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TITLE: RARE ANEMIAS DUE TO GENETIC IRON METABOLISM DEFECTS

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ABSTRACT

Anemia is defined by a deficiency of hemoglobin, an iron-rich protein that binds oxygen in the blood. It can be due to multiple causes, either acquired or genetic. Genetic alterations of proteins involved in iron metabolism may be responsible, usually at a young age, for rare forms of chronic and often severe congenital anemia. These diseases encompass a variety of sideroblastic anemias, characterized by the presence of ring sideroblasts in the bone marrow. Clinical expression of congenital sideroblastic anemia is either monosyndromic (restricted to hematological lineages) or polysyndromic (with systemic expression), depending on whether iron metabolism, and especially heme synthesis, is directly or indirectly affected. Beside sideroblastic anemias, a number of other anemias can develop due to mutations of key proteins acting either on cellular iron transport (such as the DMT1 transporter), plasma iron transport (transferrin), and iron recycling (ceruloplasmin). Contrasting with the aforementioned entities which involve compartmental, and sometimes, systemic iron excess, the iron refractory iron deficiency anemia (IRIDA) corresponds to a usually severe anemia with whole body iron deficiency related to chronic increase of plasma hepcidin, the systemic negative regulator of plasma iron. Once clinically suggested, these diseases are confirmed by genetic testing in specialized laboratories.

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Key words:

Anemia, iron, genetic defects, congenital sideroblastic anemia

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Anemia ; iron; congenital sideroblastic anemia ; DMT1 ; atransferrinemia ; aceruloplasminemia ; ferroportin ; matriptase ; IRIDA

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RARE ANEMIAS DUE TO GENETIC IRON METABOLISM DEFECTS

1 INTRODUCTION

Anemia is defined by a deficiency of hemoglobin, an erythrocyte iron-rich protein that binds oxygen and allows its delivery to the organism. Anemia can be due to multiple etiologies, either acquired (such as blood losses, hematological malignancies, aplastic anemia, chronic inflammation-, dietary iron deficiency or malabsorption, or dietary vitamin deficiency) or genetic. Genetic causes can involve quantitative or qualitative impairment of globin chain synthesis (thalassemias and sickle cell disease, respectively), or genetically related abnormalities of the erythrocyte, either at the membrane level (pyruvate kinase, glucose-6-phosphate dehydrogenase (G6PD), stomatocytosis) or concerning enzyme function (hereditary spherocytosis also known as Minkowski-Chauffard syndrome). In addition, a number of rare anemias are related to genetic defects of iron metabolism itself. These defects involve mutations of genes that play a role in systemic or cellular iron metabolism. The present review will expose the main actors involved in iron metabolism whose mutations are reported to potentially generate anemia and will then focus on the hematological consequences generated by those mutations.

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2 THE MOLECULAR CONNECTIONS BETWEEN IRON, HEMOGLOBIN AND RED BLOOD CELLS

Erythrocytes, also named red blood cells, are responsible for oxygen uptake and delivery throughout the body. For ensuring these functions, red blood cells contain hemoglobin which reversibly binds oxygen. Hemoglobin is an iron-containing protein so that perturbations in iron metabolism can result in anemia. The zebrafish model has been essential for discovering many genes involved in hemoglobin synthesis, iron metabolism and red blood cell differentiation [1].

2.1. DISTRIBUTION OF IRON

2.1.1. Intestinal absorption of iron

Dietary iron is the only source of new iron for the body. Heme and non-heme iron are the two forms of dietary iron. Both forms are absorbed at the duodenal level (review in [2][3][4]). On the one hand, uptake of heme iron by duodenal enterocytes is still unknown, but a mechanism that involves the heme carrier protein 1 (HCP1) protein, encoded by *SLC46A1* gene, has been proposed [5]. Within the enterocytes iron atoms are then released from heme by heme oxygenases. On the other hand, the non-heme ferric iron is reduced into ferrous iron by DCYTB reductase (duodenal cytochrome *b*, also known as *CYBRD1* gene) at the duodenal brush border membrane, and then taken up by the transporter DMT1 (Divalent Metal Transporter 1 also known as *SLC11A2*), that permits the ingress of iron at the apical membrane of the enterocytes to enter the cell **(Fig.1A).** Iron is then exported from

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enterocytes toward plasma through the ferroportin protein (see below) at the basolateral pole of the enterocyte. Therefore, *DMT1* mutations can potentially impair non heme iron absorption and possibly contribute to anemia by limiting dietary iron uptake.

2.1.2. Plasma iron transport

After crossing the enterocytes, iron reaches the plasma where it is taken up by its carrier protein, transferrin (*TF* gene). Ferrous iron, exported from enterocytes, is then oxidized by the protein hephaestin (*HEPH* gene) and transferred to transferrin **(Fig.1A).** Transferrin is a plasma homodimeric beta-globulin, synthesized by the hepatocyte **(Fig.1B),** and that may bind two ferric iron molecules [6]. The major target tissue of transferrin-bound iron is the bone marrow where approximately 80% of iron is delivered in order to contribute to the maturation of red blood cells. Transferrin saturation, meaning the ratio between iron occupied binding sites of transferrin and the total iron binding sites of transferrin present in plasma, reflects iron availability for developing red blood cells in the bone marrow. Mutations leading to lack of transferrin protein or to a decrease of transferrin-iron saturation may therefore cause anemia.

