Recent advances in disorders of iron metabolism: mutations, mechanisms and modifiers

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The spectrum of known disorders of iron metabolism has expanded dramatically over the past few years. Identification of HFE, the gene most commonly mutated in patients with hereditary hemochromatosis, has allowed molecular diagnosis and paved the way for identification of other genes, such as TFR2, that are important in non-HFE-associated iron overload. There are clearly several other, unidentified, iron overload disease genes yet to be found. In parallel, our understanding of iron transport has expanded through identification of Fpn1/Ireg1/MTP1, Sfxn1 and Dcytb. Ongoing studies of Friedreich’s ataxia, sideroblastic anemia, aceruloplasminemia and neurodegeneration with brain-iron accumulation are clarifying the role for iron in the nervous system. Finally, as the number of known iron metabolic genes increases and their respective functions are ascertained, new opportunities have arisen to identify genetic modifiers of iron homeostasis.

The same properties that make iron essential for basic biological processes such as transport of oxygen and electrons also make it toxic, because iron can promote oxidative damage to vital biological structures. Iron homeostasis must, therefore, be tightly regulated. Genes that maintain iron homeostasis may facilitate iron uptake, storage or egress, or the regulation of any of these processes. Recently, several genetic diseases have given new insights into the function and regulation of genes of iron metabolism. New genes have been identified that are involved in iron transport, recycling and mitochondrial iron balance. Furthermore, variable phenotypic expression of mutant genotypes in mice and man is revealing the presence of genetic modifiers.

Hereditary hemochromatosis is a common autosomal recessive disorder that results in iron overload [for review see (1)]. What was once thought to be a singular disease with varying degrees of severity is now known to be heterogeneous, resulting from defects in multiple genes. **Type 1 hemochromatosis** is associated with mutations in the HFE gene (human chromosome 6p21.3) (2). The progression of iron loading is usually slow, and affected individuals often do not present with clinical signs or symptoms until the fifth or sixth decade of life. The initial symptoms are subtle, and often include pain in the joints of the fingers, skin hyperpigmentation, fatigue and depression. As iron loading proceeds, affected patients develop liver disease, gradually progressing from fibrosis to cirrhosis. They have a greatly increased incidence of hepatocellular carcinoma. Cardiomyopathy and arrhythmias may develop from deposition of iron in the heart. Endocrine abnormalities are common, including hypogonadism and diabetes.
The normal function of the HFE protein is not understood. It is related to class I major histocompatibility proteins, and, accordingly, it forms a heterodimer with β2 microglobulin (2). It does not bind iron and it is not an iron transporter; rather, it appears to be a regulatory molecule that influences the efficiency of intestinal iron absorption. HFE has been observed to associate with the transferrin receptor (3–5) and to attenuate cellular iron uptake from its ligand, the plasma iron carrier transferrin (6–9). However, the mechanism by which the HFE and transferrin receptor complex modulates body iron homeostasis remains under investigation.

The majority of type 1 hemochromatosis patients are homozygous for a unique allele containing a cysteine to tyrosine conversion at codon 282 (C282Y) of the HFE protein (2). This mutation prevents the formation of an intramolecular disulfide bond that is critical for efficient expression of HFE (10,11), and results in a partial loss of protein function (12). Other mutations and polymorphisms [H63D (2), S65C (13), I105T and G93R (14)] have been identified in patients with hereditary hemochromatosis, but their contributions to the disease are not well understood (10,12). Despite the prevalence of HFE mutations in individuals with hemochromatosis, not all individuals with hemochromatosis carry mutations in HFE. This has led to the identification of other genes that, when mutated, also cause hemochromatosis.

**Type 2 hemochromatosis**, or juvenile hemochromatosis, is more severe than type 1 (15). While the gene responsible for this disease has not been identified, it is linked to human chromosome 1q and has been designated HFE2 (16). Type 2 hemochromatosis is characterized by rapid iron loading and clinical presentation within the second decade of life (15). Cardiac and endocrine abnormalities dominate the clinical picture, but liver disease may be significant. Because the rate of iron loading in type 2 hemochromatosis exceeds that of type 1 hemochromatosis, it is likely that HFE2 either plays a more important role than HFE within the same regulatory pathway, or is part of a distinct and more potent regulatory pathway.1

**Type 3 hemochromatosis**, which is phenotypically indistinguishable from HFE-associated hemochromatosis, is associated with mutations in TFR2 (17). This locus encodes transferrin receptor 2 (human chromosome 7q22) (18), a protein that shares significant homology with the extracellular domain of the transferrin receptor. Like the transferrin receptor, transferrin receptor 2 can bind transferrin, but it does so with much lower affinity than its homolog, and it is uncertain whether transferrin receptor 2 serves in the uptake of diferric transferrin in vivo (18,19). Transferrin receptor 2 mRNA expression is highest in the liver (18) but, unlike the transferrin receptor, transferrin receptor 2 expression does not respond to changes in cellular iron status (20). The exact role of transferrin receptor 2 in the pathogenesis of iron loading is still unknown. Recent biochemical evidence suggests that type 3 hemochromatosis may be distinct from the HFE pathway because, unlike the transferrin receptor (3,4), transferrin receptor 2 does not form a stable complex with the HFE protein in vitro (21). Whether HFE, HFE2 and Tfr2 participate in overlapping or completely independent genetic pathways awaits further investigation.
Friedreich’s ataxia (FRDA) and sideroblastic anemia represent two diseases that highlight the importance of mitochondrial iron transport and homeostasis. FRDA is a neurodegenerative disease characterized by loss of sensory neurons in the spinal cord and dorsal root ganglia [for review see (22)]. Patients show evidence of mitochondrial iron overload (23) and a loss of activity of iron–sulfur cluster-containing enzymes (24). They frequently die from cardiomyopathy. The majority of FRDA cases result from the expansion of triple nucleotide repeats within an intron of the FRDA gene (human chromosome 9q13) (25) leading to reduced expression of frataxin mRNA and protein (26). However, point mutations have also been identified in a small number of cases.

