**Genetic/metabolic effect of iron metabolism and rare anemias**

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**Abstract**

Advances in iron metabolism have allowed a novel classification of iron disorders and to identify previously unknown diseases. These disorders include genetic iron overload (hemochromatosis) and inherited iron-related anemias, in some cases accompanied by iron overload. Rare inherited anemias may affect the hepcidin pathway, iron absorption, transport, utilization and recycling. Among the genetic iron-related anemias the most common form is likely the iron-refractory iron-deficiency anemia (IRIDA), due to mutations of the hepcidin inhibitor TMPRSS6 encoding the serine protease matriptase-2. IRIDA is characterized by hepcidin up-regulation, decrease iron absorption and macrophage recycling and by microcytic-hypochromic anemia, unresponsive to oral iron. High serum hepcidin levels may suggest the diagnosis, which requires demonstrating the causal TMPRSS6 mutations by gene sequencing. Other rare microcytic hypochromic anemias associated with defects of iron transport-uptake are the rare hypotransferrinemia, and DMT1 and STEAP3 mutations. The degree of anemia is variable and accompanied by secondary iron overload even in the absence of blood transfusions. This is due to the iron-deficient or expanded erythropoiesis that inhibits hepcidin transcription, increases iron absorption, through the erythroid regulator, in untransfused beta-thalassemia. Sideroblastic anemias are due to decreased mitochondrial iron utilization for heme or sulfur cluster synthesis. Their diagnosis requires demonstrating ringed sideroblasts by Perl’s staining of the bone marrow smears. The commonest X-linked form is due to delta-amino-levulinic-synthase-2-acid (ALAS2) mutations. The recessive, more severe form, affects SLC25A38, which encodes a potential mitochondrial importer of glycine, an amino acid essential for ALA synthesis and thus results in heme deficiency. Two disorders affect iron/sulfur cluster biogenesis: deficiency of the ATP-binding cassette B7 (ABCB7) causes X-linked sideroblastic anemia/ataxia, likely impairing the activity of ferrochelatase, which is an iron/sulfur-cluster-dependent enzyme. A recessive form affects GLRX5, a protein involved in the iron/sulfur cluster biogenesis. Aceruloplasminemia is a rare recessive syndrome characterized by anemia, diabetes, retinal degeneration, ataxia and other neurological symptoms, low serum iron but high serum ferritin, due to decreased iron recycling from macrophages and other cells. The study of these rare conditions has greatly contributed to our understanding of iron transport, utilization and recycling. Their distinction is clinically essential in order to plan the best treatment.

**Introduction**

Recent advances in the biology of iron metabolism and its systemic regulation have improved our understanding of iron-related disorders, identified new genetic diseases and offered novel iron related molecules/pathways to be targeted by innovative therapeutic strategies.

Iron disorders traditionally include genetic iron overload (hereditary hemochromatosis) and inherited iron-related microcytic anemias. However, some conditions characterized by primary iron overload may manifest mild anemia and some genetic iron-related anemias are accompanied by iron overload, making the clinical distinction in some cases difficult. In addition, in a more extensive classification iron disorders may also include acquired forms of secondary iron overload, as the iron loading anemias, whose prototype is beta-thalassemia (1). Another acquired iron-related disorder is represented by anemia of chronic diseases (ACD) a common anemia related to inflammatory cytokines and immune dysregulation, in which total body iron is preserved but maldistributed, with increased iron stores and iron restricted erythropoiesis. Table 1 reports a broad classification of iron disorders.

**Hereditary hemochromatosis**

Autosomal recessive iron overload includes the classic HFE-related hemochromatosis (type 1), juvenile hemochromatosis, (type 2), due to hepcidin or hemojuvelin mutations, and TFR2-related (type 3) hemochromatosis (2). All these disorders, although with variable severity, are characterized by high transferrin saturation and high serum ferritin, iron accumulation in liver and, in severe cases, in other organs (pancreas, heart, endocrine glands) and are responsive to phlebotomy, which may prevent iron toxicity and organ failure. The prototype of these disorders and is the homozygous condition for HFE C282Y mutation that leads to an adult form of iron overload prevalent-ly in the liver and is the most common form. The study of these conditions and of the corresponding animal models has been extremely informative to understand the regulation of systemic iron homeosta-
sis, based on the hepatic hormone hepcidin. This is a small (25 amino acid) peptide produced by liver that regulates the surface expression of the sole known cell iron exporter, ferroportin and thus regulates the iron release to plasma (3). It is now clear from human and animal studies that all recessive hemochromatosis are disorders in which ferroportin production is insufficient compared to iron stores. These disorders are a model for iron overload that occurs in the iron loading anemia, exemplified by transfused beta-thalassemia intermedia.