2.1.3. Cellular iron uptake: the transferrin cycle

Cells acquire iron bound to transferrin. Soluble transferrin-iron complexes are recognized by two transmembrane receptors, transferrin receptor 1 (*TFR1* or *TFRC* official gene) and transferrin receptor 2 (*TFR2* gene) [7][8][9]. The affinity of TFR2 for iron is dramatically lower than that of TFR1 so that its role in iron uptake is likely minimal compared to TFR1. TFR1/TF-

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Fe or TFR2/TF-Fe complexes are internalized by endocytosis and retrieved in endolysosomal vesicles. Iron is then released from TFR1/TF-Fe or TFR2/TF-Fe complexes by acidification of the endolysosomal compartment [8]. Endosomal ferric iron (Fe^{3+}) is reduced into ferrous iron (Fe^{2+}) by metalloreductases, including the six-transmembrane epithelial antigen of the prostate family member 3 (STEAP3) [10] and transported into the cytoplasm by divalent metal transporter 1 (DMT1) [11] (review in [12]) **(Fig.1C)**. Of note, beyond intestinal absorption, DMT1 transporter is present in additional sites which are the ubiquitous endolysosomal vesicles. The consequence of the role of DMT1 in recycling iron from the transferrin receptor 1 cycle is that mutations affecting DMT1 will not only cause anemia by limiting dietary iron uptake but will concomitantly generate intracellular iron overload through a retention mechanism.

2.1.4. Cellular iron export and recycling

Three main proteins are implicated in iron export and recycling, hephaestin, ferroportin, and ceruloplasmin. Hephaestin, as previously mentioned, is a multicopper oxidase anchored in cell membrane of enterocytes allowing transfer of iron that comes from enterocytes toward ferroportin **(Fig.1A)**. Ferroportin (solute carrier family 40, member 1, *SLC40A1* gene) is the only known protein ensuring cellular iron export into the plasma, especially in macrophages [13]. It plays a major role in the recycling of iron from macrophages during the erythrophagocytosis process. Ferroportin iron export dysfunction affects iron release into the plasma, leading to decreased transferrin saturation and to a propensity to anemia. Ceruloplasmin (*CP* gene) is a plasma protein mainly synthetized by hepatocytes, that ensures the oxidation of iron released from the cells (especially macrophages) through the

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ferroportin channel [14] **(Fig.1D)**. This oxidation step is needed for iron uptake by transferrin. Dysfunction in ceruloplasmin oxidizing property will therefore decrease transferrin saturation by iron and expose to the risk of anemia. Moreover, by impairing ferroportin activity, ceruloplasmin dysfunction may also lead to iron overload.

2.1.5. Systemic iron metabolism regulation

Hepcidin is a protein, whose corresponding gene (*HAMP*) expression is regulated by iron [15], and is the master negative regulator of plasma iron [16] [17] [18]. This hormone is a small peptide mainly produced by hepatocytes [15] [16] **(Fig.1E)** that targets ferroportin, promoting its degradation. Thus, low hepcidin levels favor duodenal (by enterocytes) and splenic (by macrophages) iron release into plasma, leading to high plasma iron levels, iron becoming available for iron greedy organs such as bone marrow for developing red blood cells. On the contrary, high hepcidin levels induce intracellular iron retention in particular in macrophages, and decrease duodenal iron absorption. Therefore, elevated hepcidin levels result in a drop of plasma iron and transferrin saturation, and consequently, may favor anemia due to inadequate amount of iron available for differentiation of red blood cells. Body iron load is a potent inducer of hepcidin synthesis [19]. Other factors such as hypoxia, anemia or inflammation are also strong stimuli of hepcidin synthesis [3]. The signal transduction pathway involves BMP6 that interacts on cell membrane with the BMP receptors (bone morphogenetic protein receptor) together with the hemojuvelin protein, thus promoting the activation of the SMAD1,5,8 (son of mothers against decapentaplegic homologues) pathway and hepcidin gene transcription. Matriptase 2, whose corresponding gene is *TMPRSS6* (transmembrane serine protease S6), is a negative hepcidin regulator

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cleaving multiple components of the hepcidin induction pathway including Hfe, Tfr2 in mouse model or hemojuvelin that inhibits the BMP/SMAD [20] **(Fig.1E).** Therefore, *TMPRSS6* mutations lead to increase hepcidin synthesis causing hyposideremia and anemia corresponding to body iron deficiency.

2.2. INTRACELLULAR IRON METABOLISM: THE CRUCIAL ROLE OF THE MITOCHONDRION FOR HEMOGLOBIN SYNTHESIS AND RED BLOOD CELL DIFFERENTIATION

2.2.1. Relationship between serum iron, mitochondrial function and developing red blood cells

Up to 80% of serum iron, mainly carried by transferrin, will reach the bone marrow. Iron enters the erythroblasts *via* transferrin receptor 1 and will undergo the classical transferrin iron cycle. Globally, serum iron contributes to the daily production of approximately 200 billion of new red blood cells and two-thirds of body iron are contained in red blood cells within the hemoglobin molecules [21][3]. Heme plays a crucial role in erythropoiesis. Not only heme is an essential structural component of hemoglobin – so that heme synthesis and export are essential for erythropoiesis [22][23]-, but also heme regulates both the transcription and translation of globin chains [24][25]. Therefore, mitochondria, where heme synthesis takes place, are key cellular organelles for erythropoiesis and for iron metabolism (review in [26][27][28]). Within mitochondrion, a major amount of cellular iron is incorporated in two main distinct metabolic pathways, heme synthesis and iron-sulfur

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cluster biogenesis **(Fig.2).** Mutations impacting those various pathways may, directly or indirectly, favor the development of anemias. Finally, excess iron can be stored in mitochondrial ferritin; however, no significant defects have been reported in mice lacking mitochondrial ferritin [29].

