REVIEW ARTICLE

Molecular basis of inherited microcytic anemia due to defects in iron acquisition or heme synthesis

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ABSTRACT

Microcytic anemia is the most commonly encountered anemia in general medical practice. Nutritional iron deficiency and β thalassemia trait are the primary causes in pediatrics, whereas bleeding disorders and anemia of chronic disease are common in adulthood. Microcytic hypochromic anemia can result from a defect in globin genes, in heme synthesis, in iron availability or in iron acquisition by the erythroid precursors. These microcytic anemia can be sideroblastic or not, a trait which reflects the implications of different gene abnormalities. Iron is a trace element that may act as a redox component and therefore is integral to vital biological processes that require the transfer of electrons as in oxygen transport, oxidative phosphorylation, DNA biosynthesis and xenobiotic metabolism. However, it can also be pro-oxidant and to avoid its toxicity, iron metabolism is strictly controlled and failure of these control systems could induce iron overload or iron deficient anemia. During the past few years, several new discoveries mostly arising from human patients or mouse models have highlighted the implication of iron metabolism components in hereditary microcytic anemia, from intestinal absorption to its final inclusion into heme. In this paper we will review the new information available on the iron acquisition pathway by developing erythrocytes and its regulation, and we will consider only inherited microcytosis due to heme synthesis or to iron metabolism defects. This information could be useful in the diagnosis and classification of these microcytic anemias.

Key words: anemia, microcytosis, iron, red blood cells, heme.

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Introduction

Microcytic anemia is the most common form of anemia in children and adolescents. It is a very heterogeneous group of diseases that may be either acquired (mostly due to iron deficiency) or inherited. In recent years, human patients or animal models have highlighted the existence of new conditions involved in the pathogenesis of hereditary microcytic anemia.

Total body iron requirements are largely dependent on heme biosynthesis in bone marrow erythroblasts. Absorption of dietary iron by the intestine, as well as iron recycling by the macrophage, is regulated by several different physiological cues including iron load, erythropoiesis, hypoxia and inflammation. Intestinal iron absorption and iron recycling by macrophages following erythrophagocytosis and heme catabolism are the two major processes that fulfill the iron needs of mammalian organisms. Mutations of proteins involved in these iron acquisition processes can lead either to hemochromatosis diseases or to microcytic hypochromic anemia, often associated with iron overload, particularly in the liver.[1]

The presence of red cells with reduced mean corpuscular volume (MCV) reflects reduced hemoglobin synthesis, which can have several causes. It can result from defects either in iron acquisition or availability, or in heme or globin synthesis. Experimental data in mouse demonstrated that early mitotic divisions are accompanied with no reduction of MCV, whereas during differentiation mitotic events are associated with a substantial reduction of MCV.[2]

Microcytic hypochromic anemia can result from a defect in globin genes (hemoglobinopathies or thalassemias), a defect in heme synthesis, a defect in iron availability or in iron acquisition by the erythroid precursors. These microcytic anemias can be sideroblastic or not, a trait which reflects the implications of different gene abnormalities.

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In this paper we will review the new information available on the iron acquisition pathway by developing erythrocytes and its regulation, and we will consider only inherited microcytosis due to heme synthesis or to iron metabolism defects.

Iron and erythropoiesis

Iron acquisition pathway in erythroid cells

Developing erythroid cells in the bone marrow acquire iron from the plasma iron-transferrin (Tf) complex, through the transferrin receptor (TfR) mediated pathway (Figure 1). All proliferating cells express transferrin receptors on their cell surface at levels varying from 10^3 to 10^5 molecules per cell. Erythroid precursors, which have an extraordinary requirement for iron to allow for production of hemoglobin, may have over 10^6 transferrin receptors per cell.[3] The transferrin-Fe(III) complex in plasma is transported into cells principally through transferrin receptor 1 (TfR1), which is expressed on all dividing cells and is particularly abundant on erythroid precursors. TfR2, encoded by a different gene, is expressed primarily in the liver and binds the transferrin-Fe(III) complex at a much lower affinity than TfR1 and appears to be a signaling molecule rather than an iron transporter.[4] At the pH of the cell surface, TfR1 binds only diferric transferrin. The Tf-Fe(III)/TfR1 complex is internalized into a clathrin-coated pit that, assisted by an adaptor protein complex designated AP-2,[5] rapidly matures to a proton-pumping, pH-lowering endosome. At the lowered pH, iron is released from Tf and subsequently reduced to Fe(II) and transported across the endosomal membrane by Divalent Metal Transporter 1 (DMT1).[6] DMT1/Nramp2, a member of the Natural Resistance-Associated-Macrophage Protein (Nramp) family, is a proton-coupled divalent metal transporter found at the plasma membrane and in endosomes of cells of both the duodenum and peripheral tissues.[7]

Iron-depleted Tf is then returned to the cell surface where, again encountering a pH of 7.4, the protein is released for another cycle of iron transport. Recently, *Steap3* (6-transmembrane epithelial antigen of the prostate 3), an endosomal ferrireductase that facilitates Tf-mediated uptake of iron in erythroid precursors has been identified.[8] Steap3 is expressed highly in hematopoietic tissues, colocalizes with the Tf cycle endosome and facilitates Tf-bound iron uptake. Erythroid cells from mice deficient in *Steap3* (*nm1054*) are defective in Tf-dependent iron uptake, resulting in a hypochromic, microcytic anemia typical of iron deficiency[9] whereas overexpression of Steap3 stimulates the reduction of iron. Taken together, these findings indicate that Steap3 is an endosomal ferrireductase required for efficient Tf-dependent iron uptake in erythroid cells. However, *nml054* and *Steap3*$^{-/-}$ erythroid precursors retain some residual ferrireductase as well as iron uptake activity, indicating that there are other ways of reducing iron in the Tf-cycle endosome and/or that there are other endosomal iron transporters that are not restricted to reduced Fe(II). Therefore, the expression of *Steap2* and *Steap4* in erythropoietic tissues, the partial colocalization of epitope-tagged forms with Tf and TfR1, and the demonstration that they have ferrireductase activity *in vitro* indicate that Steap2 and Steap4 are reasonable candidates for redundant ferrireductases in the erythroid Tf-cycle endosome.[10]

Identification of the exocyst as an important partner of Tf cycle came from elucidation of the molecular basis of the hemoglobin-deficit mouse mutant (*hbd*).[11] These mutant mice are characterized by a hypochromic, microcytic anemia that is inherited in an autosomal recessive manner. Two independent groups have simultaneously identified the defect as being caused by the removal of an exon from the *Sec15LI* gene.[11,12] The yeast homolog of the *Sec15L1* protein is an integral member of the exocyst, a group of proteins that orchestrate transport of secreted material from the Golgi, likely involved in vesicular

