Ferroportin Disease (FD) is an autosomal dominant hereditary iron loading disorder associated with heterozygote mutations of the ferroportin-1 (FPN) gene. It represents one of the commonest causes of genetic hyperferritinemia, regardless of ethnicity. FPN1 transfers iron from the intestine, macrophages and placenta into the bloodstream. In FD, loss-of-function mutations of FPN1 limit but do not impair iron export in enterocytes, but they do severely affect iron transfer in macrophages. This leads to progressive and preferential iron trapping in tissue macrophages, reduced iron release to serum transferrin (i.e. inappropriately low transferrin saturation) and a tendency towards anemia at menarche or after intense bloodletting. The hallmark of FD is marked iron accumulation in hepatic Kupffer cells. Numerous FD-associated mutations have been reported worldwide, with a few occurring in different populations and some more commonly reported (e.g. Val192del, A77D, and G80S). FPN1 polymorphisms also represent the gene variants most commonly responsible for hyperferritinemia in Africans. Differential diagnosis includes mainly hereditary hemochromatosis, the syndrome commonly due to either HFE or TfR2, HJV, HAMP, and, in rare instances, FPN1 itself. Here, unlike FD, hyperferritinemia associates with high transferrin saturation, iron-spared macrophages, and progressive parenchymal cell iron load. Abdominal magnetic resonance imaging (MRI), the key non-invasive diagnostic tool for the diagnosis of FD, shows the characteristic iron loading SSL triad (spleen, spine and liver). A non-aggressive phlebotomy regimen is recommended, with careful monitoring of transferrin saturation and hemoglobin due to the risk of anemia. Family screening is mandatory since siblings and offspring have a 50% chance of carrying the pathogenic mutation.

Introduction

The name Ferroportin Disease (FD) refers to a clinical entity that differs from all other known forms of hereditary iron overload, including hemochromatosis (HC) [synonymous for hereditary hemochromatosis (HH)], i.e. the syndrome due to either HFE or non-HFE hemochromatosis gene mutations. In humans, a number of genetic disorders associate with systemic iron overload (Table 1) while others are caused by iron misdistribution and are associated with the regional accumulation of iron in subcellular compartments (e.g. mitochondria in Friedreich ataxia) or certain cell types and organs (e.g. basal ganglia in neuroferritinopathy) (Table 1). In strict terms, the latter disorders may not all qualify as true iron-overload states, as the total body iron content may not be increased. Ferroportin Disease (phenotype MIM number 606069, gene/Locus; MIM number 604653; https://www.omim.org/entry/606069?search=ferroportin%20dis-
ease&highlight=ferroportin%20disease) is due to pathogenic (usually missense) mutations of the ferroportin1 gene (FPN1; SLC40A1) which encodes the only iron exporter so far identified in mammals,2,4 lack-of-function mutations impair the iron-export capability of FPN1, particularly in cells with high iron turnover, such as tissue macrophages. Unlike the mutations causing FD, other rare mutations of FPN1 (such as N144H, C326Y, C326S and C326F),5,6 do not impair protein expression at the cell membrane or its iron export capability, but make FPN1 resistant to the inhibitory effect of hepcidin, the physiological FPN1 inhibitor (see below under Pathogenesis). This causes unchecked iron export activity of FPN1; the resulting clinical disorder is different from FD and indistinguishable from other forms of hereditary HC (Tables 1 and 2).