Autosomal dominant hemochromatosis, also called ‘ferroportin disease’, is due to mutations of the SLC40A1 gene, encoding ferroportin and is characterized in most cases by iron loading of Kupffer cells, normal transferrin saturation and high serum ferritin. Few mutations of SLC40A1 cause hepcidin resistance and lead to a phenotype indistinguishable from hemochromatosis. Recessive hemochromatosis is not characterized by anemia; instead, patients show high tolerance towards repeated phlebotomies. On the contrary, some patients with ferroportin disease show marginal anemia or phlebotomy intolerance, indicating a disorder borderline between iron overload and iron deficient erythropoiesis. The issue of reduced tolerance to phlebotomy should be taken into account when planning iron depletion therapy in ferroportin disease.

### Inherited iron-related anemias

Rare inherited anemias may affect the hepcidin pathway and thus iron absorption, or iron transport and utilization by the erythron and macrophage iron recycling. All these disorders are characterized by microcytic-hypochromic erythrocytes because of reduction of heme/hemoglobin formation.

### Table 1. Classification of inherited iron-related disorders

| HEREDITARY HEMOCHROMATOSIS | Gene | Inheritance | Phenotype |
|-----------------------------|------|-------------|-----------|
| Type 1 (classic form)       | HFE  | AR          | Iron overload (liver, other organs) slowly progressive |
| Type 2 (Juvenile form)      | HAMP, HJV | AR | Iron overload (liver, other organs) early onset, rapidly progressive |
| Type 3                      | TFR2 | AR          | Iron overload (liver, other organs) early onset, slowly progressive |
| Ferroportin disease         | FPN  | AD          | Iron overload (macrophages) |
| Type 4 Hemochromatosis      | FPN  | AD          | Iron overload (liver, other organs) |

| IRON RELATED ANEMIAS | Gene | Inheritance | Phenotype |
|----------------------|------|-------------|-----------|
| Defective iron absorption | Tmprss6 | AR | Microcytic anemia. Iron deficiency |
| Defective iron transport | TF | AR | Microcytic anemia. Iron overload |
| Defective transferrin receptor cycle | SLC11A2 | AR | Microcytic anemia. Iron overload |
| STEAP3 defects (single family) | STEAP3 | AR | Transfusion-dependent microcytic anemia |
| Defective iron utilization | Ala57 | X-L | Microcytic anemia. Iron overload |
| X-linked sideroblastic anemia | SLCE25A38 | AR | Blood transfusion anemia. Iron overload |
| Sideroblastic anemia due to Fe/S cluster deficiency | GLRX5 | AR | Microcytic anemia (mild). No iron overload |
| Aceruloplasminemia | CP | AR | Anemia, iron overload, diabetes, retinal degeneration, neuropathy |

**Iron-refractory iron-deficiency anemia (IRIDA)**

Among the inherited anemias due to abnormal iron metabolism one of the most important, likely the most common, is the recessive Iron-refractory iron-deficiency anemia (IRIDA), due to mutations of Tmprss6. The disease was first described in the Mask mouse, which shows high hepcidin levels and iron deficiency, unresponsive to oral iron. This mouse allowed the cloning of the Tmprss6, the gene that encodes matriptase 2, a transmembrane serine protease that suppresses hepcidin production in vivo (4). The Mask mouse was shown to have a homozygous deletion of the serine protease domain of Tmprss6 and has the same phenotype of the mouse with the homozygous deletion of the Tmprss6 gene (Tmprss6 null mouse)(5). Several patients have been described homozygous or compound heterozygous for Tmprss6 mutations, that affect not only the serine protease, but also other protein structural domains (6,7). They are usually children or adults with longstanding anemia, show mild-moderate degree of anemia, but extremely low MCV, MCH and transferrin saturation, while serum ferritin levels may be within the normal range or only slightly decreased. Anemia seems not to be present at birth but develops in the first months of life. Hepcidin serum levels are especially high considering that in iron deficiency hepcidin levels are usually low/undetectable. Since hepcidin blocks ferroportin export activity in duodenal cells, patients are resistant to oral and only partially responsive to parenteral iron. The latter finding is explained by a less efficient iron release from macrophages since ferroportin is degraded by the inappropriately high hepcidin levels.

Other forms of anemia are due to decreased iron transport or erythroblast iron uptake. These disorders include deficiency of the iron carrier transferrin (Tf) and of proteins of the Transferrin Receptor (TfR) cycle and are characterized by anemia and iron overload without
the typical features of sideroblastic anemia. They include ataxferri-
nemia and molecular defects of divalent metal transporter 1 (DMT1)
and 6-transmembrane epithelial antigen of the prostate 3 (STEAP3).

**Atransferrinemia**

Atransferrinemia (or hypotransferrinemia) is a genetic recessive
anemia, extremely rare, well known since the last century. It is the pro-
totype of disorders of TIR cycle because of deficiency of the transferrin
receptor ligand. It presents with severe anemia and iron overload that
involves several tissues, including liver, pancreas and heart.