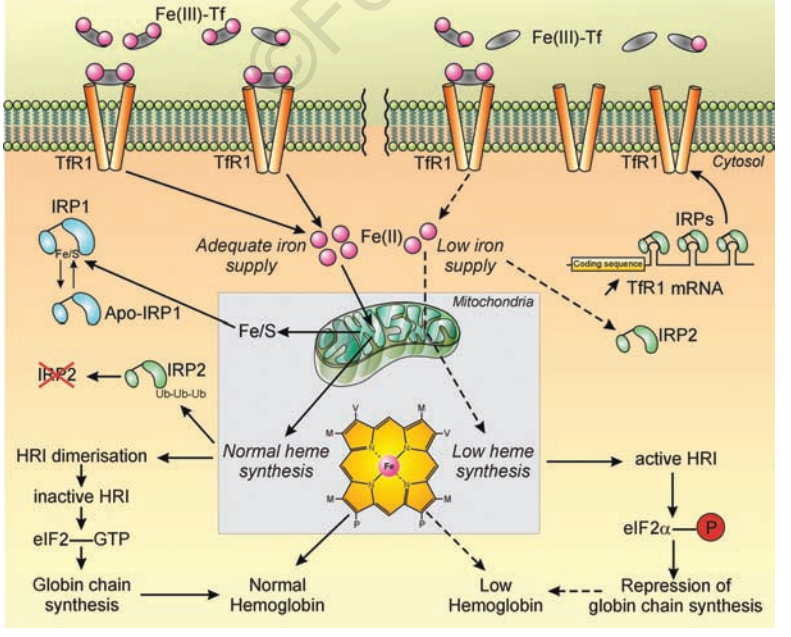

Figure 1. The iron acquisition pathway in developing erythroblasts The Fe(III)-Tf complex binds to specific receptors present on the cell surface, inducing the formation of an endocytic vesicle. Acidification of the endosome will release iron from transferrin and Steap3, an erythroid-specific reductase, will reduce iron to its ferrous Fe(II) state. DMT1/Nramp2, a proton and Fe(II) co-transporter present in the endosomal membrane (see putative structure in lower insert) will export iron into the cytosol. Most of the iron will be targeted to mitochondria to participate in heme synthesis or in Fe/S cluster assembly. Mitoferrin is part of the mitochondrial iron transport machinery. Ferrochelatase, the last enzyme of the heme biosynthetic pathway, catalyzes the insertion of Fe(II) into PPIX to form heme (right insert) *Illustration by Jean-Pierre Laigneau.*

fusion.[13] Tf-Fe(III) complex appears to bind to the TfR in *hbd* reticulocytes and to enter the cells by the endosomal pathway[14] but Lim *et al*. hypothesized that the *hbd* mutation leads to premature exocytosis of Fe-containing vesicles, thus reducing iron bioavailability in the cells,[11] while White *et al*. suggested that the consequences of *Sec15L1* mutation involve a reduction in the efficiency of vesicular trafficking, docking, fusing, and/or cargo delivery.[12] Similar data were obtained by Zhang *et al*. since they observed a decrease in 125I-Tf cycling in hbd reticulocytes.[14] The *hbd* mutant suffers a hypochromic, microcytic anemia but has no other phenotypic defects. This suggests that Sec15L1 must have a specific function since no tissues other than erythroid cells appear to be affected.

The entire Tf-TfR cycle is completed within a few minutes, and 100-200 such cycles are experienced by Tf during its lifetime in the circulation.[15]

TfR1 plays a critical role in iron homeostasis by serving as a gatekeeper regulating iron uptake from Tf. Homozygous TfR1 mutant mice were not viable beyond embryonic day 12.5 and had severe anemia with hydrops as well as diffuse neurological abnormalities.[16] It seems that inadequate iron led to neuronal apoptosis, but that tissues other than erythrocytes and neurons can obtain sufficient iron for growth and development through mechanisms independent of the transferrin cycle. Haploinsufficiency for the transferrin receptor resulted in microcytic, hypochromic erythrocytes; normal hemoglobin and hematocrit values were due to compensatory increase in red cell numbers. Although iron saturation of serum transferrin was indistinguishable from that of wild type, heterozygotes had significantly less tissue iron. Recently, reduced expression of TfR1 was found in a mouse model with deficient Stat5 expression (*Stat5a/b*$^{-/-}$) and the expression of constitutively active STAT5A in an erythroid cell line induced Tfr1 levels. Tissue specific deletion of *Stat5a/b* in hematopoietic cells induced hypochromic microcytic anemia.[17] Moreover, STAT5A/B is an important regulator of erythroid progenitor iron uptake via its control of *IRP2* expression, its defect resulting in more than 2-fold lower cell-surface expression of TfR-1 and thus strongly reduced iron uptake in erythroid cells.[18]

Mitochondrial iron

From the endosome to the mitochondria

In erythroid cells, most of the iron leaving the endosome will be targeted to mitochondria to participate in heme synthesis and iron-sulfur cluster assembly.[19] The precise chemical form of iron in transit between the endosome and the mitochondria is still a matter of debate.[20]

Mitoferrin (SLC25a37), a protein belonging to the family of mitochondrial carrier proteins expressed in the inner mitochondrial membrane, is thought to be implicated in shuttling iron across mitochondrial membranes. The zebrafish mutant *frascati* with a mutated mitoferrin gene shows profound hypochromic anemia and maturation arrest at the stage of immature erythroblasts.[21] Mouse erythroblasts derived from embryonic stem cells with a defective mitoferrin gene show a complete inhibition of iron incorporation into heme as compared to wild type cells. There are two paralog genes in mammals, one ubiquitously expressed and the other one expressed exclusively in developing erythroblasts. Deletion or overexpression of the two yeast homologs Mrs3 and Mrs4 affects incorporation of iron into protoporphyrin IX and the efficacy of iron sulfur cluster assembly.[22]

Heme biosynthesis

Heme is the prosthetic group of various types of proteins, such as hemoglobin, myoglobin, cytochrome p450, catalase and peroxidase. Most of these have important functions in erythropoiesis and erythrocytes. In mammals, eight enzymes are involved in the heme biosynthetic pathway, located successively in the mitochondria, the cytosol and the mitochondria. The first enzyme of the pathway, 5-aminolevulinate synthase (ALAS), which catalyzes the condensation of glycine and succinyl CoA to form 5-aminolevulinic acid (ALA), plays a critical role in controlling the heme biosynthetic pathway. It is encoded by two different genes, ALAS1, ubiquitously expressed and ALAS2, present on the X chromosome and expressed only in erythroid cells. Heme exerts a negative control over ALAS1 expression, especially in the liver, by inhibiting transcription of the gene, translation of the mRNA and mitochondrial targeting of the enzyme,[23] whereas regulation of ALAS2 expression in erythroid cells is only dependent on iron (see subheading "the iron sensing pathway").