**Definition and classification**

The OMIM database classifies the two forms of hereditary iron overload due to FPN1 mutations within the same taxonomic category as “hemochromatosis type 4” (https://www.omim.org/entry/606069?search=ferroportin%20disease). Similar terminology has then been adopted by Orphanet with the inclusion of two subcategories: hemochromatosis type 4A (referring to classic FD due to lack-of-function FPN1 mutations) and hemochromatosis type 4B (referring to FD due to gain-of-function FPN1 mutations) (https://www.orpha.net/consort/cgi-bin/OC_Exp.php?Lng=EN&Expert=139491). These classifications have been incorporated into recent publications, with some variants.8,9 Disease naming and classification (taxonomy) can vary depending on different criteria, such as pathogenic genes, mechanisms, clinical manifestations, etc. Ideally, disease taxonomy (and names) should also help clinicians to recognize, diagnose, and cure diseases. In this context, the taxonomy adopted by OMIM and Orphanet, by embracing two pathogenically and clinically different disorders caused by mutations in the same gene under the term “hemochromatosis”, may fail to reach those objectives. Over the past decades, the term hemochromatosis has been inconsistently used in the literature and in clinical practice to imprecisely refer to: i) any form of body iron overload; ii) tissue iron overload causing organ damage and disease; iii) genetically determined iron overload; and, recently, iv) HFE-related iron overload.11 Recent discoveries in the field have shown that, regardless of the underlying genetic defect, a number of hereditary iron loading disorders (i.e. those due to loss-of-function mutations of HFE, TFR2, HVJ, HAMP and gain-of-function mutations of FPN1) belong to the same syndromic entity as they share the pathogenic basis (lack of hepcidin function-activity), biochemical expressivity (high transferrin saturation and high serum ferritin), liver pathology features (iron accumulation in parenchymal cells with iron-spared Kupffer cells until late stage), damage and disease of distinct target organs (liver, heart, endocrine glands, joints), and the therapeutic approach with optimal response to phlebotomy.12 As discussed in the following sections, each individual feature reported above is different in classic FD. Therefore, using the term “hemochromatosis” for the classic FD or the term “Ferroportin Disease” for FPN1-associated HC, is misleading, particularly for clinicians, since clinical suspicion, diagnostic strategy and management differ profoundly. Based on these considerations, and on our present understanding of the pathogenesis and clinical manifestations of these disorders, it is proposed that the disorder due to lack-of-function mutations of FPN1 is termed “Ferroportin Disease”, as originally described,1 and the disorder due to gain-of-function FPN1 mutations is termed “FPN-1 associated hemochromatosis” (Table 1). Instead, in analogy with other protein-related classifications (e.g. ferritinopathies; hemoglobinopathies), both disorders due to lack- and gain-of-function mutations of FPN1 may well be included in a broader taxonomic category named “ferroportinopathies”.

**Historical aspects**

In 1996, the HFE hemochromatosis gene, whose C282Y homozygote mutation is responsible for most cases of HH in Caucasians, was identified.12 Soon after, it became apparent that not all hereditary iron overload disorders could be explained by HFE mutations, particularly in Southern Europe, where an active search for other genes linked to genetic iron overload flourished. From 2000 to 2004, all known non-HFE genes associated so far with HC, namely transferrin receptor 2 (TFR2),13 FPN1,14 hepcidin (HAMP),15 and hemojuvelin (HVJ)16 were identified. A few years earlier, in 1999, a distinct and somehow unusual phenotype had been reported in a large family with hereditary iron overload from Italy: Selective iron

| Table 1. Human hereditary disorders associated with iron overload and iron mis-distribution. |
|-------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Disorder/cause | Iron overload Pattern of iron accumulation | Disorder/cause | Iron mis-distribution Pattern of iron accumulation |
| Ferroportin Disease | Hereditary hemochromatosis (HFE-TFR2-, HJV, HAMP, FPN1-associated) | X-linked sideroblastic anemias | Systemic (mitochondria) |
| Acenuloplasminemia | Systemic (preferential iron accumulation in macrophages) | Friedreich ataxia | Systemic (mitochondria) |
| Atransferrinemia | Systemic | Neuroferritinopathy | Regional (brain) |
| DMT-1 deficiency | Regional (mainly liver) | Systemic | |
| Hereditary iron-loading anemias with inefficient erythropoiesis | Systemic (early iron accumulation in hepatocytes due to increased iron absorption) | | |

Ferroportin Disease
loading of liver macrophages, hyperferritinemia co-existing with normal-low transferrin saturation, and tendency to anemia after intense phlebotomy were the hallmarks of the disorder. In 2001, all affected family members were reported to be heterozygous for a c. 230 C → A substitution resulting in the replacement of alanine 77 with aspartate in FPN1. This entity was subsequently named FD. On the other hand, FPN1-related HC, due to a gain-of-function mutation of FPN1 (p.N144H), was first reported by Njajou et al. in 2001. Yet, it is worth mentioning that the first clinical description of an “autosomal dominant” form of classic HC had been already reported by Eason et al. in a Melanesian kindred in 1990. In this same population, Arden et al. have later linked the HC phenotype to the N144T gain-of-function mutation of FPN1.