2.2.2. Entry of iron into the mitochondria

Several iron import pathways across the outer mitochondrial membrane, which has high metabolite-permeability relative to the inner membrane, have been proposed. They include passage through voltage-dependent anion channels (VDAC) or DMT1, directly from the iron labile pool or assisted by chaperones or through direct interaction of the endosome with the mitochondria (for review, see Lane et al., 2015). Iron enters mitochondrial matrix via a highly conserved transporter called mitoferrin (*MFRN1/2*), which has been first identified following functional studies of the zebrafish mutant *frascati* that showed profound hypochromic anemia and erythroid maturation arrest due to defects in mitochondrial iron uptake [30]. *Mfrn1* knock-out mice showed a similar phenotype of anemia [31]. While *MFRN2* is ubiquitously expressed in different tissues, *MFRN1* (also known as *SLC25A37*) is specifically expressed in red blood cell precursors. ABCB10 physically interacts with MFRN1 to enhance its stability and function [32].

2.2.3. Heme synthesis pathway

Heme synthesis is a multi-step enzymatic cascade, with the first and three last steps occurring within the mitochondrion and intermediary steps within cytoplasm **(Fig.2A)**. We

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provide here a brief overview of the heme synthesis pathway, and propose the following recent and comprehensive review for readers willing to understand the full picture of current knowledge on heme synthesis (review in [28]). Briefly, during the first step, glycine, which is transported into mitochondrion by SLC25A38, is condensed to succinyl CoA to produce 5-aminolevelunic acid (ALA). This enzymatic step is catalyzed within the mitochondrion by aminolevulinic acid synthases, ALAS1 or ALAS2. *ALAS1* is ubiquitously expressed while *ALAS2* is specific to erythroblasts. ALA is then exported out of the mitochondrion, possibly assisted by ABCB10 [33], and enters the cytoplasm where it will undergo 4 additional modifications producing coproporphyrinogen III. This intermediate will be then transported back into mitochondria possibly by ABCB6 [34] [35] [36] and subjected to two successive modifications by coproporphyrinogen oxidase and protoporphyrinogen oxidase producing protoporphyrin IX. The final enzymatic reaction in heme synthesis will be the incorporation of ferrous iron by ferrochelatase (*FECH* gene), an iron-sulfur cluster containing enzyme, into protoporphyrin IX to generate heme. Heme is finally exported possibly by Feline leukemia virus subgroup C receptor 1b (Flvcr1b, a mitochondrial isoform of Flvcr)[37] out of mitochondrion and enters cytoplasm where heme will be incorporated into proteins.

Heme is a crucial prosthetic group for efficient enzymatic functions of many proteins including hemoglobin, myoglobin, cytochrome p450, respiratory cytochromes. Deficiency in iron incorporation into protoporphyrin IX will result in lack of heme production, and subsequently in the lack of hemoglobin that will impede the production of mature red blood cells.

Inherited mutations in most of those genes involved in heme synthesis will result either in anemias (by deficiency of heme-containing hemoglobin production and consequently

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production of mature red blood cells) or in porphyrias (by accumulation of heme precursors such as protoporphyrin IX leading to skin lesions and acute neurovisceral attacks) **(Fig.2B)**. Description of the various porphyrias is out of the scope of the present review; for further information, we recommend to read [38]. Inherited anemias originating from mutations in heme synthesis pathways are further described in section 3.2.

2.2.4. Fe/S cluster biogenesis

Fe/S clusters (iron-sulfur cluster or ISC) are inorganic cofactors that mainly participate in electron transfer reaction, catalysis or stabilization of protein structure. Fe-S proteins fulfill important functions in the mitochondria, as components of the respiratory complexes, but also in the cytosol, the endoplasmic reticulum and the nucleus (DNA polymerases, DNA helicases) [39]. Fe/S clusters are also key elements of proteins involved in iron metabolism including ferrochelatase, responsible for incorporation of mitochondrial iron into protoporphyrin IX, or IRP1 (iron responsive protein 1) protein that is implicated in intracellular iron sensing. This wide distribution of iron-sulfur cluster proteins makes this moiety crucial for mitochondrial respiration and cofactor synthesis, and numerous extra-mitochondrial pathways including nuclear DNA synthesis and repair, ribosomal protein synthesis, nucleotide metabolism and cellular iron regulation [39].

Fe/S cluster biogenesis is a multi-step process conserved from yeast to mammalian [39]. In this review we briefly summarize the main steps and actors that could be further associated with anemia clinical presentation. For more details on the Fe/S cluster protein assembly, we recommend referring to [39][40] **(Fig2A).**

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Mitochondrial Fe/S protein assembly, which relies on 18 proteins, can be dissected in three major steps. First, a [2Fe-2S] cluster is generated *de novo* on a scaffold protein [39]. This process requires mitochondrial ferrous iron, possibly delivered by frataxin (*FRX* gene), and a sulfur source provided by a cysteine desulfurase complex Nfs1-Isd11 which cooperates with ferredoxin-ferredoxin reductase, to synthesize an [2Fe-2S] cluster on the scaffold protein ISCU (review in [41]). In the second step, the Fe/S cluster is released from the scaffold protein ISCU and transiently transferred toward apoproteins with the help of a dedicated multi-functional Hsp70 chaperone system and the glutaredoxin Glrx5 (review in [41][39]). Finally, in the third step, specific Fe/S cluster targeting factors assist in the generation of [4Fe-4S] clusters and insert the Fe/S cluster into mitochondrial apoproteins (review in [39][40]).

Assembly of extra-mitochondrial Fe/S proteins involves the mitochondrion and a dedicated cytosolic machinery, called CIA (for cytosolic Fe/S cluster assembly machinery). A sulfur-containing compound generated by the core mitochondrial ISC machinery is exported out of the mitochondria *via* the ABC transporter ABCB7 [42][43], assisted by the sulfhydryl oxidase ALR and glutathione. *ABCB7,* whose defect or mutations lead to defective cytosolic Fe/S proteins in different species [44][43][45], is essential for hematopoiesis [46]. The CIA machinery, which relies on 11 proteins, allows the synthesis and insertion of Fe/S clusters in client proteins in the cytosol and the nucleus (review in [40]).

