

Genetic disorders of iron overload and the novel "ferroportin disease"

A positive iron balance inevitably leads to iron overload.¹ Excluding red blood cell transfusion, iron loading usually reflects an altered mucosal regulation of iron absorption, observed both in genetic conditions not associated with anemia and in iron-loading anemias.^{2,3}

The best characterized form of genetic iron overload is a common recessive HLA-linked disorder, initially called idiopathic hemochromatosis and more recently reported as hereditary or genetic hemochromatosis.⁴ Patients with this disorder have excessive iron absorption combined with decreased macrophage iron retention, and undergo progressive parenchymal iron loading with appearance of clinical manifestations in the fifth decade of life, predominantly in males.⁵ In 1996, HFE was identified as the HLA-related hemochromatosis gene by positional cloning.⁶

For years, hereditary hemochromatosis has been considered by most investigators as a single genetic entity, and also as a typically WASP disorder, presumably of Celtic origin. While few reports denying this have appeared in the past two decades, several molecular studies in the last few years have definitely shown that the one-disease dogma is totally untrue. A large body of evidence clearly indicates that, although the HFE-related condition is highly prevalent in Caucasians, on a worldwide basis hereditary hemochromatosis must be considered a genetically heterogeneous syndrome of parenchymal iron overload with variable prevalence in different ethnic groups and with variable clinical expression.

HFE-related genetic hemochromatosis in Caucasians (HFE, OMIM 235200)

Most patients with HFE-related genetic hemochromatosis are homozygous for a Cys282→Tyr (C282Y) mutation and others are compound heterozygotes for C282Y and a second mutation, His63→Asp (H63D). Homozygosity for C282Y is found in more than 90% of North European⁹ and more than 80% of American patients of European origin clinically diagnosed as having genetic hemochromatosis.¹⁰ Several studies have shown lower frequencies (64–76%) for C282Y homozygosity in severely iron-loaded patients belonging to populations of Southern Europe^{11–13} or including individuals of such origin.¹⁴ According to Beutler *et al.*¹⁵ the penetrance of the C282Y mutation is low, with less than

1% of homozygotes developing frank clinical hemochromatosis.

Additional HFE mutations have been reported. For instance, Mura *et al.*¹⁶ described the S65C missense substitution in a study of a large series of hemochromatosis probands and controls. The S65C substitution accounted for 7.8% of hemochromatosis chromosomes that were neither C282Y nor H63D. Other uncommon HFE exon and intron mutations may be occasionally discovered among hemochromatosis patients who have atypical HFE genotypes. For instance, the E168X mutation can be found in hemochromatosis patients of Northern ancestry with an incomplete HFE genotype.¹⁷

Mechanisms by which C282Y and H63D may disrupt the normal functioning of HFE have been suggested, but the role of HFE in the process of normal iron metabolism has yet to be clearly defined. Recent studies, however, suggest a major role for hepcidin.^{18,19}

HFE-unrelated genetic hemochromatosis

In the last few years, Italian and Greek studies^{20–22} have clearly shown that significant iron loading can occur in Caucasians with no evidence of HFE mutations and no identifiable cause of secondary iron overload, indicating that genes other than mutant HFE genes have a role in causing hereditary hemochromatosis syndromes.

Juvenile genetic hemochromatosis (HFE2, OMIM 602390)

Fifteen years ago we described juvenile genetic hemochromatosis as a distinct nosological entity.⁷ In the juvenile variant of the disease, males and females appear to be equally affected. Patients present with hypogonadotropic hypogonadism, and, unless proper treatment is started, die early because of cardiac dysfunction.²³

We studied 2 Italian families with cases of juvenile GH. Affected individuals showed body iron distribution patterns similar to those found in adult patients, but presented severe iron overload around the age of 20. The rate of iron accumulation proved to be particularly fast (from 3.2 to 3.9 mg per day) compared with that in adult HFE-related GH (from 0.8 to 1.6 mg per day), thereby explaining why these young individuals develop parenchymal iron overload in excess of 200 mg/kg already in their second decade of life. The remarkable quantitative difference in iron over-procurement between classical and juvenile GH (about 1 versus 3–4 mg per day) suggests that a completely different pathogenetic defect is

Table 1. Genetic disorders of iron overload.