Ferroportin biology and physiology and FD

FPN1, the product of the FPN1 (SLC40A1) gene, transfers iron from the external milieu (i.e. maternal blood or intestinal lumen) and from internal sites of iron storage and recycles it into the bloodstream. In fact, it is highly expressed in liver and spleen macrophages, the luminal site of enterocytes and placental syncytiotrophoblasts.

FPN1 is regulated at different levels by a number of factors, including transcriptionally by heme, translationally by the iron-regulatory proteins (IRPs), and posttranslationally mainly by hepcidin, the iron hormone. Hepcidin is produced by the liver in response to iron, inflammation, and a variety of stressors. Hepcidin binds to the extracellular loop of FPN1 and triggers its ubiquitylation on lysine residues located in the intracellular domain leading to internalization and degradation in lysosomes. This mechanism allows a finely-tuned control of iron efflux from enterocytes and macrophages toward the bloodstream when more iron is needed during active erythropoiesis (in this case, hepcidin synthesis is inhibited by erythropoietic signals, or blood iron must be controlled due to pathogen proliferation/growth or incipient iron overload (here hepcidin synthesis is induced by inflammatory or iron mediators, respectively) (reviewed by Drakesmith, Nemeth and Ganz).

Table 2. Main features of Ferroportin Disease and other hereditary iron overload disorders in humans.

| Disorder | Affected gene (symbol / location) | Known or postulated gene product function | Epidemiology | Genetics | Mechanism for increased cellular iron deposits | Clinical onset (decade) | Main clinical manifestation | Clinical course |
|----------|----------------------------------|------------------------------------------|--------------|----------|-----------------------------------------------|-------------------------|----------------------------|-----------------|
| I. Ferroportin Disease | Solute carrier family 40 (iron-regulated transporter), member 1 (SLC40A1 / 2q32) | Iron exporter from cells including macrophages, enterocytes, syncytiotrophoblasts | Affects Caucasians and non Caucasians | Autosomal recessive | Increased iron retention due to decreased iron export | Any | • Liver disease | Mild |
| II. Hemochromatosis | Hemochromatosis gene (HFE / 6p21.3) | Hepcidin regulator | Affects Caucasians of North European descent | Autosomal recessive | | 4th-5th | | |
| | Transferrin-receptor 2 (TIR2 / 7q22) | Hepcidin regulator | Affects Caucasians and non Caucasians | Autosomal recessive | | | | |
| | Solute carrier family 40 (iron-regulated transporter), member 1 (SLC40A1 / 2q32) | Iron exporter from cells including macrophages, enterocytes, syncytiotrophoblasts | Affects Caucasians and non Caucasians | Autosomal recessive | | | | |
| | Hepcidin antimicrobial peptide (HAMP / 1q9.13.1) | Degradation of ferroportin and downregulation of iron efflux from cells | Affects Caucasians and non Caucasians | Autosomal recessive | | | | |
| | Hemojuvelin (HJV / 1p12) | Hepcidin regulator | Affects Caucasians and non Caucasians | Autosomal recessive | | | | |
| III. Aceruloplasminemia | Ceruloplasmin (CP / 3q23-q25) | Iron efflux from cells | Affects Caucasians and non Caucasians | Autosomal recessive | Decreased iron efflux | 2nd-3rd | • Neurological manifestations | Severe |
| IV. A (hypo)transferrinemia | Transferrin (TF / 5q21) | Iron transport in the bloodstream | Affects Caucasians and non Caucasians | Autosomal recessive | Increased iron influx | 1st-2nd | Anemia | Severe |
tions (Figure 1A). The selective binding of hepcidin to the outer-facing conformation would, therefore, guarantee that FPN1 degradation can occur only when intracellular iron is abundant and actively pumped through the channel (Figure 1B). Recently, the crystal structures of a bacterial homolog of FPN1, BbFPN, has been resolved in both the outward- and inward-facing states, and a homology model with human FPN1 has been developed. According to the Au, FPN1 has 12 TM helices, as previously predicted, and is divided into two halves, one forming the N lobe, the other the C lobe connected by a long cytosolic loop, with a central cavity between the lobes that is open towards the extracellular side and not accessible from the intracellular side (Figure 1A). FPN1 undergoes an intra-domain conformational rearrangement during the transport cycle. When hepcidin enters the central cavity between the N and C lobes, and interacts with the hepcidin-binding site located on the C lobe, it elicits two effects: a) it increases the accessibility of the intracellular loops that harbor the ubiquitination sites to the ubiquitin ligases; and b) it arrests the conformational transition of FPN1 from the outward-facing state to the inward-facing state, inhibiting the access of iron from the cytoplasm to the substrate-binding site within the intracellular gate (Figure 1B).