Ferrochelatase, the last enzyme of the pathway, is located in the mitochondrial inner membrane and catalyzes the insertion of Fe(II) iron into protoporphyrin IX (PPIX) (Figure 1). In iron deficiency anemia, PPIX will accumulate in erythroid cells in the form of a zinc chelate, whereas free PPIX accumulates in conditions of ferrochelatase deficiency (see subheading "Anemia and porphyria"). Following its synthesis, heme is exported out of the mitochondria to be associated to globin chains and apocytochromes. Heme export from the mitochondria is thought to be mediated by ATP-Binding Cassette (ABC) transporters, with several possible candidates such as ABC-me[24] or ABC-G2.[25]

Iron sulfur cluster assembly

Another important utilization of iron in the mitochondria is to ensure the synthesis of the iron-sulfur clusters (ISC). These ancient modular protein cofactors consist of iron cations (Fe^{2+} or Fe^{3+}) and sulfide anions (S^{2-}). These ISC are associated with specific sites in the acceptor proteins through co-ordination by the sulfur of cysteine residues. Fe/S proteins are among the most important electron carriers in nature and are found in the mitochondria, the cytosol and the nucleus. They are integral components of respiratory and photosynthetic electron transfer chains, in which groups of ISC function as a linear series of redox centers or electron reservoirs. Eukaryotic machineries for the biogenesis of Fe/S proteins comprise the ISC assembly machinery in the mitochondria, the mitochondrial ISC export system and the cytosolic Fe/S protein assembly machinery.[26] Regulations of these machineries in the erythroid cells and control of the distribution of iron between heme synthesis and the ISC machinery are not known but molecular defects in some of these components can lead to microcytic anemia (see subheading "new form of sideroblastic anemia).

Heme export

The feline leukemia virus, subgroup C, receptor (FLVCR) was originally cloned as a cell surface protein that served as a receptor for feline leukemia virus, subgroup C and subsequently found to be a heme exporter.[27,28] Cats viremic with FeLV-C develop pure red cell aplasia, characterized by a block in erythroid differentiation at the CFU-E-proerythroblast stage, reticulocytopenia, and severe anemia. Studies with chimeric retroviruses suggest that the surface unit of the FeLV-C envelope protein induces this phenotype by blocking FLVCR function. This transporter belongs to the family of MFS (major facilitator superfamily) proteins that transport small solutes across membranes by using the energy of ion-proton gradients. However, unlike cells that express HCP1 (heme carrier protein 1), cells engineered to express FLVCR showed significant reduction in cellular heme content, suggesting that FLVCR is involved in heme transport.[28]

Mice with neonatal inactivation of FLVCR develop a severe macrocytic anemia with proerythroblast maturation arrest, which suggests that erythroid precursors export excess heme to ensure survival.[29] Thus, the trafficking of heme, and not just elemental iron, facilitates erythropoiesis and systemic iron balance.

Co-ordinated regulation of iron acquisition and heme synthesis in erythroid cells (HRI, IRPs)

Iron plays a crucial role in many facets of mitochondrial metabolism.[19,30] Trafficking and storage of iron in the mitochondria must be tightly regulated as disruption of these pathways can be catastrophic. For example excess free iron promotes the generation of harmful reactive oxygen species[31] whereas an inadequate supply of iron impairs the metabolic and respiratory activities of the organelle and prevents hemoglobin formation in developing erythroid precursors. Thereby, several mechanisms are operative to co-ordinate iron acquisition and heme synthesis, especially in erythroid cells[32] (Figure 2).

The iron sensing pathway

There are two Iron Regulatory Proteins (IRP1 and IRP2) that act as iron sensors in mammalian cells and regulate translation or stability of mRNAs encoding proteins of iron metabolism.[33] In their native conformation, both IRPs have a high binding affinity for short hairpin structures called Iron Responsive Element (IRE) present in the mRNAs of their target genes. Binding of IRPs to the IRE present in the 5' untranslated region of the H and L ferritin mRNAs, as well as in the ferroportin and in the ALAS2 mRNA, repress translation. IRPs binding to the multiple IREs present in the 3' untranslated region of TfR1 mRNA stabilize the mRNA. IRP1 is an ISC protein that loses its RNA binding activity in iron-replete conditions through acquisition of a 4Fe-4S cluster whereas IRP2 is oxidized, ubiquinated and subsequently degraded by the proteasome. This regulation explains the increase observed in the expression of TfR1 in erythroid cells in conditions of iron deficiency.[34] However, the respective roles of IRP1 and IRP2 in erythroid cells is not clear since IRP1 knock-out mice have no erythroid phenotype[35] whereas IRP2 knock-out mice develop microcytic hypochromic anemia probably resulting from decreased TfR1 expression in bone marrow cells.[36,37]

This standard regulation of iron metabolism seems to have an alternative model during terminal erythroid differentiation. Schranzhofer *et al.*[38] demonstrated that during terminal differentiation of primary mouse erythroid progenitors, ferritin mRNA translation is massively impaired, whereas translation of *ALAS2* mRNA proceeds unimpeded. This dual mechanism has not been fully understood but in developing erythroblasts it contributes to maintaining the high flux of incoming iron available for heme synthesis rather than being sequestered into the ferritin molecules.

The heme sensing pathway

Besides its function as prosthetic moiety in heme proteins, heme itself can influence gene expression at the

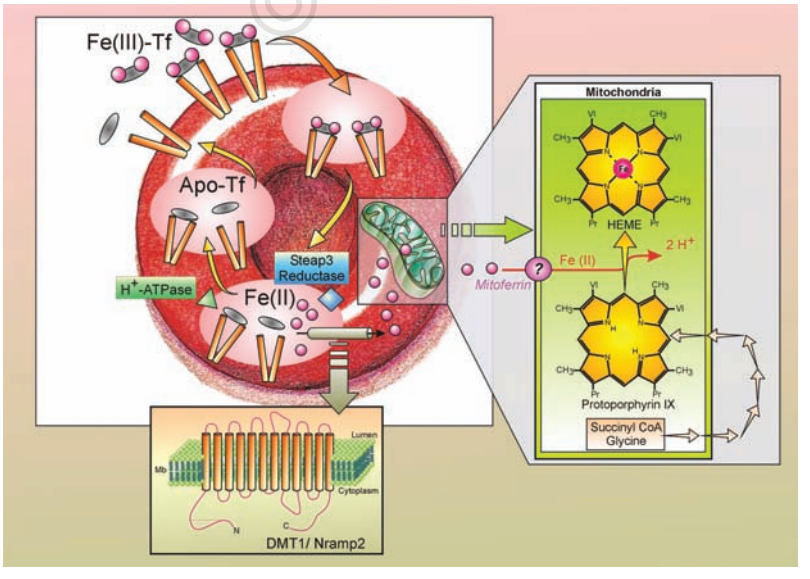

Figure 2. Iron and heme sensing pathway in developing erythroblasts. The left part of the figure shows that when iron supply is adequate, heme and ISC are normally synthesized. Available Fe/S clusters will inactivate IRP1, stimulating ALAS2 synthesis and heme will inactivate HRI, allowing a full rate of globin synthesis and hemoglobin formation. *Uncommitted* heme or *free* iron will contribute to ubiquitination and degradation of IRP2 to prevent excessive iron uptake. The right part of the figure shows the effect of low iron supply on IRP2 activation and stabilization of TfR1 mRNA as well as activation of HRI that will phosphorylate eIF2α, leading to repression of globin chain translation.

level of transcription, protein synthesis, miRNA processing or post-translational modifications. Heme is a potent inducer of heme oxygenase 1 (*HO1*), a cytoprotective and anti-inflammatory molecule which catalyzes heme degradation. Heme inactivates the transcriptional repressor Bach1, thereby allowing the binding of Nrf2 to Maf recognition elements (MAREs) present in the regulatory regions HO1.[39] MAREs are also present in the enhancer of the H ferritin gene[40] or in the β globin Locus Control Region.[41] Although HO1 expression has not been extensively studied in erythroid cells, it has been shown that HO1 mRNA decreases following erythroid differentiation of Friend erythroleukemia cells, while mRNAs coding for the enzymes of the heme biosynthetic pathway increase.[42]