**Sideroblastic anemia**

Inherited sideroblastic anemia includes several forms of anemias
due to defective erythroblast iron utilization and characterized by iron
accumulation in mitochondria. The classification of sideroblastic and
non-sideroblastic anemia takes relies on the presence of ringed sider-
oblasts (more than 15%) in the bone marrow erythroblasts after Perl’s
staining. Sideroblastic anemia is characterized by reduced iron utiliza-

**Acquired iron related disorders**

Untransfused beta-thalassemia is the prototype of Iron loading ane-
mias, because a huge ineffective erythropoiesis in this condition drives
increased iron absorption and release from macrophage operating on
hepcidin suppression. Indeed whereas after transfusion hepcidin levels
increase in thalassemic patients they are low/undetectable in tha-
lassemia intermedia in basal conditions due to transcriptional suppres-
sion likely operated by hypoxia and by the negative signal form the
expanded erythropoiesis, a signal that should also be present in iron
deficiency. One potential candidate to this effect is GDF15, although its
real role is still debated (3).

ACD is an acquired disorder that occurs in chronic infections, immu-
ne dysregulation and cancer. It is characterized by relative defi-
ciency of erythropoietin, decreased bone marrow response to anemia
and iron maldistribution with increased iron stores especially in
macrophages and hepatocytes and decreased iron in the bone marrow
at the erythropoietic (17) level. The pathophysiology of the disease and
of iron abnormalities relies on excessive cytokine production, especial-
ly IL6 and IL1beta. It is a common form of mild-moderate anemia, char-
acterized by high hepcidin production. Treatment is addressed against
the primary disorder, although some patients may respond to i.v. iron,
or erythropoietin.

**Diagnostic approaches to rare iron-related anemias**

The precise diagnosis of rare iron related anemias requires family
studies, measurement of all iron parameters, the peripheral blood

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smeared and, in selected cases, bone marrow examination after Perl's staining. In cases with iron overload the evaluation of tissue (liver) iron is mandatory either using magnetic resonance imaging (MRI) or, in selected cases, liver biopsy. A first distinction should be done between conditions with increased transferrin saturation and increased serum ferritin (signs of iron overload) and those with low transferrin saturation, as IRIDA (7). In the first group are included sideroblastic and non sideroblastic conditions. To diagnose sideroblastic anemia requires examination of the bone marrow smear after Perl’s staining for the search of ringed sideroblasts. They could be seen also in peripheral blood smear as coarse iron inclusions (Pappenheimer bodies). In sideroblastic anemia search for mutations by DNA analysis may be less relevant than in other cases. Differential diagnosis especially when dealing with adult patients is with the acquired forms. Non sideroblastic forms are related to defective uptake of iron by the erythron and due to DMT1, STEAP3 or other proteins of the TIR cycle mutations.

Except for atransferrinemia and aceruloplasminemia all these cases require DNA sequencing of the correspondent genes. Second level tests include soluble transferrin receptor, which is a sign of expanded erythropoiesis and serum hepcidin dosage (18) in the case of IRIDA. In most cases of IRIDA nucleotide sequencing of TMPRSS6 gene may provide the precise diagnosis. For these reasons index cases should be referred for second level diagnosis to selected expert centers.

**Differential diagnosis**

Rare genetic anemias with iron overload need to be differentiated by other congenital microcytic anemia as thalassemia, whereas IRIDA implies a differential diagnosis with anemia of chronic diseases (ACD). The mechanism of iron loading is similar in all iron loading anemias and is due to the expanded erythropoiesis that drives iron absorption through hepcidin suppression. However, the defective globin chain synthesis of beta- and alpha-thalassemia, especially thalassemia intermedia may be easily recognized based on family studies, hemoglobin and/or gene analysis.

IRIDA may be differentiated by ACD that show common features, because of high hepcidin production secondary to cytokines stimulation. If the inflammatory disorder is evident distinction is easy; in other cases the determination of the CRP level may help to exclude inflammatory clinically silent conditions. Usually microcytosis and hypochromia are absent/borderline and transferrin saturation is less decreased unless ACD is longstanding, especially severe or is accompanied by true iron deficiency.

**Treatment**

Different approaches are required according to the defects. Atransferrinemia requires regular plasma infusions. DMT1 deficiency has been treated with erythropoietin in cases of severe anemia in order to avoid transfusions. Sideroblastic anemia due to ALAS2 mutations are worth to be treated with pyridoxine, although not all cases are responsive. The other forms of sideroblastic anemia are not responsive to B6. Few cases of severe sideroblastic anemia due to SLC25A38 have received a allelogenic bone marrow transplantation. Severe cases require blood transfusions and iron chelation treatment. The latter approach has been reported to ameliorate the degree of anemia in few patients. There is not established treatment of aceruloplasminemia: iron chelation may worsen anemia but for the limited experience available cannot reverse neurological symptoms.

Animal models are available for most of the described disorders. New therapeutic strategies are under development to manipulate the hepcidin pathway in order to reduce excessive hepcidin production (e.g. in IRIDA and ACD) or to increase hepcidin activity (hemochromatosis and thalassemia) (19). These approaches are aimed either to contrast the protein effect or interfere with protein synthesis in the first case and to substitute the missing hormone or increase its production in the second case. A change of our approaches to treatment of the described disorders may be foreseen in the near future.

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