Molecular pathogenesis

The general pathophysiological basis of FD is well defined and relies on the impaired iron export from the iron storage/recycling site (particularly macrophages) towards the bloodstream. Figure 2 shows the basic iron transport defect of the FD as opposed to FPN1-associated

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Figure 1. Biology of ferroportin and postulated pathobiology of Ferroportin Disease (FD). (A) Structure-function relationship of iron-export ferroportin activity. Putative mechanisms of hepcidin binding to FPN and its degradation. (C) Postulated basis for FD. (Upper panel) In cells undergoing relatively low iron flux, such as enterocytes, the product of the FPN wild-type allele is able to reach the plasma membrane and export iron. For clarity, mutated FPN1 was not depicted at the cell surface: based on previous in vitro work, it has been postulated that some mutant FPN1 can still reach the cell surface and preserve some iron-transport competence, but this is still controversial. (Lower panel) In cells undergoing high iron turnover, such as macrophages, increased requests for iron export impose high demands on FPN traffic leading to a ‘traffic jam’ within the endocytic/plasmamembrane and degradation compartments and inappropriately low wild-type allele product targeting to the cell membrane. (D) Postulated effect of FPN mutations that affect formation of the intracellular gate and access to the iron binding site.
that are exposed such as enterocytes (Figure 1C).54 On the contrary, in cells mutated and degraded upon exposure to hepcidin. Based on these observations, the basic defect can be addressed in the context of the autosomal dominant trait of FD. However, other studies from different groups have provided experimental evidence to support the opposite conclusion and showed that Fpn1 is a monomer in cultured cells62,59,72,73 and in vivo.50 More recently, Sabelli et al.54 using for the first-time cultured macrophages from FD patients, found that endogenous FPN1 shows a similar localization to that in donor macrophages, except for greater accumulation in lysosomes, suggesting a higher degradation rate of mutant FPN1. Unexpectedly, and contrary to previous studies using over-expressed mutant protein in cell lines, FPN1 in FD macrophage circulates in the early endocytic compartment, does not multimerize, it reaches the plasma membrane, is iron-transport competent (although to a lesser extent than normal macrophages), and is promptly internalized and degraded upon exposure to hepcidin. However, when FD macrophages are exposed to large amounts of heme iron, in contrast to donor macrophages, FPN1 can no longer reach the cell surface, leading to marked intracellular iron retention. Based on these observations, a model of FD has been proposed in which FPN1 monomers, in spite of the fact that half proteins are mutated, can still reach the cell surface and export iron in cells that are exposed in vivo to a relatively low flux of iron, such as enterocytes (Figure 1C).54 On the contrary, in cells undergoing high iron turnover in vivo, such as tissue macrophages, sufficient FPN1 is prevented from reaching the plasma membrane, possibly due to a ‘traffic jam’ in the degradation and/or endocytic cycling pathways. This model is consistent with the clinical manifestation of FD characterized by early iron accumulation in hepatic Kupffer cells and normal transferrin saturation, indicating that mutant FPN1 activity does not limit intestinal iron transfer; the latter becomes critically low in young females at menarche or after aggressive phlebotomy, when high iron demands for erythropoiesis likely impose increased FPN1 traffic/cycling within tissue macrophages.5,57 (See under Clinical Manifestations and Diagnosis and Treatment). This study did not address the question as to whether mutant or only wild-type FPN1 reaches the plasma membrane and whether mutant-FPN1 is transport competent. Previous studies have not been conclusive. Exogenously expressed p.A77D and p.Val162del FPN1 mutants have been found to be iron-transport incompetent in all studies, but able to reach the cell membrane in some,49,50,54,41,45 and not in others.10,31 The p.G80S FPN1 mutant has been localized at the cell surface in two published studies,50,55 and found iron transport competent in one49 and incompetent in the other.48

According to Taniguchi et al.,39 the mutation sites associated with FD are mainly mapped onto the inter-lobar interface, mostly on the intracellular side, and form the intracellular gate. These mutations would, therefore, destabilize the inter-lobar interactions, thereby affecting the stable formation of the intracellular gate and reducing the iron transport activity of FPN1 (Figure 1D). It is possible that different mutants differently affect iron transport capability of FPN1; while this may be better overcome by the normal allele product in cells with low iron turnover such as enterocytes or hepatocytes, it may be further hampered in cells like macrophages where the additional ‘traffic jam’ in the endocytic-plasmamembrane compartment will aggravate the basic defect (see above).