By contrast, heme deficiency will repress protein translation, and especially globin synthesis. During erythroid differentiation, it is essential to have a well balanced production of α globin and β globin chains as well as of the heme molecules, to ensure the formation of the hemoglobin (2α-2β-4heme) molecule. Since globin chains tend to misfold and precipitate in the absence of proper binding to heme, heme deficiency activates a stress protein kinase named heme regulated inhibitor (HRI) that phosphorylates eIF2a.[43,44] When eIF2 is phosphorylated, it remains bound to its binding protein, thereby preventing the regeneration, of GDP into GTP and shutting down mRNAs translation. HRI deficient mice present hyperchromic normocytic anemia with erythroid hyperplasia and erythrocytes loaded with multiple globin inclusions.[45]

Additional regulatory roles of heme have been demonstrated, although not specifically in erythroid cells. *Uncommitted* heme has been shown to down-regulate IRP2 by ubiquitination and degradation, thereby inducing destabilization of TfR1 mRNAs and reduced iron uptake.[46] Very recently a new and very important role has been attributed to heme in microRNA processing. Faller *et al.*[47] demonstrated that heme is involved in pre-miRNA processing suggesting that the gene-regulation network of miRNA and signal-transduction pathways involving heme might be connected.

Therefore, during maturation of erythroid precursors there is an interplay of positive and negative regulations to maintain sufficient iron supply for heme synthesis and to prevent formation of heme in excess of globin chains.

Erythrophagocytosis and recycling of heme iron

The amount of iron required for the daily production of 300 billion red blood cells is provided mostly by recycling of heme iron by macrophages, following phagocytosis of senescent erythrocytes. This process allows the recycling of about 20-25 mg of iron per day, corresponding mostly to what is needed daily for bone marrow erythropoiesis.[48] In normal conditions, iron absorbed from the diet by duodenal enterocytes (1-2 mg) is required to compensate for daily losses, resulting from desquamation of epithelial cells and minor blood losses. Throughout their life span, circulating erythrocytes will accumulate biochemical changes at their surface, such as peroxydation of membrane-bound lipoproteins, loss of sialic acid residues and formation of senescence neoantigens, such as modified Band 3 molecules.[49] Eryptosis, a particular programmed cell death characteristic of red blood cells characterized by cell shrinkage and externalization of phosphatidyl-serine, will also contribute to the removal of senescent erythrocytes[50] through recognition by CD36, the phosphatidyl-serine receptor on macrophages. These modifications will allow tissue macrophages from bone marrow, spleen and liver to identify the red blood cells to be eliminated through interactions with specific receptors, such as the Fc receptor or the scavenger receptor. After this initial recognition step, the red blood cell is internalized by phagocytosis and the phagosome undergoes a series of fusions with intracellular vesicles and with the endoplasmic reticulum to acquire the machinery necessary for the degradation of red cell constituents.[51] The heme molecule is catabolized by an enzymatic complex anchored in the endoplasmic reticulum membrane and comprising an NADPH-cytochrome c reductase, HO1 and biliverdin reductase. Iron released from heme catabolism is either recycled back to the plasma through ferroportin, a membrane-bound Fe (II) export molecule, or retained within the ferritin molecules, to be released at later stages. Erythrophagocytosis induces changes in macrophage gene expression, including HO1, ferroportin and ferritin, by several mechanisms. Heme is a potent transcriptional activator of HO1[52] and ferroportin genes whereas iron released from heme catabolism regulates ferroportin and ferritin mRNA translation[53] through the IRE/IRP system. However, the actual amount of iron exported from macrophages to the plasma is directly controlled by hepcidin through its interaction with ferroportin.[54,55] Modifications in erythrophagocytosis contribute to the pathogenesis of several disorders. Increased eryptosis of erythrocytes has been described in iron deficiency,[56] in sickle cell disease,[57] or in patients with non-alcoholic steatohepatitis,[58] contributing to reduced life span of red blood cells, severity of the anemia and abnormal liver iron deposits. Activation of macrophages by cytokines and increased erythrophagocytosis are hallmarks of the anemia of chronic diseases and hemophagocytic syndrome.[59] Therefore, erythrophagocytosis and recycling of heme iron appears as a central process in iron homeostasis.

Hepcidin

Hepcidin, a major regulator of iron homeostasis

In 2001, hepcidin was isolated and purified simultaneously by two groups who were trying to identify new antimicrobial peptides.[60,61] It was subsequently shown to be inducible by iron[62] and essential in the prevention of parechymal iron overload.[63] Hepcidin is synthesized in the liver as a pre-pro-peptide of 84 AA, including a N-Terminal signal peptide, a pro-region, and the C-terminal mature peptide of 25 AA. This mature peptide is secreted to the plasma and eliminated in urine. It is now well established that hepcidin reduces the quantity of

circulating iron by preventing its exit from the cells, especially from enterocytes and macrophages. To limit cellular iron egress, hepcidin binds to ferroportin, thereby inducing its internalization and degradation.[55,64] In the absence of hepcidin, increased intestinal iron absorption associated with increased iron efflux from macrophages leads to parenchymal iron overload.[1] This mechanism of hepcidin action accounts for the rapid reduction in plasma iron levels which follows direct hepcidin injection into mouse,[65] transgenic hepcidin gene induction[66] or hepcidin stimulation by IL-6 infusion.[67]

Regulation of hepcidin gene expression

Regulation of hepcidin gene expression is very complex and depends on multiple pathways. Iron overload and inflammation stimulate hepcidin gene expression whereas anemia, iron deficiency and stimulation of erythropoiesis repress its expression.[68] Basal hepcidin expression depends on hemojuvelin (HJV) that acts as a BMP (Bone Morphogenic Receptor) co-receptor and stimulates hepcidin gene transcription by signal transduction through Smad proteins.[69] HJV is expressed in liver, heart and skeletal muscle and is inserted into the plasma membrane through a GPI anchor. A soluble form of HJV (sHJV) produced in response to hypoxia or iron deficiency by a furin-mediated cleavage represses the BMP-signaling pathway and hepcidin gene expression.[70] It has been proposed that the iron-sensing system is based on the concentration of holotransferrin that could be detected by HFE and TfR2[71] but a more recent study shows that iron stimulates the expression of proteins of the BMP/Smad pathway.[72]

Inflammation, and especially IL6[67] and IL-1β,[73] stimulates hepcidin expression through a *Stat3* dependant pathway. The characteristic features of the inflammation anemia (reduction in serum iron, retention of iron in macrophages and blocking of intestinal iron absorption) are all compatible with the consequences of an increase in the production of hepcidin.[74] Conversely, anemia, hypoxia, bleeding and erythropoietin injections also inhibit the synthesis of hepcidin through mechanisms which have not been clarified. Furthermore, paradoxically, in dyserythropoietic states such as thalassemia intermedia or myelodysplastic syndromes, hepcidin expression is repressed and leads to iron overload, despite the anemia; therefore these conditions are called iron loading anemias. Recently, it has been proposed that the elevated serum concentrations of GDF15, a member of the transforming growth factor-β superfamily, found in patients with thalassemia, were responsible for the inhibition of hepcidin expression.[75] However in thalassemia major, transfusions suppress endogenous erythropoiesis and hepcidin levels tend to be high.[76] Recently, some of us have shown that stimulation of erythropoiesis by repeated blood samplings or erythropoietin injections represses hepcidin expression in a mouse model of chronic inflammation, despite the presence of elevated IL6 expression.[77] Thus, the erythroid signaling pathway dominates over the iron sensing pathway or over the IL6/Stat3 signaling. Hepcidin appears as the major, if not the only, regulator of iron delivery to the plasma.