Genetic mutations in a number of Fe/S assembly genes lead to severe neurological, hematological and metabolic diseases [39], underlying the crucial role of Fe-S proteins for fundamental processes, including erythropoiesis **(Fig.2B).**

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2.2.5. Role of mitochondria in the regulation of cellular iron homeostasis

One aspect of Fe/S clusters biogenesis should be stressed to understand cellular iron perturbation observed in diseases resulting from defects of the core mitochondrial Fe/S cluster biogenesis. Functional defects, as mutations could generate, of the core Fe/S cluster assembly machinery are signaled to cytosolic or nuclear iron regulatory systems resulting in increased cellular iron acquisition and mitochondrial iron accumulation (review in [41]). This signaling relies on the cytosolic iron regulatory proteins IRP1 and IRP2, which act in a post-transcriptional fashion to adjust the cellular needs for iron (for review see [47]). IRP1 and IRP2 both recognize Iron Responsive Elements (IRE) located in 5'UTR or 3'UTR of specific mRNA transcripts of proteins involved in the trafficking (DMT1, TFR1, ferroportin), storage (ferritin) and utilization (ALAS2, FECH) of iron. In low iron, IRPs bind to 5' IRE in ferritin and ferroportin mRNAs with high affinity to repress translation, and to 3' IREs in TFR1 mRNA to block its degradation. In contrast to IRP2, IRP1 activity is directly influenced by iron availability. In iron-replete conditions, IRP1 acts as an [4Fe-4S] aconitase, while in iron deficient cells, the lost of the Fe/S cluster induces a conformation change that makes IRP1, in its apo-form, able to bind mRNA. The involvement of a cytosolic Fe/S protein in cellular iron homeostasis provides a direct connection between mitochondria and cellular iron homeostasis [48].

2.2.6. Mitochondrial iron accumulation

Mitochondrial iron accumulation in congenital sideroblastic anemia (CSA) reflects a strong disturbance of cellular iron homeostasis. The exact mechanisms by which iron accumulates

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in erythroblast mitochondria in the bone marrow of CSA patients remain to be clarified and may depend on the genetic origin of the corresponding disease. Nevertheless, different scenari can be considered.

In ALAS2-related CSA, heme, which is a negative regulator of iron uptake, is deficient and iron may not be properly used in erythroblast mitochondria due to a decreased supply of PPIX [49][50]. Consequently, iron, which normally exits mitochondria within heme, would be sequestered into the mitochondrion.

In humans, disturbance of the core ISC assembly, as in GLRX5-related CSA, or the mitochondrial export system (ABCB7) may affect cellular iron homeostasis. Loss of Fe/S cluster assembly in the *GLRX5* deficient *shiraz* zebrafish mutant was shown to activate IRP1 and to block heme biosynthesis [51]. Subsequently, an increase in IRP1's IRE binding activity has been reported in peripheral blood mononuclear cells from a GLRX5-related CSA patient [52], and *GLXR5* deficiency was shown to cause sideroblastic anemia by impairing heme biosynthesis and depleting cytosolic iron [53]. RNA silencing of *ABCB7* causes an iron-deficient phenotype with mitochondrial iron overload [54], a feature of XLSA/A. Disturbance of iron homeostasis in both GLRX5-related CSA and XLSA/A may be due to a deficit of Fe-S cluster insertion into IRP1. Improperly matured IRP1 would lead to increase TFR1 protein levels, favoring iron uptake, but also prevent ALAS2 translation. Moreover, any disruption of the mitochondrial ISC synthesis can affect assembly of ferrochelatase, a Fe/S protein, which in turn affect heme synthesis.

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3 ANEMIAS WITH SYSTEMIC OR COMPARTMENTAL IRON OVERLOAD OF GENETIC ORIGIN

Numerous iron-related proteins, scattered from the digestive tube (iron absorption) to the bone marrow (hemoglobin synthesis) and macrophages (iron recycling) via the plasma (iron transport), are implicated in plasma iron concentration and red blood synthesis. When mutated, those genes may lead to anemia through failure of iron to reach the bone marrow or impossibility for the bone marrow iron to contribute to hemoglobin synthesis **(Fig.3).**

3.1 DEFECT IN SYSTEMIC PLAYERS OF IRON METABOLISM

3.1.1 Defect in iron uptake: DMT1-related anemia (OMIM entry 600523)

Due to mutations of the *SLC11A2* gene (solute carrier family 11, member 2, also known as DMT1), this disease associates hypochromic microcytic anemia and visceral iron overload [55] [56] **(Table 1).** This clinical profile is related to the dual role of DMT1 in dietary iron uptake at the apical membrane of the duodenal enterocyte and in iron egress from cytosolic endosomes.

One biochemical peculiarity is that plasma ferritin elevation is relatively mild in regard of body iron excess. Chelation therapy is poorly effective. Erythropoietin could be of interest [57].

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3.1.2 Defect in iron transport: Atransferrinemia-related anemia (OMIM entry 209300)

Due to mutations of the transferrin gene (*TF*), this autosomal recessive rare disease (a dozen of reported cases) associates microcytic anemia (due to marked reduction of iron delivery to the bone marrow) and severe hepatic, pancreatic, and cardiac iron overload. Iron excess is due to the presence of plasma non-transferrin bound iron [58][59], exacerbated by enhanced intestinal iron absorption due to decreased hepatic hepcidin production [60] **(Table 1).** Infusions of fresh frozen human plasma and apotransferrin may be beneficial but have only a transient effect [61][62].

