furthermore, Canavese et al. observed an increased prevalence of patients capable of showing Hb levels within the normal range without erythropoietin therapy in C282Y-mutated patients.[44] Therefore, *HFE* mutations may attenuate the effects of inflammation on iron metabolism, including reduced iron absorption, iron sequestration in macrophages, and erythroblast resistance to erythropoietin, and protect against iron-related damage by favoring the delivery of intravenously administered iron to the erythron.

The presence of *HFE* mutations was also possibly associated with a reduced mortality. Indeed, in carriers of *HFE* mutations, we also observed a lower mortality due to sepsis, previously associated with a higher iron dosage.[39,47] A lower mortality due to cardiovascular disease, possibly linked to hypertension and thromboembolic events related to erythropoietin[48,49] or to oxidative stress related to iron,[6] was also observed in CHD patients carrying *HFE* mutations.[14]

The relationship between *HFE* mutations and protection from cardiovascular risk in CHD patients remains however controversial, since it has been demonstrated that increased ferritin levels associate with the presence of *HFE* mutations and represent a risk factor for advanced cardiovascular damage in CHD patients[6]. Bi et al. also highlighted the association between H63D mutation, when present in homozygosity, and cardiovascular disease in a large cohort of CHD patients from China.[41] It is, therefore, possible that the impact of *HFE* genotype depends on body iron stores: when increased iron stores accumulate in carriers of at risk genotypes, these can outweigh the possible benefit on iron inflammation and iron utilization for erythropoiesis. As for mortality, a recent cross-sectional study carried out among more than 1000 patients affected by end-stage renal disease pointed out controversial findings. The authors report in fact significant differences in the frequencies of *HFE* H63D and C282Y genotypes between HD patients and healthy controls, possibly suggesting an unfavorable impact of these polymorphisms on renal failure development. A bias in patients' selection however could have occurred, since



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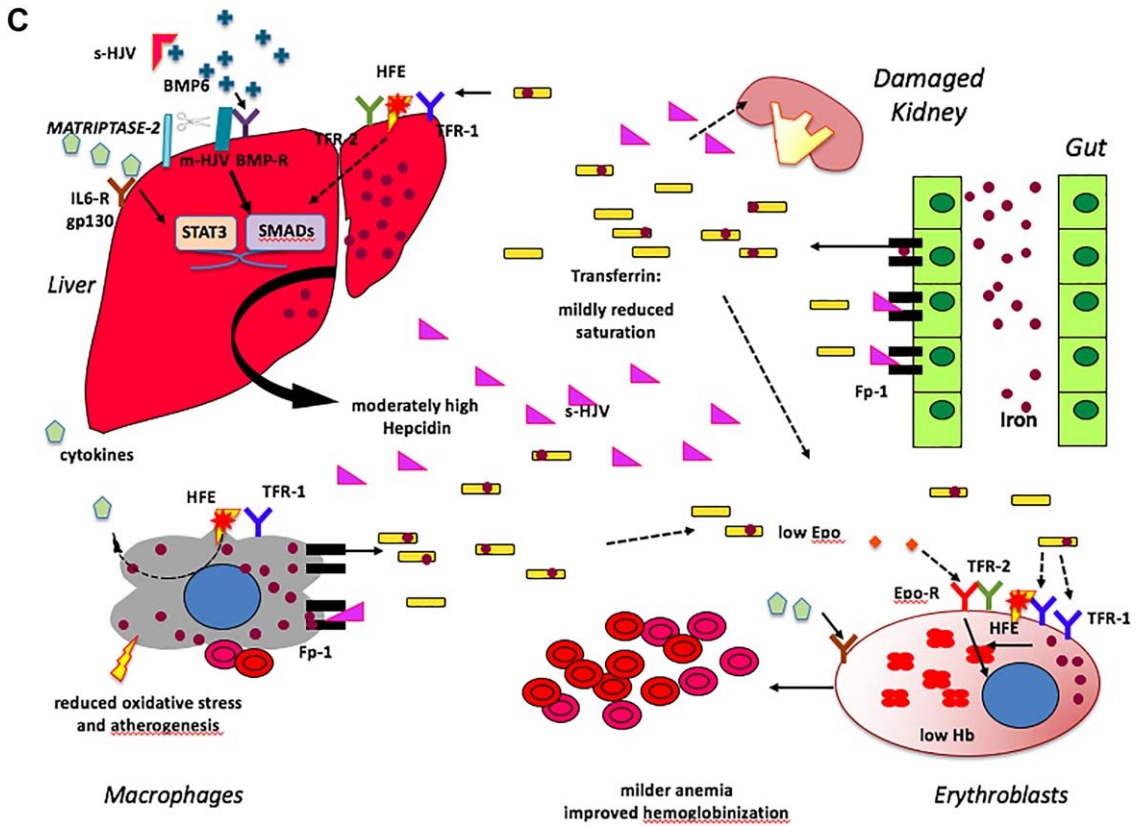