Genetic microcytic anemia

Sideroblastic anemia (ALAS2, ABCB7, GRX5)

Sideroblastic anemia is a group of disorders characterized by a variable population of hypochromic red cells in the peripheral blood and by ringed sideroblasts in the bone marrow. The latter are not to be confused with the sideroblasts in the marrows of normal subjects (red cell precursors that contain stainable iron granules). Ringed sideroblasts are found only in pathological states. The iron granules are denser and more abundant and form a partial or complete ring around the nucleus of the cell. As seen on an electron microscopy, ringed sideroblasts are normoblasts with iron-laden mitochondria.[78] Retention of iron in the mitochondria and the associated hypochromic anemia appear to reflect a defect in mitochondrial heme synthesis.[79,80]

X-linked sideroblastic anemia due to ALAS2 mutations

The most frequent form of inherited sideroblastic anemia is X-linked sideroblastic anemia (XLSA), caused by mutations in the erythroid-specific *ALAS2* gene located at chromosome Xp11.21.

The zebrafish mutant *sauternes (sau)* has a microcytic, hypochromic anemia, suggesting that hemoglobin production is perturbed.[81] Using positional cloning techniques, it was established that *sau* encodes ALAS2. A mouse model of ALAS2 deficiency was also created. An intriguing difference between the human disease and the mouse model is the absence of ring sideroblasts in the mouse model.[82] However, introduction of a low expressed ALAS2 gene in the mutant animals allowed formation of ring sideroblasts.[83]

Hemizygous males have microcytic anemia and iron overload, so that XLSA belongs to the group of iron loading anemias. Various mutations in ALAS2 have been reported leading to defective ALAS2 activity in bone marrow erythroblasts, to impaired heme biosynthesis and to insufficient PPIX to use all the available Fe.[80] Most of the Fe deposited in perinuclear mitochondria of ringed sideroblasts is stored in mitochondrial ferritin.[84] This accumulation leads to increased ROS and shortening of cell life.

The features of the X-linked disorder include: (i) detectable disease in newborns but not always present clinically until mid-life or, rarely, later; (ii) death from hemochromatosis at a relatively young age, with the number of transfusions inadequate to account for the iron overload; and (iii) abundance of siderocytes in peripheral blood after splenectomy.

Most ALAS2 mutations are missense mutations[79] and in most cases, the mutation in this gene appears to affect the affinity of the enzyme for its cofactor, pyridoxal 5'-phosphate;[85] these patients are, to some extent, responsive to pyridoxine which is metabolized to pyridoxal 5'-phosphate. In other cases, the ALAS2 gene mutation either decreases the processing or the stability of the enzyme, leading to reduction of its level,[86] or abrogates its interaction with protein partners,[87] thus rendering patients resistant to pyridoxine treatment.

X-linked sideroblastic anemia with ataxia

X-linked sideroblastic anemia with ataxia (XLSA/A) is a rare syndromic form of inherited sideroblastic anemia of early onset, associated with non- or slowly progressive, predominantly truncal, spinocerebellar ataxia and severe, selective cerebellar hypoplasia. The anemia of XLSA/A is typically mild and may be overlooked until after the neurological presentation. This form of sideroblastic anemia is due to defects in ABCB7, functional homolog of yeast Atm1p.[88] ABCB7 deficiency causes the mitochondrial accumulation of Fe, elevated free erythrocyte PPIX levels and mild hypochromic microcytic anemia. This deficiency induces a disruption in the maturation of cytosolic ISC, indicating that ABCB7 is an essential component of the ISC export machinery.[89] The ataxia observed in ABCB7-deficient patients may be related to the mitochondrial damage generated by iron loading in neural cells. Interestingly in Friedreich ataxia iron plays an important role in neurological damage; a recent study showed that iron chelator treatment was able to reduce neuropathy and ataxic gait in the youngest patients, with no obvious hematologic or neurological side effects.[90]

New form of sideroblastic anemia

Sideroblastic anemia due to mutation in Glutaredoxins (GRXs or GLRXs) is a new entity which has been recently described.[91,92] GRXs are small ubiquitous disulfide oxidoreductases known to use reduced glutathione (GSH) as electron donor and are part of the ISC assembly machinery. Loss of grx5 function in yeast leads to mitochondrial iron accumulation and production of reactive oxygen species (ROS). *Grx5* deficient zebrafish (*shiraz*, sir) showed severely hypochromic red blood cells, deficient definitive hematopoiesis, and embryonic lethality between days 7 and 10 post-fertilization.[91] The authors predicted that a block in ISC assembly in *sir* caused more active IRP1 to be present, inappropriately reflecting a low-iron state and causing the repressed synthesis of some target genes such as ALAS-2.

Very recently Camaschella *et al.*[92] described the first human mutant of *GRX5*. The patient had a homozygous (c.294A>G) mutation that interferes with intron 1 splicing and drastically reduces *GLRX5* mRNA. He suffers with a mild microcytic anemia associated with iron overload and a low number of ringed sideroblasts. Surprisingly hemoglobin level progressively declined with age until the patient became transfusion dependent. Anemia was partially reverted by iron chelation. This clinical case allows the proposal of a model of iron and IRP regulation in GLRX5-deficient non-erythroid and erythroid cells. In non-erythroid cells, impaired ISC synthesis activates IRP1 leading to high cellular iron import and high iron levels degrade IRP2. Iron levels are increased to a similar extent both in cytosol and the mitochondria. On the other hand, in erythroid cells, IRP1 activation occurs as in non-erythoid cells but the imported iron is directed to mitochondria yet is not utilized due to PPIX deficiency secondary to ALAS2 repression. Low heme allows high IRP2 activity, stabilizion of TfR1 mRNAs and further increase in iron uptake. The defective ISC assembly leads to iron deposition in mitochondria and formation of ring sideroblasts. This vicious circle may be relieved by DFO treatment, which shifts iron to the cytosol, modulating IRP2 activity, improving erythrocyte hemoglobinization and decreasing ringed sideroblasts. Finally, several cases of genetic sideroblastic anemia not related to *ALAS2, ABCB7* or *GRX5* are known (*A. Iolascon and C. Beaumont, personal observations, 2008*), suggesting that other genes implicated in this disease remain to be identified. Genes encoding enzymes of the ISC assembly are possible candidate genes since mutation in any of these genes in yeast lead to abnormal deposition of iron in mitochondria. However, these enzymes are still poorly characterized in mammals, and have not been studied in erythroid cells.