3.1.3 Defect in iron egress from endosome: STEAP3/TSAP6-related anemia (OMIM entry 615234)

A nonsense mutation in the *STEAP3/TSAP6* gene has been described in one family presenting with hypochromic and (mildly) microcytic anemia **(Table 1).** A tendency to spontaneous iron overload (*i.e.* prior to any transfusions) was observed. This phenotype resembles that of mouse models with STEAP3/TSAP6 inactivation and is in keeping with the protein function. Indeed, STEAP3/TSAP6 is a ferrireductase acting at the level of endocytic vesicles to reduce ferric iron into ferrous iron that can be exported through DMT1 into the cytosol [63].

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3.1.4 Defect in iron export

3.1.4.1 Aceruloplasminemia-related anemia (OMIM entry 604290)

Hereditary aceruloplasminemia is due to mutations of the ceruloplasmin gene (*CP*) [64] **(Table 1).** The phenotype associates, in adulthood, microcytic anemia with very low plasma iron and transferrin saturation levels, contrasting with marked hyperferritinemia. Visceral iron overload affects mainly the liver and the basal ganglia. The latter location explains neurological symptoms such as extrapyramidal syndrome. Iron excess is classically attributed to the lack of ceruloplasmin-related ferroxidase activity that, in turn, would cause an upstream defect in the cellular iron export function of ferroportin [65]. However, the absence of spleen iron overload does not favor this explanation when considering that ferroportin is particularly expressed in this organ [3].

Once evoked, the diagnosis relies first on the detection of very low plasma ceruloplasmin levels (together with low plasma ferroxidase activity), subsequently confirmed by genetic testing whose diagnostic interest concerns not only the patient but also his/her family.

Iron overload is partially reversed by parenteral (desferrioxamine) or oral (deferasirox, deferiprone) chelation therapy possibly associated with fresh frozen plasma therapy [66][67][68].

3.1.4.2 Ferroportin-related anemia (OMIM entry 606069)

The ferroportin disease is due to mutations of the ferroportin gene (*SLC40A1* gene). In its most frequent form, also named type 4A hemochromatosis, the ferroportin disease is a dominant form of genetic iron overload affecting adults but occasionally also younger

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subjects [69][70] **(Table 1).** Iron excess is not due, like in hepcidin-deficient forms of hemochromatosis, to an excessive iron entry into the parenchymal cells but to iron retention mainly within the macrophages (in the spleen and the liver). The development of body iron excess despite an expected decrease of intestinal iron absorption may be related to the fact that digestive egress of iron from enterocytes is less affected than iron egress from macrophages, due to a lower enterocyte iron flux compared to macrophages [71].

From the hematological viewpoint, there is no anemia in the basal state (although plasma iron and transferrin saturation may be decreased). However, anemia can occur during venesection therapy due to defective iron recycling process. This usually leads to alleviate the venesection program. Oral chelation (deferasirox) has been occasionally reported to be beneficial [72].

3.2 ALTERATION OF MITOCHONDRIAL PATHWAYS: CONGENITAL SIDEROBLASTIC ANEMIAS (CSAs): THE COMMON DENOMINATOR: MITOCHONDRIAL IRON OVERLOAD

Congenital Sideroblastic Anemias (CSAs) are a heterogeneous group of diseases, characterized by anemia of varying severity, which share a common feature: the presence of excessive iron within the mitochondria of erythroblasts **(Table 1).** This compartmental iron excess corresponds to the presence, in the bone marrow, of the characteristic ring sideroblasts that consist of iron deposits, visualized by Perls' staining, which surround the erythroblast nucleus. Ring sideroblasts must be differentiated from normal sideroblasts containing stainable iron granules. Most of this iron is present in the form of mitochondrial ferritin [73].

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CSAs are associated with germline mutations leading to defects in mitochondrial heme synthesis, iron-sulfur (Fe/S) cluster biogenesis, or protein synthesis.

Most CSAs closely related to iron-sulfur cluster biogenesis and/or heme synthesis impairment are essentially expressed by hematological features, a profile usually defined as "non syndromic" CSAs. Actually, this expression corresponding to a true hematological syndrome, it may be preferable to use the term "monosyndromic CSAs" in contrast to situations where this syndrome is associated with extra-hematological syndromes and which could therefore be named, instead of syndromic CSAs, "polysyndromic CSAs".

3.2.1 CSAs related to genes whose products are directly involved in iron or heme metabolism: mainly "monosyndromic" CSAs

3.2.1.1 X-linked sideroblastic anemia (XLSA) due to ALAS2 mutations (OMIM entry 300751)

X-linked sideroblastic anemia (XLSA) is one of the most frequent hereditary CSA anemias, with nearly 80 different mutations reported to date (HGMD website). The *ALAS2* (5-aminolevulinate synthase 2) gene, only expressed in erythroid cells, encodes the mitochondrial protein ALAS. ALAS is the first enzyme of the heme biosynthetic pathway and plays a critical role in this process. *ALAS2* gene mutations lead to defective heme synthesis in erythroid lineage. The zebrafish mutant *sauternes*, mutated in *ALAS2* orthologous, develops a closely related hematological condition [74]. Ineffective erythropoiesis occurs and is

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responsible for systemic iron overload through the decreasing effect on hepatic hepcidin synthesis of increased erythropoietic activity [75]. Most *ALAS2* mutations are missense mutations which impact the enzyme affinity for its cofactor pyridoxal-5'phosphate, so that patients may be responsive to pyridoxine. The nonsense mutations would be responsible for pyridoxine unresponsiveness in other patients.

The phenotypic expression of XLSA is very variable between families and within relatives of a given affected family [76]. XLSA leads, in neonates or adults, to microcytic and hypochromic anemia, usually moderate and not requiring transfusions. Significant body iron overload can develop. Once evoked, the diagnosis involves two steps: detection of ring sideroblasts in the bone marrow by Perls'staining, and identification of the *ALAS2* mutations.