Genetics and epidemiology

A list of published mutations associated with FD and FPN1-related HC is reported in Table 3.5-8,17,43,56-102 Numerous mutations of the FPN1 gene have been identified so far in probands with the classic FD phenotype of French-Canadian, Melanesian, Thai, Japanese and European heritages. A few common FPN1 mutations have been reported in independent pedigrees, in different countries (e.g. Val192del46,58,70,17,79,77 A77D,17,59,60 G80S,43,55,56,61-63 It is now believed that the most frequently reported FPN1 mutations, such as the p.Val162del, are more frequently identified than other SLC40A1 mutations because they have occurred multiple times in isolated populations rather than occurring once and spreading to different populations, as indicated by the identification of a de novo p.Val162del variant in an isolated case of FD.79

FPN1 variants are highly prevalent in African populations. The first prevalent FPN1 variant reported in Africans and Black Americans was the common Q248H polymorphism (p.Gln248His).82,83,100-102 Interestingly, global analysis of variants in the SLC40A1 gene (which includes mutations associated with both the FD and FPN1-associated HH) revealed an allele frequency of 0.0364%, giving a predicted pathogenic genotype carrier rate of 1 in 1373, a figure that approaches the frequency of HFE-HC.102 This was largely due to the relatively high allele frequencies for two SLC40A1 variants (p.Asp270Val and p.Arg371Trp) in the African populations; the predicted SLC40A1 pathogenic genotype carrier rate of these two variants is 1 in 197 among the African population.102 The Q248H,102,103 the p.Asp270Val and the p.Arg371Trp and other FPN1 polymorphic variants may also predispose to iron overload; but no clear evidence for this has been provided (e.g. lack of functional studies), while the possibility remains that, because of the small sample size, these observations could...
Table 3. Disease-associated mutations of the FPN1 gene.