DMT1 mutants

DMT1 mutations were first described in microcytic anemia (*mk*) mice[93] and belgrade (b) rats,[94] both models carrying the same *G185R* in transmembrane domain (TM) 4 of this protein. Both animals exhibit severe microcytic, hypochromic anemia due to a defect in iron uptake in the intestine but also in iron acquisition and utilization in peripheral tissues, including RBC precursors.[6] In the zebrafish *chardonnay* (*cdy*) mutant, DMT1 was identified as the mutated gene, through a combination of positional and candidate cloning approaches.[95] Like in rodent *DMT1* mutants, cdy animals are viable and have a hypochromic, microcytic anemia.

Up to now, three human patients have been reported with *DMT1* mutations. These mutations cause microcytic hypochromic anemia due to decreased erythroid iron utilization but, in contrast to animal models, lead to increased liver iron stores. Also, transferrin saturation is high and serum ferritin is mildly elevated. Normal to low urinary hepcidin levels with regards to the levels of iron stores have been found in these patients, probably accounting for the increased intestinal iron absorption.[96] In the *DMT1* deficient animals, the absence of heme in the diet probably prevents the onset of iron overload. The anemia is present from birth and its severity is variable.[97] Functional studies of the mutant proteins suggest some phenotype/genotype correlation.

The mildest anemia was found in a Czech patient with 1285G>C mutation affecting the last nucleotide of exon 12, and leading to an E399D replacement and preferential skipping of exon 12.[97] Functional studies demonstrated that the E399D protein is normally expressed and fully functional[98] but 90% of DMT1 transcripts in the patient's erythroid cells missed exon 12 and encoded a shorter and probably unstable version of the protein.[97] Shortly thereafter, a patient suffering from severe microcytic anemia from birth was reported as compound heterozygote, including a 3-bp deletion (delCTT) in intron 4 that partially impaired normal splicing and an amino acid substitution (R416C) at a conserved residue in TM9 of the protein.[99] Thus, a quantitative reduction in DMT1 expression could be the cause of this patient's severe anemia. Although no functional studies were performed

on the mutated proteins from the third French patient, it can be speculated that the milder phenotype reflects residual transport activity of the rather conservative G212V mutation in TM5, whereas the *delVal114* is likely to disrupt the TM structure.[100]

Chelation therapy aiming at reducing the liver iron burden was either ineffective or led to a prompt drop in hemoglobin level and thus was interrupted (*G. Tchernia and C. Beaumont, unpublished data, 2007*). All 3 patients described up to now, appeared to respond to erythropoietin (Epo) administration.[96,101] Because mean corpuscular volume and mean cell hemoglobin did not change during Epo treatment, it was concluded that Epo did not improve iron utilization of the erythroblasts, but likely reduced the degree or intensity of apoptosis, affecting erythropoiesis. Based on the clinical course of patient 1, the Epo treatment maintained for four years did not prevent liver iron accumulation. However, serum ferritin levels were drastically reduced after three months of Epo therapy.

TMPRSS6 mutants

Several studies on a mouse model and human patients have very recently highlighted the unexpected role of membrane-bound serine protease type 6 (TMPRSS6) in the control of hepcidin regulation in response to iron deficiency.

TMPRSS6 is a member of type 2 transmembrane serine proteases family with unique structural features including (i) a serine protease domain, (ii) a transmembrane domain, (iii) a short cytoplasmic domain, and (iv) a stem region containing an LDL receptor class A (LDLRA) and a scavenger receptor cysteine-rich (SRCR) domain.[102] In both humans and mice, the major site of TMPRSS6 expression is the liver. By positional cloning, Du *et al.* were able to ascribe the Mask mouse phenotype to a splicing error in the Tmprss6 gene.[103] Mask mice have microcytic anemia due to iron deficiency, caused by inefficient iron absorption, and regional alopecia of truncal hair, which is corrected with iron supplementation. In contrast to typical iron deficiency in which the iron regulator hepcidin is greatly decreased, Mask mice have elevated hepcidin and profound iron deficiency. Very recently, Finberg *et al.* identified TMPRSS6 mutations in individuals with iron-refractory iron deficiency anemia (IRIDA).[104] These patients are characterized by hypochromic, microcytic anemia and very low serum iron values, always below 2-5 μmol/L and normal or slightly elevated hepcidin levels despite the severe anemia. These patients are refractory to a treatment with oral iron and show abnormal iron utilization characterized by a sluggish, incomplete response to parenteral iron. In most cases, anemia was not detected at birth.[104] As more cases are being found, the frequency and the full clinical pictures of these patients will be more clearly defined.[105,106] The finding of inappropriately elevated urinary hepcidin levels in individuals with IRIDA provides insight into the pathophysiology of the disorder. It may explain the failure to absorb dietary iron despite systemic iron deficiency, as well as the coexistent failure to respond to parenteral iron complexes, which must be processed and exported by macrophages before utilization.[107] Because overexpression of TMPRSS6 blocks activation of diverse pathways for upregulation of hepcidin, the overexpression or dysregulation of TMPRSS6 might cause iron overload, whereas mutational inactivation of TMPRSS6 (as in Mask mice) would lower body iron content.[103] This transcriptional effect might be achieved by cleaving a protein that up-regulates a pathway that normally represses hepcidin transcription, or that down-regulates a pathway that normally activates hepcidin transcription. Furthermore, the finding that TMPRSS6 regulates hepcidin levels in humans may have potential applications for treatment of iron disorders. For example, inhibition of the putative protease function might be a potential treatment for disorders in which hepcidin is inappropriately low, such as primary hemochromatosis and iron loading anemia. Similarly, treatment with agonists or with the endogenous substrate of TMPRSS6 might be employed in the anemia of chronic disease, in which hepcidin is inappropriately high.[104]

Anemia and porphyria

Porphyrias are a heterogeneous group of diseases resulting from a partial deficiency of an enzyme of the heme biosynthesis pathway,[108] with the exception of mutations in ALAS2 which give rise to sideroblastic anemia. In patients with porphyrias, heme synthesis usually remains normal since these enzyme deficiencies are only partial and the principal clinical manifestations result from accumulation of porphyrins or their precursors (ALA and PBG). Porphyrias are classified as hepatic or erythropoietic, depending on the major production site of porphyrins. There are two erythropoietic porphyria, namely congenital erythropoietic porphyria (CEP) due to mutations in Uroporphyrinogen III synthase, and erythropoietic protoporphyria (EPP) due to mutations in ferrochelatase. Both diseases are associated with anemia, although the features of these anemias differ between the two pathological conditions. Animal models for these two diseases have been developed and have helped to define these features.

Congenital erythropoietic porphyria

Congenital erythropoietic porphyria (CEP) is transmitted as an autosomal recessive trait. Patients with CEP are either homozygotes or compound heterozygotes for mutations of the *UROS* gene at 10q25.2-q26.3 that encodes Uroporphyrinogen III synthase (UROS). The associated hemolytic anemia is generally not microcytic.