Treatment may consist of pyridoxine supplementation, iron removal by chelating agents or adapted venesections. Genetic counseling, based on molecular diagnosis, is essential.

3.2.1.2 SLC25A38-related CSA (OMIM entry205950)

It is, with XLSA, one of the most frequent congenital CSA diseases [77] [76]. It is related to the *SLC25A38* gene, encoding the erythroid-specific mitochondrial carrier protein [78], and whose mutations lead to impaired heme synthesis. The mode of transmission is autosomal recessive.

Patients present, at a very young age (usually from a few months to adolescence), severe microcytic anemia requiring regular transfusions. Iron overload develops, due not only to transfusions but also to dyserythropoiesis [79].

The diagnostic approach should first exclude thalassemia major before resorting to *SLC25A38* sequencing.

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Conservative treatment consists of iron chelation therapy. The only curative therapy is stem cell transplantation.

3.2.1.3 Glutaredoxin 5 (GLRX5)-related CSA (OMIM entry 609588)

GLRX5 is a mitochondrial protein highly expressed in erythroid cells. It is part of the assembly machinery of iron-sulfur clusters and ensures the maintenance of normal mitochondrial and cytosolic homeostasis [53].

Glutaredoxin deficiency in the zebrafish mutant *shiraz* develops severe microcytic-hypochromic anemia [51]. Two human cases of GLRX5 deficiency have been reported so far. The first case relates to a middle-aged man who had a homozygous mutation interfering with intron 1 splicing and drastically reducing GLRX5 mRNA. He presented a microcytic anemia of progressive aggravation with a relatively low number of ring sideroblasts, associated with pronounced body iron overload. Unexpectedly, anemia was worsened by blood transfusions and partially reversed by iron chelation [52]. The second case was a compound heterozygote for two missense mutations in *GLRX5*. This male patient suffered from severe anemia, with mild erythroid hyperplasia and 19% ring sideroblasts (Liu et al., 2014).

3.2.1.4 FECH (ferrochelatase) erythropoietic protoporphyria (EPP) (OMIM entry 612386)

Mutations of the *FECH* gene, whose protein product catalyses the final step of heme biosynthesis, may lead, beside photosensitivity and hepatic failure, to microcytic anemia

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(present in 20-60% of the patients). One case of a child presenting EPP and CSA has been reported [80].

3.2.2 CSAs related to genes indirectly involved in iron and heme metabolism: mainly "polysyndromic" CSAs

Mutations of those genes impact mitochondrial functions and mainly lead to "polysyndromic" CSAs.

3.2.2.1 X-linked sideroblastic anemia with ataxia (XLSA/A) (OMIM entry 300135)

XLSA/A is due to missense mutations in the ATP binding cassette *ABCB7* [81] [44] which disturb the export machinery of a sulfur-containing compound required for the assembly of extra-mitochondrial Fe/S proteins. Only four families, each with a distinct missense mutation, have been reported so far [82]. XLSA/A causes, early in life, mild hypochromic microcytic anemia and ataxia. Ataxia may be due to mitochondrial iron overload in neural cells through a mechanism close to that of Friedreich ataxia.
There are neither systemic iron overload nor response to pyridoxine supplementation.

3.2.2.2 CSA with myopathy and lactic acidosis (MLASA)

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MLASA is an autosomal recessive disorder, due to mutations of three genes located on chromosome 12. MLASA1 is caused by homozygous mutations of the *PUS1* (pseudouridylate synthase 1) gene [83] (OMIM entry 600462), MLASA2 by mutations of the *YARS2* (tyrosyl tRNA synthetase 2) gene [84][85], and MLASA3 by mutations of *MTATP6* (mitochondrially encoded ATP synthase 6) [86]. How these different mutations lead to a similar phenotype remains largely unknown. It is however considered that MLASA is an oxidative phosphorylation disorder, and it has been recently shown, in a mouse model of mitochondrial myopathy with sideroblastic anemia, that alterations in muscle metabolism related to mitochondrial content and oxidative capacity may account for the reduced exercise capacity [87].

3.2.2.3 CSA with marrow-pancreas syndrome (Pearson syndrome) (OMIM entry 557000)

Due to deletions and rearrangements of mitochondrial DNA (mtDNA) leading to a defect of oxidative phosphorylation, this is a rare and severe disease leading often to death before 4 years of age. Beside CSA, multiple organ damages develop, related to energy shortage likely due to imbalance between ATP production and requirement. Visceral damage concerns the pancreas (insulin-dependent diabetes), the liver (hepatic dysfunction), the kidney (renal tubulopathy), and the neurological system (muscle weakness, hypotony, development delay). Lactic acidosis is very frequently present [88].

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3.2.2.4 CSA with B cell immunodeficiency, periodic fevers, and development delay (SFID) (OMIM entry 616084)

This autosomal recessive disorder has been reported to be caused by biallelic mutations in *TRNT1*, the gene encoding the CCA-adding enzyme required for the maturation of cytosolic and mitochondrial transfer RNAs (tRNAs) [89][90]. The precise links between *TRNT1* mutations and the clinical manifestations of the disease remain to be elucidated. It has been proposed that clinical severity of the phenotypes was related to the degree of CCA-adding enzyme loss of function [89]. Moreover, it has been recently reported that the levels of serum inflammatory cytokines (mainly IL-6) were elevated, and TNF (tumour necrosis factor) and IL-1ß were present in biopsies of patients presenting an active inflammatory disease, with promising results of anti-TNF therapy [91].