| Gene SLC40A1 (RefSeq NM_014585.5, NP_055400.1) | Amino acid change | Type of variation* | Phenotype                  | Reference                  |
|-----------------------------------------------|-------------------|--------------------|-----------------------------|----------------------------|
| A. Ferroportin disease                        |                   |                    |                             |                            |
| Nucleotide change                             |                   |                    |                             |                            |
| 1. c.134C>A                                   | p.Ala45Glu        | Missense           | ferroportin disease         | 56                         |
| 2. c.205G>A                                   | p.Ala68Thr        | Missense           | ferroportin disease         | 57                         |
| 3. c.206C>T                                   | p.Ala69Val        | Missense           | ferroportin disease         | 56                         |
| 4. c.212C>T                                   | p.Ser71Phe        | Missense           | ferroportin disease         | 56                         |
| 5. c.214G>T                                   | p.Val72Phe        | Missense           | ferroportin disease         | 58                         |
| 6. c.239C>A                                   | p.Ala77Asp        | Missense           | ferroportin disease         | 17,59,60                   |
| 7. c.238G>A                                   | p.Gly80Ser        | Missense           | ferroportin disease         | 43,55,56,61-63              |
| 8. c.239G>T                                   | p.Gly80Val        | Missense           | ferroportin disease         | 64                         |
| 9. c.252C>G                                   | p.Asp84Glu        | Missense           | ferroportin disease         | 91                         |
| 10. c.262A>G                                  | p.Arg88Gly        | Missense           | ferroportin disease         | 56, 65                     |
| 11. c.263G>C                                  | p.Arg88Thr        | Missense           | ferroportin disease         | 66                         |
| 12. c.388T>C                                  | p.Leu129Pro       | Missense           | ferroportin disease         | 67                         |
| 13. c.454A>T                                  | p.Ile152Phe       | Missense           | ferroportin disease         | 68                         |
| 14. c.469G>A                                  | p.Asp157Ain       | Missense           | ferroportin disease         | 58                         |
| 15. c.469G>T                                  | p.Asp157Tyr       | Missense           | ferroportin disease         | 69                         |
| 16. c.470A>C                                  | p.Asp157Aa        | Missense           | ferroportin disease         | 70                         |
| 17. c.470A>G                                  | p.Asp157Gly       | Missense           | ferroportin disease         | 56, 69, 71                 |
| 18. c.473G>T                                  | p.Trp158Leu       | Missense           | ferroportin disease         | 56                         |
| 19. c.474G>T                                  | p.Trp158Cys       | Missense           | ferroportin disease         | 47                         |
| 20. c.484_486del                              | p.Val162del       | Deletion           | ferroportin disease         | 56, 60, 72-79, 92           |
| 21. c.521A>T                                  | p.Asn174Ile       | Missense           | ferroportin disease         | 61                         |
| 22. c.522C>G                                  | p.Asp175Gly       | Missense           | ferroportin disease         | 77                         |
| 23. c.533G>A                                  | p.Asp176Gln       | Missense           | ferroportin disease         | 56, 65                     |
| 24. c.539T>C                                  | p.Ile180Thr       | Missense           | ferroportin disease         | 66                         |
| 25. c.542A>T                                  | p.Asp181Val       | Missense           | ferroportin disease         | 56, 64, 69                 |
| 26. c.546G>T                                  | p.Gln182His       | Missense           | ferroportin disease         | 56, 71                     |
| 27. c.553A>G                                  | p.Asn185Asp       | Missense           | ferroportin disease         | 56, 80                     |
| 28. c.554A>C                                  | p.Asn185Thr       | Missense           | ferroportin disease*        | 56                         |
| 29. c.560G>A                                  | p.Asp185Ser       | Missense           | ferroportin disease*        | 56                         |
| 30. c.689C>A                                  | p.Thr230Asn       | Missense           | ferroportin disease         | 69                         |
| 31. c.695C>A                                  | p.Ala232Asp       | Missense           | ferroportin disease         | 81                         |
| 32. c.698T>C                                  | p.Leu233Pro       | Missense           | ferroportin disease         | 68, 69                     |
| 33. c.744G>T                                  | p.Gln248His       | Missense           | ferroportin disease         | 82, 83, 100-102             |
| 34. c.797T>C                                  | p.Met266Thr       | Missense           | ferroportin disease         | 69                         |
| 35. c.800G>A                                  | p.Asp267Asp       | Missense           | ferroportin disease         | 64                         |
| 36. c.809A>T                                  | p.Asp270Val       | Missense           | ferroportin disease         | 84, 85                     |
| 37. c.968G>T                                  | p.Gly323Val       | Missense           | ferroportin disease         | 71                         |
| 38. c.1035G>C                                 | p.Leu345Phe       | Missense           | ferroportin disease         | 69                         |
| 39. c.1051A>G                                 | p.Ile351Val       | Missense           | ferroportin disease         | 69                         |
| 40. c.1111C>T                                 | p.Arg371Ile       | Missense           | ferroportin disease         | 56                         |
| 41. c.1112G>A                                 | p.Arg371Gln       | Missense           | ferroportin disease*        | 56                         |
| 42. c.1328C>T                                 | p.Pro443Leu       | Missense           | ferroportin disease         | 69                         |
| 43. c.1402G>A                                 | p.Gly468Ser       | Missense           | ferroportin disease*        | 86                         |
| 44. c.1466G>A                                 | p.Arg489Lys       | Missense           | ferroportin disease         | 46                         |
| 45. c.1467A>C                                 | p.Arg489Ser       | Missense           | ferroportin disease         | 87                         |
| 46. c.1468G>A                                 | p.Gly490Ser       | Missense           | ferroportin disease         | 56, 65, 69                 |
| 47. c.1469G>A                                 | p.Gly490Asp       | Missense           | ferroportin disease         | 88, 69                     |
| 48. c.1520A>G                                 | p.His507Arg       | Missense           | ferroportin disease         | 89                         |
| 49. c.1681A>G                                 | p.Arg561Gly       | Missense           | ferroportin disease         | 90                         |

*continued on the next page*
be attributable to chance or that the polymorphisms identified may be in linkage disequilibrium with other disease-causing loci. Yet, taken together, the collected data make FPN1 the gene most frequently associated with hereditary hyperferritinemia in Africans.