Very recently, a 3-year old boy with the clinical phenotype of CEP was described.[109] The anemia was similar to that of thalassemia intermedia. Fetal hemoglobin (Hb F) was dramatically increased and moderate thrombocytopenia was present. No mutation or rearrangement in *UROS* or in the globin genes or in their major regulatory elements were identified. Instead, a novel R216W germ line mutation in the X-linked erythroid-specific transcription factor GATA binding protein 1 (GATA-1)

was observed. Mutations in several genes encoding transcription factors (eg, *ATRX*, *TFIIH*, and *GATA1*) have proven causative in rare cases of thalassemia. This case represents the first description of a *trans*-acting mutation associated with CEP, and the first association of CEP with a collective hematologic phenotype of microcytic anemia. The R216 residue is located in a region of the N-terminal zinc finger of the GATA-1 protein that is highly conserved. Five germ line mutations of *GATA1* causing X-linked disorders have been described, and all cluster in the N-terminal zinc finger. All are associated with altered affinity of GATA-1 for either its cofactor, Friend of GATA-1 (FOG1), or with palindromic DNA GATA recognition sites.[109] The mutation might impair the function of the alternative erythroid-specific promoter of the UROS gene that contains several GATA-1 binding sites.[110] Interestingly, a pathogenic mutation in one of these GATA-1 binding elements was found in a CEP patient.[111]

Erythropoietic protoporphyria

Erythropoietic protoporphyria (EPP) is caused by decreased activity of the enzyme ferrochelatase (FECH), the terminal enzyme of the heme biosynthetic pathway, which catalyzes the insertion of iron into PPIX to form heme. The *FECH* gene has been cloned, sequenced and mapped to the long arm of chromosome 18. EPP is characterized clinically by photosensitivity to visible light commencing in childhood, and biochemically by elevated red cell PPIX levels. In rare cases, the disorder is transmitted as an autosomal recessive trait with typically a residual lymphocyte FECH activity < 10% of normal. In most cases, the inheritance of EPP is described as an autosomal dominant disorder with incomplete penetrance. In a family, affected siblings usually share a hypomorphic allele that is common in the general population in *trans* to a rare loss-of-function allele. A common intronic SNP, IVS3-48C, is responsible for the low expression of the hypomorphic allele.[112]

FECH deficiency leads to accumulation of PPIX in normoblasts, erythrocytes, plasma, skin, and liver, causing lifelong acute photosensitivity and, in approximately 2% of patients, severe liver disease. Rademakers *et al.*[113] conducted an ultrastructural examination of the bone marrow and consistently found finely dispersed electron-dense deposits (iron) localized in mithocondria of erythroblasts in patients with EPP, although these observations have never been confirmed. As molecular mechanisms continue to be identified, phenotype/genotype correlations should become apparent, and it may eventually be possible to identify those patients at risk of developing hepatic failure. Microcytic anemia occurs in 20-60% of patients.[114] In contrast to other inherited disorders of erythroid heme biosynthesis, the anemia is not dyserythropoietic, there is no iron overload, and there is evidence for iron deficiency without iron loss. A mouse model of EPP, the homozygous $Fech^{m1Pas}$ mutant, develops a similar microcytic anemia.[115] Although it is probable that anemia of EPP reflects limitation of heme formation by FECH deficiency, its incidence, mechanism, and relationship to disordered iron metabolism remain unclear.

Other forms of anemia

Hereditary atransferrinemia

Hereditary atransferrinemia, also known as congenital hypotransferrinemia, is a very rare disorder characterized by microcytic anemia and iron overload. To date, it has been reported in only 10 patients from 8 families. However, the molecular basis of atransferrinemia has been determined in only 4 human cases.[116] The predominant clinical features of this deficiency are pallor, fatigue and increased sensitivity to infections.[117] Because transferrin functions to deliver iron to the developing erythron, as well as to other tissues, atransferrinemia results in reduced delivery of iron to the bone marrow erythroid precursors and reduced hemoglobin synthesis. Some iron is present in the plasma in a non-transferrin bound form and results in severe iron overload of liver, pancreas, heart. This is exacerbated by increased intestinal iron absorption due to decreased hepcidin production.[118] Correction of the anemia and partial reduction of the iron overload have been obtained with monthly infusions of fresh-frozen human plasma[117] or apo-transferrin.[119] Congenital atransferrinemia is very uncommon and only a few patients have been characterized on a molecular basis until now, so it is rather difficult to make comments on phenotype/genotype correlations.

Hereditary aceruloplasminemia is a rare autosomal recessive disease characterized by anemia, iron overload and progressive neurodegeneration. The disease is caused by the absence of ceruloplasmin (Cp), a multicopper ferroxidase, which catalyzes the oxidation of ferrous to ferric iron, a change required for binding of iron to plasma transferrin. Cp knock-out mice display increased iron content in liver and spleen macrophages as well as in hepatocytes.[120] Ferrokinetic studies in $Cp^{+/+}$ and $Cp^{-/-}$ mice revealed a striking impairment in the movement of iron out of reticuloendothelial cells and hepatocytes, suggesting that Cp co-operates with ferroportin to allow iron export. Interestingly, another multi-copper ferroxidase called hephaestin is expressed in duodenal enterocytes and present at the plasma membrane as a GPI-anchored protein. Hephaestin mutation in sla (sex-linked anemia) mice results in reduced iron efflux from duodenal enterocytes and development of moderate microcytic anemia.[121] Therefore, Cp seems important for mobilization of iron stores and hephaestin for intestinal iron absorption. No hephaestin mutations have been found in human patients whereas hereditary aceruloplasminemia results in iron deposition in the liver, pancreas, basal ganglia, and other organs. Patients develop diabetes mellitus, retinal degeneration, ataxia, and dementia late in life.[122] A mild-to-moderate degree of anemia with low serum iron and elevated serum ferritin is a constant feature. Aceruloplasminemia has been described mainly in Japanese patients and rarely in whites.[123]

Pim-1

The human *pim-1* gene encodes a serine/threonine kinase, which belongs to the group of calcium/calmodulin-regulated kinases (CAMK). It contains a characteristic kinase domain, a so-called ATP anchor and an active

Table 1. Classification of inherited microcytosis due to defects in iron metabolism or heme synthesis (human diseases and animal models).