3.2.2.5 Thiamine-responsive megaloblastic anemia (TRMA) (OMIM 249270)

Thiamine-responsive megaloblastic anemia is due to mutations of the *SLC19A2* gene whose protein product is a high-affinity thiamine transporter. The development of CSA and associated syndromes, such as diabetes and deafness, may be related to impaired heme synthesis (due to lack of succinyl-CoA) and to defective protein synthesis (due to decreased ribose synthesis) [92]. The disorder responds to thiamine supplementation.

3.2.2.6 Others

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Autosomal recessive CSA caused by mutations in the *HSPA9* gene (heat shock protein A9) (OMIM 600548), a *HSP70* homolog involved in mitochondrial iron-sulfur biogenesis, has been reported [93].

CSA is also observed in patients presenting a recurring mutation in the *NDUFB11* gene, a mitochondrial respiratory complex I- associated protein [94].

A number of CSAs remain to be genetically identified [77].

For most polysyndromic CSAs, an important question raises about the correlation between genotype and phenotype. Iolascon *et al.* has proposed that diffuse abnormalities of protein biosynthesis, related to tRNA dysfunction, and impacting cell viability, may be responsible for the phenotypic pleiotropy but further studies are needed to elucidate the underlying intimate mechanisms [90].

3.3 THE CASE OF CONGENITAL DYSERYTHROPOIETIC ANEMIAS (CDAS)

Although the genetic defects responsible for congenital dyserythropoietic anemias (CDAs) are not primarily on genes involved in iron transport and utilization (for instance CDA type II, the most frequent form of CDA, is due to mutations of the gene encoding the secretory COPII component SEC23B [95][96][97]), this type of anemia may be associated with iron overload and mimics many of the diseases presented in this review. Iron overload in CDA is likely related to hepcidin suppression due to ineffective erythropoiesis possibly through erythroferrone or other mediators [98].

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4 ANEMIA WITH BODY IRON DEFICIENCY OF GENETIC ORIGIN (OMIM entry 206200)

This disorder refers to iron refractory iron deficiency anemia (IRIDA). It is an autosomal recessive disease, due to mutations in the *TMPRSS6* gene that encodes matriptase-2, a member of type 2 serine protease family [99]. Matriptase-2 physiologically decreases hepcidin synthesis by the liver. Acting as "antimatriptase-2", *TMPRSS6* mutations strongly enhance hepcidin production and therefore cause both a decrease in duodenal iron absorption and a decreased release by the spleen of iron originating from normal erythrophagocytosis. The global result at the plasma level is a marked decrease in iron concentration and transferrin saturation, leading to hypochromic and microcytic anemia that can be severe.

Several clues support the diagnosis: i) the absence of acquired causes for iron deficiency such as no dietary iron deprivation, malabsorption syndrome, or excessive digestive or gynecological blood losses ; ii) the contrast between, on the one hand, very low levels of plasma iron and transferrin saturation, on the other hand ferritin levels in the normal or moderately elevated range (whereas very low levels would be expected in case of acquired iron deficiency) ; iii) CRP is normal whereas it would be elevated in chronic inflammatory anemia ; iv) anemia is typically resistant to iron oral supplementation (and partially resistant to parenteral supplementation) ; v) high plasma hepcidin levels, whereas in acquired iron deficiency anemia, pronounced hypohepcidinemia is expected.

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Genetic testing will confirm the diagnosis and provide a key indicator for subsequent family study.

In fact, IRIDA appears to be a genotypically and a phenotypically heterogeneous disorder, at least partially due to the interference of genetic and acquired factors [100][101][102].

Current treatment is mainly based on parenteral iron supplementation and should, in the future, benefit of manipulating the hepcidin pathway with the goal of inhibiting it [103].

5 CONCLUSION

In conclusion, a variety of rare anemias are directly or indirectly related to genetic defects of iron metabolism. On the contrary to iron-deficiency anemia from dietary origin, therapeutic options for congenital anemia remain globally limited, and are mainly based on symptomatic treatment of systemic iron excess (relying on chelation treatment) or deficiency (mainly transfusions or parenteral iron supplementation). Stem cell transplantation and manipulation of the hepcidin pathway represent, mainly for the future, promising therapeutic alternatives. In light of the myriad of proteins involved in this metabolism, it is more than likely that novel mutations remain to be discovered and will permit to increase our knowledge and understanding of this very specific hematological field.

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FIGURE LEGENDS

FIGURE 1. Cellular and systemic iron trafficking and regulation.

(A) *Intestinal iron absorption.* DMT1 (divalent metal transporter 1) plays a major role in intestinal non-heme iron absorption, allowing iron uptake into enterocytes. DCYTB (Duodenal cytochrome B) reduces iron prior to enterocyte uptake. *DMT1* mutations inhibit iron absorption, favoring the development of anemia. Ferroportin and hephaestin are responsible for iron export from enterocytes to plasma, where iron is carried by transferrin (TF). Fe^{3+} : ferric iron (oxidized iron) ; Fe^{2+}: ferrous iron (reduced iron).

(B) *Plasma iron transport.* Transferrin, synthetized by the hepatocytes, is the physiological plasma iron carrier. Mutations of the transferrin gene cause anemia due to lack of transferrin-iron, and iron overload due to non-transferrin bound iron.

(C) *Cellular iron uptake and intracellular iron release*. Transferrin-bound-iron is internalized after binding to TFR1. Iron is released from TF into endolysosomes where iron is reduced by STEAP3 and exported within the cytoplasm by DMT1. TF: transferrin ; TFR1: transferrin receptor 1 ; DMT1: divalent metal transporter 1 ; STEAP3: six-transmembrane epithelial antigen of prostate3.

(D) *Cellular iron export and recycling*. Recycling of senescent erythrocytes by macrophages is the major source of recycled iron. Mutations of the ceruloplasmin gene, whose protein product allows iron export, lead to hyposideremia.