	<i>Classic HFE-related genetic hemochromatosis (OMIM 235200)</i>	<i>Juvenile genetic hemochromatosis, HFE2A (OMIM 602390)</i>	<i>Juvenile genetic hemochromatosis, HFE2B (OMIM 602390)</i>	<i>Hemochromatosis type 3 (HFE3, 604250)</i>	<i>Hemochromatosis type 4 (HFE4, OMIM 606069), or ferroportin disease</i>
Gene	HFE, which maps to 6p21.3	Unknown gene, which maps to 1q21	HAMP on 19q13, coding for hepcidin antimicrobial peptide	TFR2, which codes for transferrin receptor 2 and maps to 7q22	SLC11A3 gene, which maps to 2q32 encodes ferroportin
Mutations	C282Y (highest phenotypic expression), H63D (minor if any phenotypic expression, one third of the population is heterozygous), S65C, E168X, others	Unknown	93delG resulting in frameshift, 166C-T changing arginine at position 56 to a stop codon (R56X)	A few private mutations	Several mutations, including the Val 162 deletion, which likely represents a recurrent mutation due to slippage mispairing
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal dominant
Frequency	In Caucasians, the frequency of individuals heterozygous for the C282Y mutation is about 5%; the frequency of homozygotes is 2-3%	Rare disease	Rare disease	Rare disease	Likely less rare than thought
Penetrance	Highly variable estimates available. According to Beutler <i>et al.</i> (Lancet 2002;359:211-8) less than 1% of homozygotes develop frank clinical hemochromatosis	Full penetrance	Full penetrance	Unknown	Full penetrance
Pathogenesis	Increased iron absorption and reticuloendothelial iron release	Highly increased iron absorption and reticuloendothelial iron release; iron accumulation in the order of 3-4 mg/day	Animal models have indicated that hepcidin is a key regulator of iron absorption in mammals, and that absence of this peptide results in a typical hemochromatosis phenotype.	Unknown	Reduced iron release from reticuloendothelial cells in all instances. In addition, reduced release from parenchymal cells associated with particular mutations
Body iron status	Early increase in serum iron and transferrin saturation (>45%), later increase in serum ferritin	Increase in serum iron and transferrin saturation already during first decade of life	Increase in serum iron and transferrin saturation already during first decade of life	Early increase in serum iron and transferrin saturation (>45%), later increase in serum ferritin	Early increase in serum ferritin with normal serum iron and transferrin saturation (isolated hyperferritinemia). Some mutations are associated with pure reticuloendothelial iron overload, while others involve also parenchymal iron overload.
Clinical onset	4 th -5 th decade	2 nd decade	2 nd decade	4 th -5 th decade	Hyperferritinemia already present in the first decades of life, parenchymal iron overload develops later in some patients
Clinical manifestation	Liver disease, diabetes arthropathy, hypogonadism, cardiopathy	Patients present with hypogonadotropic hypogonadism, and, unless treatment is started, die of cardiac dysfunction around the age of 20	Patients present with hypogonadotropic hypogonadism, and, unless treatment is started, die of cardiac dysfunction around the age of 20	Liver disease, diabetes, arthropathy, hypogonadism, cardiopathy	No clinical manifestations in pure reticuloendothelial iron overload. Manifestations of hemochromatosis in patients with concomitant parenchymal iron overload.

involved.²⁴ In a study of 7 Italian patients belonging to 5 unrelated families with juvenile hemochromatosis, Camaschella *et al.*²⁵ excluded the HFE gene as responsible for this condition. Later they performed a genomewide search to map the juvenile GH locus in 9 families, 6 of whom had consanguineous parents while 3 showed multiple affected patients.²⁶ Linkage analysis showed linkage to markers of chromosome 1q with a lod score of >3.0, and

homozygosity mapping defined the limits of the candidate region in the < 4 cM interval between D1S442 and D1S2347. Analysis of the genes mapped in this interval excluded obvious candidates. Thus the juvenile GH locus does not correspond to the chromosomal localization of any known iron protein gene.