Disease	Gene	Human findings	Animal models
Heme synthesis			
Sideroblastic anemias			
X-linked sideroblastic anemia (XLSA)	Aminolevulinic acid synthase type II (*ALAS2*)	Microcytic anemia and iron overload	*Sauternes* (sau) zebrafish
X-linked sideroblastic anemia with ataxia (XLSA/A)	Adenosine triphosphate-binding cassette protein (*ABCB7*)	Predominantly truncal, spinocerebellar ataxia, accompanied by severe, selective cerebellar hypoplasia	
Sideroblastic-like microcytic anemia	Glutaredoxin 5 (*GLRX5*)	Hypochromic microcytic anemia, hepatosplenomegaly, and iron overload	*Shiraz* zebrafish
Erythropoietic porphyria			
Erythropoietic protoporphyria (EPP)	Ferrochelatase (*FECH*)	Photosensitivity, and occasionally, acute hepatic failure, and microcytic anemia (20-60% of patients)	Homozygous *Fechm*^{1Pas} mouse, and homozygous *drc*^{m248} zebrafish
Congenital erythropoietic porphyria (CEP)	Uroporphyrinogen III synthase (*UROS*) GATA binding protein 1 (*GATA-1*)	Photosensitivity, red urine, a and hirsutism soon after birth Hematologic phenotype of β-thalassemia intermedia, markedly elevated Hb F levels, and thrombocytopenia	*Urosmut*^{248} knock-in mouse
Iron metabolism deficiency			
Iron deficiency anemia	Divalent metal transporter (*DMT1*; also *NRAMP2*, *DCT1*)	Microcytic hypochromic anemia and iron overload	*mk* (mice), *b* (rats), and *chardonnay* (*cdy*) zebrafish
?	Duodenal cytochrome b (*DCYTB*)		*Cybrd1-/-* mice (not anemic, and their hematologic parameters were indistinguishable from wild-type mice)
Ferroportin disease	Ferroportin/*IREG1/MTP1*	Mild anemia has been reported in some patients with loss-of-function ferroportin mutations	*weißherbst* (weh) zebrafish (severe hypochromic anemia, poor viability)
?	Hephaestin (Hp)		Sex-linked anemia (*sla*) mice (moderate to severe microcytic hypochromic anemia)
Hereditary atransferrinemia	Transferrin (Tf)	Pallor and fatigue, some patients have mild hepatomegaly, iron overload and hypochromic anemia	*hpx* mice, gavi^{HE067} and gavi^{IT029} zebrafish
?	Transferrin receptor 1 (TfR1)		Chianti (*cia*) zebrafish (hypochromic, microcytic anemia)
?	*Sec15l1*		Hemoglobin deficit (*hbd*) mice (hypochromic anemia)
?	Transmembrane epithelial antigen of the prostate 3 (*STEAP 3*)		*Steap3*$^{-/-}$ mice (hypochromic, microcytic anemia)
Hereditary aceruloplasminemia	Ceruloplasmin (Cp)	Hepatic iron overload, diabetes, peripheral retinal degeneration, dystonia, dementia, or dysarthria microcytic anemia, low serum iron and elevated serum ferritin	*Cp* knockout (mice)
I RIDA	Type 2 transmembrane serine protease 6 (*TMPRSS6*)	Hypocromic, microcytic anemia	*Mask* mice (iron deficiency and regional alopecia of truncal hair)

"?" indicates that there is no human counterpart of the given gene defect.

Table 2. **Biological characteristics of human inherited microcytosis due to defects in iron metabolism or heme synthesis.**

	XLSA	XLSA-A	GLRX5 def	EPP	DMT1 def.	aTrans-ferrinemia	Acerulo-plasminemia	TMPRSS6 Def. (IRIDA)
MCV (fL) (n=80-85)	60-75	60-75	55-70	60-75	45-55	50-60	60-75	49-60
Serum Fe	N or +	N or +	N	+	++	+	N	low
TFRsat.	+	+	+	+	++	+	+	low
sTFR	+	+	+	+	++	N	N	+
Sideroblasts	+++	++	+	no	no	no	no	no
PPIX	N or -	N or -	N	+++	+	N	N	
Liver iron	++	++	++	+	+++	+++	++	N
Neonatal appearance	no	no	no	yes	yes	yes	yes-no	no
Oral Fe effect	–	–	–	–	no	no	+	Refractory
IV iron effect	–	–	–	–	no	no	+	+/-
Transmission	X-linked	X-linked	AR	D /R	AR	AR	AR or AD	AR
Proposed therapy	vitB6	vitB6	chelation	B-carot	Epo	Plasma or apo-Tf	chelation	

N: normal value; +: increased; –: decreased values.

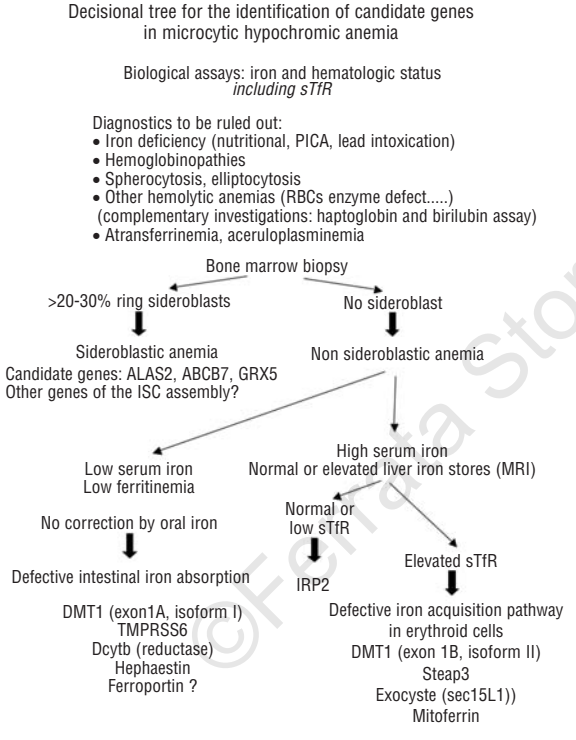

Figure 3. **Decisional tree for the choice of candidate genes to sequence for the molecular diagnosis of genetic microcytic anemia. This decisional tree is based on the authors' own experience and liable to modifications following the discovery of new forms of genetic microcytic anemia. Some genes are proposed based on mouse models but have not yet been found to be implicated in human diseases (Table 1). DMT1 has several isoforms and according to the position of the mutation can theoretically give rise solely to defective intestinal iron absorption or to defective iron uptake by erythroid precursors. Some additional diagnostic tools are recommended such as sTfRs, e-PPIX and serum hepcidin. The soluble form of TfR1 (sTfR) is formed by proteolytic cleavage of TfR1 ectodomain and sTfR levels correlate with the total mass of erythroid precursors and increase during iron deficiency. Similarly, accumulation of Zn-PPIX in erythrocytes is a good marker of defective heme synthesis due to limited iron supply or acquisition. Finally, several hepcidin assays are now available and likely to be helpful for the choice of genes to sequence. MRI: Magnetic Resonance Imaging.**

site. In mice and humans, two Pim-1 proteins are produced from the same gene by using an alternative upstream CUG initiation codon, a 44 kD and another, shorter 34 kD form that both contain the kinase domain. Expression of Pim-1 is widespread and ranges from the hematopoietic and lymphoid system to prostate, testis and oral epithelial cells. To understand the function of Pim-1 and its role in hematopoietic development, Laird *et al.*[124] generated mice deficient in Pim-1 function. Pim-1-deficient mice were ostensibly normal, healthy, and fertile; however, detailed analysis demonstrated a correlation of Pim-1 deficiency with erythrocyte microcytosis, whereas overexpression of Pim-1 in transgenic mice resulted in erythrocyte macrocytosis.

Conclusions

The frequency of genetic microcytic anemia due to defective intestinal iron absorption or iron acquisition by erythroid cells has remained underestimated up to now by the lack of good candidate genes. However, the discovery of new genes implicated in these disorders such as Grx5, DMT1 or TMPRSS6, in addition to the genes identified in mice for which no human counterparts have been identified so far (Table 1), suggest that these conditions are more frequent than initially thought. Since the molecular diagnosis of these anemias is within easy reach, the difficulty lies in the identification of the proper candidate gene to sequence in these patients. Based on their own experience, the authors would like to propose a summary of the expected biological findings in the different known forms of genetic microcytic anemia (Table 2) as well as a decisional tree (Figure 3) that can be implemented with the discovery of new genes of interest.

Authorship and Disclosures

AI and CB prepared the manuscript; LDF performed literature data analysis and data from animal models.

The authors reported no potential conflicts of interest.

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