Clinical manifestations and diagnosis

As discussed, FD is caused by loss-of-function mutations in FPN1. These mutations impair iron export, particularly from reticuloendothelial macrophages. The result is iron accumulation in macrophages of the spleen, liver, and bone (reflected by high levels of SP) (Figure 3). At liver histology, parenchymal cells of these organs are largely spared (Figure 3), but discrete hepatocytic iron deposits are also appreciable, due to defective FPN1 activity in hepatocytes, even at early stages. Clinical presentation appears heterogeneous, but overall expressivity is milder than classic HC, and the associated liver disease is usually not as severe (Table 2 and Figure 3). As occurs in classic forms of HFE HC, also in the FD host factors (menses, blood loss, etc.), co-inheritance of mutations of other iron-transporting or other pro-oxidant damaging activity of iron in parenchymal cells may also contribute to disease progression.

Unlike HFE-HC, the pattern of inheritance of FD is autosomal dominant. Therefore, either parent carries the pathogenic mutation of FPN1 and presents with unexplained hyperferritinemia. In addition, the proband carries a 50% risk of having an affected child. The disease must be suspected in any individual with unexplained hyperferritinemia and low-normal transferrin saturation (TS), or non-parenchymal cell siderosis at liver biopsy or liver and spleen iron accumulation at MRI (Table 4).

Hyperferritinemia in FD appears very early in life, and unexplained hyperferritinemia with normal TS in a child should prompt MRI evaluation to evaluate iron accumulation in liver, spleen, and bone marrow (see below).
diagnosis of FD (Figure 4). First, common causes of hyperferritinemia, such as metabolic disorders, inflammation, cancer, etc., should be considered. If they are not found, or if the hyperferritinemia persists after their treatment, the next step depends on whether or not anemia is present. In the absence of overt anemia, if liver and spleen iron content are increased at MRI or liver biopsy shows prominent Kupffer cell iron load, FD disease should be considered and genetic testing performed for confirmation of diagnosis (Figure 4). Another common cause of hereditary hyperferritinemia with normal TS associated with iron accumulation and anemia is Gaucher disease, usually associated with hepatosplenomegaly, cytopenia, abnormal coagulation, bone disease, and neuropathic manifestations.107

In the absence of body iron accumulation, but in the presence of elevated SF levels and normal TS, autosomal dominant hyperferritinemia with cataract (due to mutations of the iron responsive element in the 5' untranslated region of the L ferritin mRNA108) or without cataract,109 should be considered. If overt anemia is present, but TS is normal/low, aceruloplasminemia should be suspected, a rare autosomal recessive disease due to loss of function mutations in ceruloplasmin (CP) and resulting in iron overload in the liver and pancreas, and progressive neurodegeneration, diabetes and retinal degeneration.110 Brain MRI with typical iron accumulation in basal ganglia and thalamus may help confirm the diagnosis. As mentioned above, another rare genetic disease presenting with hyperferritinemia and anemia is atransferrinemia/hypotransferrinemia111 which, however, is characterized by increased transferrin saturation due to extremely low serum transferrin levels.

Differential diagnosis mainly includes the classic (HFE) and non-classic (TfR2, HAMP, HJV and FPN1) forms of HH, all characterized by early and progressive increase of TS followed by elevation of serum ferritin as iron accumulation increases in parenchymal cells of the liver, pancreas, heart and other organs (Table 2). As discussed, unlike HH, in FD clinical expressivity is milder.

Abdominal MRI is a useful non-invasive tool to categorize and diagnose the disorder, as it can differentiate patients with FD, characterized by the SSL triad (spleen, spine, liver) iron retention (Figure 5B), from all other forms of HH, including FPN1-HC, associated with liver iron overload.
overload but normal spleen and bone marrow iron content (Figure 5D).112