Frataxin is localized to the mitochondrion (27–29). When frataxin levels decrease, as is the case in FRDA, iron accumulates within mitochondria, leading to increased oxidative stress and decreased activity of iron–sulfur cluster-containing proteins. Although a complete knockout of murine frataxin is embryonic lethal (30), recently developed conditional knockout mouse models of FRDA suggest that the effects of mitochondrial iron accumulation vary among different cell types (31). Two models have been generated: mice that lack frataxin in neurons and mice that lack frataxin in striated muscle. Both of these mice recapitulate features of the human disease.

Oxidative damage is thought to precipitate the neuron loss in FRDA. Experiments in yeast show that iron is redistributed to the mitochondria of Yfh (yeast frataxin homolog)-deficient yeast and that this iron accumulation precedes oxidative damage, arguing that iron accumulation is more likely to be the cause of oxidative damage to the yeast mitochondrion than the result (32). Recently, the crystal structure of the frataxin protein was solved (33). Frataxin shows structural similarity to the iron storage protein ferritin, suggesting that frataxin might mediate mitochondrial iron homeostasis by maintaining iron stores or facilitating their efficient turnover. Future experiments will determine whether frataxin is involved in mitochondrial iron storage and egress, iron–sulfur cluster biogenesis or iron–sulfur cluster transport.

Sideroblastic anemia is another disorder associated with aberrant mitochondrial iron homeostasis. Although the genetic defects leading to sideroblastic anemia are heterogeneous, they all affect the efficiency of heme production within erythroblast mitochondria, leading to iron-overloaded mitochondria that form a characteristic ring around the cell nucleus. There are two forms of X-linked sideroblastic anemia, distinguished by the presence or absence of ataxia. Mutations in ALAS2 (human chromosome Xp11.21) (34) reduce the efficiency of a critical enzyme in the heme biosynthetic pathway, -aminolevulinic acid synthetase (35). Many patients respond to pharmacological doses of pyridoxine, a co-factor for ALAS2. Sideroflexin 1, a novel gene encoding a mitochondrial membrane protein, has recently been identified because it is mutated in mice with siderocytic anemia (36). The striking similarity between mice lacking sideroflexin and mice deprived of pyridoxine (37) suggests that sideroflexin might facilitate transport of pyridoxine or another ALAS2 cofactor into the mitochondrion, but this has not yet been shown experimentally.
ABC-7 (human chromosome Xq13.1–q13.3) (38) is a gene mutated in X-linked sideroblastic anemia with ataxia. Individuals with mutations in ABC-7 present with hypochromic, microcytic anemia, ringed sideroblasts and non-progressive spinocerebellar ataxia (39,40). The functions of ABC-7, and its connection to sideroblastic anemia, have not been definitively established. However, experiments in mutant yeast cells have shown that ABC-7 can substitute for a homologous protein, Atm1p, which is involved in transport of iron–sulfur clusters from their site of synthesis in mitochondria to the cytoplasm (41). The link between iron–sulfur cluster biogenesis and ataxia in Friedreich’s ataxia and sideroblastic anemia with ataxia offers clues to the role of iron metabolic genes in the control of neuronal cell survival and function, but the mechanisms remain uncertain.

Aceruloplasminemia is an autosomal recessive disease of iron overload that results from loss of function mutations in the ceruloplasmin (Cp) gene (human chromosome 3q23–q24) (42–45). While the iron overload associated with hemochromatosis results from increased iron absorption, the iron overload associated with aceruloplasminemia results from aberrant iron distribution. Ceruloplasmin is a serum protein and a multi-copper oxidase [for review see (46)]. Without this serum oxidase activity, iron cannot be efficiently recycled from storage sites in the liver and a seemingly paradoxical constellation of iron-related symptoms develops. Serum ferritin is elevated, but serum iron remains low because iron is not efficiently loaded onto transferrin. Iron accumulates in the parenchymal and reticuloendothelial cells of the liver and pancreas, but anemia results because iron is not efficiently delivered to red blood cell precursors. Ceruloplasmin must not be essential for export of iron from all cells of the body because dietary iron can still cross the intestine to enter the blood of individuals with aceruloplasminemia. This is probably due to the copper oxidase activity of the sla gene product, hephaestin (human chromosome Xq11–q12) (47), which has significant homology to ceruloplasmin in its structure and presumed function.