Recently Roetto and co-workers described a Greek and an Italian family with juvenile genetic hemochromatosis which was not linked to chro-

mosome 1q21. They took advantage of the recent identification of the antimicrobial peptide hepcidin (HAMP; OMIM 606464) as a key regulator of iron absorption in mammals.²⁷ This led to the identification of two mutations (93delG and 166C→T) in HAMP on 19q13 in the two families with juvenile hemochromatosis (Table 1).

Transferrin receptor 2 (TfR2) and hemochromatosis type 3 (HFE3, OMIM 604250)

A homolog of the transferrin receptor has been identified as transferrin receptor 2.²⁸ TfR2 is a member of the *transferrin-like receptors* family with an unknown function, characterized by a restricted pattern of expression in the liver. Its gene maps to chromosome 7q22. In a few families with non-HFE related hereditary hemochromatosis Camaschella and co-workers found that affected individuals were homozygous for point mutations in the gene coding for TfR2, suggesting that this may represent the molecular basis for this familial iron overload syndrome.²⁹⁻³¹ The TfR2 mutations responsible for hemochromatosis type 3, or HFE3 (OMIM 604250) appear to be private mutations.

Hemochromatosis type 4 (HFE4; OMIM 606069), or ferroportin disease

Ferroportin1 – or, more simply, ferroportin – is a newly discovered molecule³² that plays an important role in iron export. It is also known as IREG1³³ or MTP1.³⁴ In nearly simultaneous reports, a Dutch and an Italian group recently described pedigrees with atypical hemochromatosis inherited as an autosomal dominant trait. The two groups found different missense mutations in the ferroportin (SLC11A3) gene. This dominant type of genetic reticuloendothelial iron overload is defined as hemochromatosis type 4, or HFE4 (OMIM 606069). Mutations in the human ferroportin gene have since been reported independently by other investigators³⁷⁻⁴¹ and substantial clinical differences exist between the reported families. In this issue of *Haematologica*, Rivard and co-workers⁴² report a new family with autosomal genetic iron overload due to a novel ferroportin mutation.

Some families with this novel *ferroportin disease* show both parenchymal and reticuloendothelial iron overload, while other families show a typical genetic reticuloendothelial iron overload. We studied a family with autosomal dominant hyperferritinemia in whom the proband showed selective iron accumulation in Kupffer cells on liver biopsy.⁴⁰ Sequence analysis of the ferroportin gene (SLC11A3) in four individuals with hyperferritinemia singled out a three base pair deletion in a region that contains four TTG repeats. This mutation removes a TTG unit from 780 to 791, and predicts the loss of one of three sequential valine residues 160-162. SLC11A3 polymorphism analysis

indicates that this likely represents a recurrent mutation due to slippage mispairing. Affected individuals may show a marginally low serum iron and transferrin saturation, and young women may have marginally low hemoglobin concentration. Serum ferritin levels are directly related to age, but 10-20 times higher than normal. The Val 162 deletion in the ferroportin gene appears, therefore, to represent a loss-of-function mutation that mainly impairs reticuloendothelial iron metabolism, while not having a major impact on intestinal iron absorption in heterozygous individuals. In turn, heterozygosity for the ferroportin Val 162 deletion represents the prototype of selective reticuloendothelial iron overload. It is at present unclear why other mutations, such as those described by Rivard and co-workers, result in both parenchymal and reticuloendothelial iron overload.⁴²

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ZAP-70 expression in chronic lymphocytic leukemia: a new parameter for an old disease

Chronic lymphocytic leukemia (CLL) has a variable clinical course. Although the median survival of patients with this form of leukemia is around 10 years, in individual patients the prognosis is extremely variable, ranging from a very short to a normal lifespan.¹ Thus, some CLL patients will have an excellent prognosis and will never require treatment, whereas in others the prognosis is poor and prompt treatment is required. The clinical staging systems, independently developed by Rai *et al.*² and Binet *et al.*³ in the early 80's, based on easily obtainable biological and clinical variables, are the most useful prognostic parameters. These staging systems not only facilitate the treatment of patients according to individual prognosis, but also make it possible to conduct and to compare trials based on the risk of the disease. However, these systems are not accurate enough to identify subgroups of patients with progressive CLL and mechanisms causing cytopenias are not taken into consideration.