Figure 1 Mechanism by which HFE mutations impact iron metabolism and erythropoiesis in chronic hemodialysis patients. Role of mediators implicated in iron homeostasis and impact of *HFE* protein mutations in renal failure. (A) *HFE* wild-type, healthy controls; (B) *HFE* wild-type, in chronic renal failure; (C) *HFE* mutated, in renal failure. Bone morphogenetic protein 6 (BMP-6) binds to the co-receptor membrane hemojuvelin (m-HJV) and to the bone morphogenetic protein receptor (BMP-R) on the membrane of hepatocytes. This promotes phosphorylation of small mother against decapentaplegic (SMAD) proteins. After nuclear translocation, these initiate hepcidin transcription. An alternative pathway leading to hepcidin expression involves Interleukin (IL)−6/gp130 signaling via signal transducer and activator of transcription 3 (STAT3) nuclear translocation. The proteolytic processing of membrane-HJV by matriptase-2 (TMPRSS6) leads to negative regulation of the BMP-HJV-hepcidin pathway. Further negative regulation of hepcidin transcription is achieved by soluble-HJV, acting as an antagonist of the BMP pathway by competing with m-HJV for BMP-6 binding. The hepcidin signaling pathway is moreover regulated by HFE, which binds either to transferrin receptor 1 (TFR-1) or transferrin receptor 2 (TFR-2) depending on serum transferrin saturation: in the presence of a high transferrin saturation HFE dissociates from TFR-1 and binds TFR-2 forming an iron sensing complex influencing hepcidin expression via SMAD/ERK signaling. Liver-secreted hepcidin secreted binds to the extracellular region of ferroportin 1 (Fp-1) on the basolateral membrane of duodenal enterocytes leading to Fp-1 internalization, ubiquitination and degradation, and consequently hampered intestinal iron absorption. EPO = erythropoietin; Epo-R = erythropoietin receptor; s-HJV = serum hemojuvelin; TS = transferrin saturation.

subjects affected by renal failure carrying *HFE* mutations might be the ones showing long term survival in CHD,[42] which could provide an alternative explanation for the enrichment of at risk genotype in this setting.

In the hypothesis that altered regulation of hepcidin release by iron stores might explain the apparent protective role of the *HFE* mutations on cardiovascular complications and on the response to eryrthopoietin,[9] it was next investigated whether the effect of *HFE* gene mutations on hepcidin-25, the active form of the hormone, could be involved in the pathophysiology of the alterations of iron metabolism and anemia. Increased levels of hepcidin-25 were observed in CHD patients compared to healthy controls,[8,9,50] and a preserved regulation of hepcidin-25 by iron stores and inflammation in CHD, as demonstrated by the very strong correlation with serum ferritin and CRP levels.[51] Interestingly, there was also a negative correlation between hepcidin-25 and

serum iron, and in a subgroup of patients with stable disease, selected to avoid the confounding effect of the frequent presence of acute inflammation, blood losses, cancer, and recent variation in the dosage of therapy, hepcidin-25 negatively correlated with hemoglobin levels. Since anemia and hyposideremia should rather decrease hepcidin levels, these findings suggest that hepcidin-25 plays a causal role in determining anemia by reducing iron availability to the erythron, thus implying that in CHD excessive iron administration may paradoxically hamper iron utilization for erythropoiesis by increasing hepcidin, and that the effect of inflammation on altered iron metabolism and erythropoiesis may be partly mediated by increased hepcidin levels. As a consequence, pharmacological downregulation of hepcidin may be beneficial for anemia control in CHD.[52]

However, the protective effect of *HFE* mutations might not be limited to enhanced erythropoiesis due to decreased hepcidin levels. Indeed, HFE is expressed in erythroblasts, where it plays a role in the regulation of erythroid differentiation, and HFE deficiency is associated with increased erythropoiesis partly due to enhanced iron absorption, and partly due to a direct effect of HFE on the modulation of iron uptake in erythroid cells.[53] Moreover, TFR-2 is associated to the erythropoietin receptor (EpoR) in the EpoR complex in erythroblasts, and is required for efficient erythroid differentiation and erythropoiesis.[54] These data indicate that the TFR-2/HFE complex is directly involved in the regulation of erythropoiesis independently of hepcidin levels, and thus that genetic variations, or pharmacological modulation, of HFE and TFR-2 may influence the production of red blood cells and anemia in conditions characterized by reduced iron availability, such as CHD (Fig. 1 B-C).