Treatment

Venesection is the cornerstone of therapy also in FD, but it may not be tolerated equally in all patients, and low TS with anemia may be rapidly established despite SF still being elevated.7 Macrophage iron overload is very resistant to iron withdrawal in this disorder, even in patients who are apparently well-treated (Figure 5C). Therefore, unlike HH, not only serum ferritin, but especially TS should be carefully monitored during therapy. In addition, therapy should not aim at reaching the usual HH targets for iron depletion (TS below 20%, SF 50 ng/L or slight anemia) but be more conservative. There are no studies on the optimal phlebotomy schedule in FD. In practical terms, a monthly/bi-monthly phlebotomy session for 1-2 years, depending on the underlying mutation, allows an acceptable state of iron depletion to be reached, while maintenance therapy (usually a phlebotomy session every 4-6 months) should be continued for life. A reasonable target for therapy is an SF level of 100-200 ng/mL. In certain cases, such Ft values may still reflect some iron loading of tissue macrophages (Figure 5C), but the associated clinical risk is negligible. Ideally, the optimal target is the lowest acceptable ferritin level for TS and hemoglobin levels not below the lower limit of normal. The (controversial) dietary restrictions sometimes recommended for patients with HH (avoiding vitamin C or iron-rich or enriched foods) do not apply to FD due to the different pathogenic basis as compared to HH: normal/sufficient enterocyte iron absorption and normal/marginally increased iron.

**Table 4. Suspecting and diagnosing Ferroportin Disease.**

| Sex    | Ethnicity | Age, y | When to suspect signs                                                                 | Essential for diagnosis                                                                 |
|--------|-----------|--------|---------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Either | Any       | 10-80  | • Unexplained hyperferritinemia and normal or inappropriately low transferrin saturation  
• Isolated hyperferritinemia in father or mother  
• Sinusoidal (Kupffer cell) iron overload at liver biopsy or spleen (and liver), iron accumulation at MRI in patients with unexplained hyperferritinemia and normal or inappropriately low transferrin saturation | Heterozygosity for FPN mutation and hyperferritinemia with normal or inappropriately low transferrin saturation and Kupffer cell iron overload at liver biopsy |

y: years; MRI: magnetic resonance imaging

Figure 3. The different stages and outcomes of “iron retention” in Ferroportin Disease versus “iron accumulation” in FPN1-associated hemochromatosis (HC). Liver histology pictures are reproduced with the permission of Sabelli et al.54
accumulation in parenchymal cells in the FD versus increased iron absorption and marked iron accumulation in parenchymal cells in HH.

Iron chelation may be an option in selected cases. Siblings of patients with FD, like their offspring, must undergo screening since they have a 50% chance of being susceptible.

Conclusions

FPN1 is a multipass membrane iron-exporter that has evolved in mammals to assure sufficient iron delivery from the external milieu and internal sites of iron storage and recycling to the bloodstream, mainly to support the erythron activity. Overall, the FPN1/SLC40A1 gene is essential for humans and total loss (homozygote mutation) of its product is incompatible with life. Loss-of-function of one FPN1 allele in humans results in FD, characterized by a preserved intestinal iron export activity but compromised iron export from tissue macrophages. This leads to progressive iron retention in liver, spleen and bone marrow macrophages, resulting in inappropriately low iron delivery to circulating transferrin and marginal iron-restricted erythropoiesis that may result in overt anemia when bone marrow demands are increased (e.g. menarche, aggressive phlebotomy regimen). Gain-of-function mutations of FPN1 preclude the inhibitory activity of hepcidin, thereby leading to unrestricted iron transfer to the bloodstream and causing a rare form of HH.

The pathogenic, biochemical and clinical signatures of FD are symmetrical and opposite to HFE and non HFE-HH: normal/sufficient enterocyte iron absorption, marked iron accumulation in non parenchymal cells in...
the FD versus increased iron absorption and marked iron accumulation in parenchymal cells in HH; hyperferritine- 
emia with normal/low transferrin saturation in FD versus hyperferritinemia and high transferrin saturation in HH; intolerance to aggressive phlebotomy regimens in FD versus optimal response to intense phlebotomy in HH; mild and benign clinical course in FD versus potentially severe clinical expressivity in HH; vertical hereditary  
transmission and presentation at each generation of FD versus recessive transmission of most forms of HH (except FFNI-HH).

While the molecular pathogenesis of FD is becoming more and more defined, the long-term effect of massive iron retention in tissue macrophages in the setting of chronic inflammatory/infectious or degenerative disor- 
ders is still unclear.

Today, isolated or unexplained hyperferritinemia represents one of the commonest reasons for referral. Knowing that FD is one of the most frequent genetic causes of hyperferritinemia, regardless of ethnicity, it is important to maintain a high diagnostic suspicion for this disorder.

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