OTHER GENETIC FACTORS: ROLE OF TMPRSS6

The *TMPRSS6* gene encodes for Matriptase-2, a membrane-bound protease that downregulates hepcidin transcription by cleaving hemojuvelin. Rare loss-of-function germline mutations of TMPRSS6 cause iron-refractory iron-deficiency anemia, characterized by very high hepcidin levels, whereas the common rs855791 polymorphisms leading to the p.A736V substitution is an important determinant of iron status in healthy subjects. In particular, the p.736V allele has been associated with lower serum iron, higher hepcidin levels, and decreased Hb in the general population.[25,32] Moreover, it has been demonstrated that the p.A736V polymorphism influences iron overload in hereditary hemochromatosis and nonalcoholic fatty liver disease.[27,28]

Since hepcidin is one of the major determinants involved in the pathogenesis of anemia of CHD, the role of the TMPRSS6 A736V polymorphism on erythropoiesis and iron parameters per se and in combination with HFE was evaluated in this subset of patients.[10]

Indeed, in CHD patients the A736V TMPRSS6 polymorphism modulates serum hepcidin, being the TMPRSS6 736V variant associated with higher levels of the iron hormone.

The combined HFE (presence or absence of *HFE* mutations) and TMPRSS6 A736V genotypes also played a role in this respect: patients negative for *HFE* mutations had higher hepcidin than patients with 736A/A and positive for *HFE* mutations. Furthermore, in patients carrying *HFE* mutations, those with the 736V/V genotype showed higher hepcidin than those with the 736A/A genotype. Importantly, *HFE* and A736V TMPRSS6 genotypes also predicted serum hepcidin independently of inflammation.

In line with previous results, in patients without acute inflammation and overt iron deficiency the 736V TMPRSS6 variant was also associated with higher erythropoietin maintenance dose.

All these findings validate the role of *HFE* on iron metabolism in CHD, but also show the importance of other genetic factors such as *TMPRSS6* per se and in combination with *HFE*. Evaluation of the effect of the *TMPRSS6* genotype on clinical outcomes in prospective studies in CHD may be in fact useful to predict the outcomes of hepcidin manipulation, and to optimize anemia management and treatment in these patients.[10]

CONCLUSIONS

The CHD setting is characterized by chronic inflammation and increased hepcidin levels with consequent reduction in iron absorption, recycling, and availability. This frequently leads to severe anemia, impairing the response to Epo and iron therapy.

The common polymorphisms C282Y and H63D of the *HFE* gene play a major role in iron metabolism: these genetic factors act by hampering hepcidin upregulation in hepatocytes in response to increased iron stores, thereby leading to reduced serum hepcidin. However, in CHD patients *HFE* mutations may cause an adaptive benefit by decreasing hepcidin release in response to iron and inflammation, thus improving iron availability to erythropoiesis, anemia control, and the response to ESA and iron. This favorable shift in iron metabolism balance may possibly result in mortality reduction, in particular

because of cardiovascular and infective causes. Evidence is also accumulating that *HFE* mutations directly promote erythroblast maturation and hemoglobinization independently of hepcidin.

Other genetic determinants such as the *TMPRSS6* A736V per se or in combination with *HFE* mutations have also been shown to influence iron metabolism in CHD patients, most likely acting through hepcidin regulation.

Despite these findings, iron metabolism in chronic hemodialysis patients remains a controversial topic, whose main aspects are still under definition. In fact, most of the currently available studies show some limitations often due to limited sample size and lack of well-characterized multicenter cohorts with long time follow-up and non-European populations evaluation.

Further studies are, therefore, needed to clarify the role of genetic determinants and hepcidin regulation on iron metabolism in CHD patients in order to develop new approaches to optimize anemia management, and to guide treatment personalization.

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