(E) *Systemic iron regulation.* Hepcidin is the master negative regulator of plasma iron. Hepcidin synthesis is positively regulated by HJV and BMP, and negatively regulated by TMPRSS6. Mutations of *TMPRSS6* can lead to chronic hyperhepcidinemia and therefore to anemia. HJV: hemojuvelin; BMP Bone morphogenic protein.

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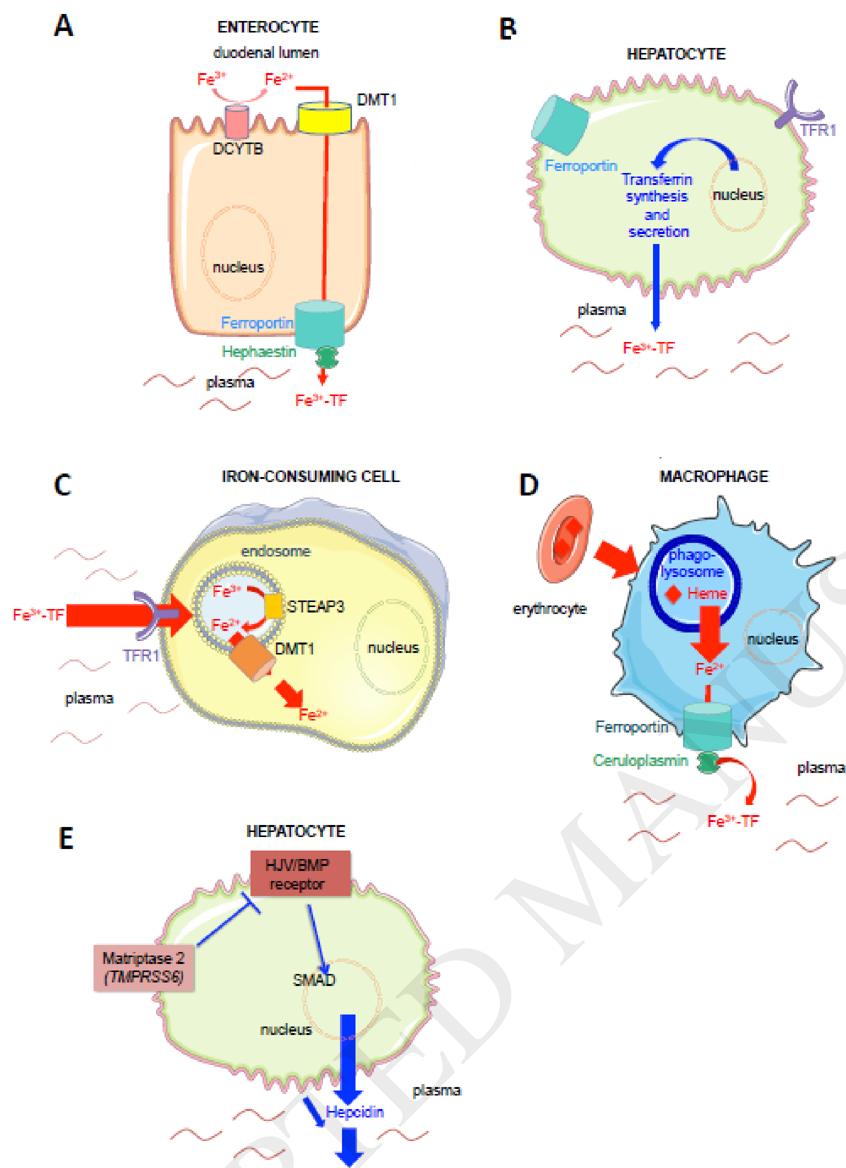

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Figure 2. Iron and the mitochondrion.

(A) Mitochondrial iron is mainly used by two pathways : heme synthesis and iron-sulfur cluster synthesis. CoA: Co-enzymeA; ALA: 5-aminolevelunic acid; ALAS2/1: aminolevulinic acid synthases; CoPIII: coproporphyrinogen III; PIX : protoporphyrin IX; M-ferritin: mitochondrial ferritin, X-S : a sulfur-containing compound transported by ABCB7.

(B) Mutations affecting these pathways lead to either mitochondrial iron overload which is the common denominator of sideroblastic anemias, or to porphyria. Pathologies due to mutations in the corresponding genes shown in panel A are indicated in boxes.

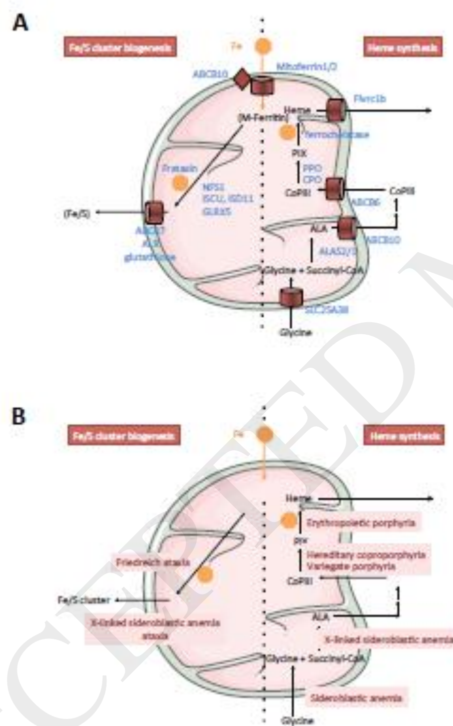

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Figure 3. Schematic overview of the mechanisms underlying the development of anemias related to genetic defects of iron metabolism. Red lightning flash corresponds to mutations in named genes ; names in italics represent genes. Red cross represents consequence of the mutations or elevated hepcidin (often a blockage or a decrease in a normal mechanism). Orange arrow may represent a biological or causative link between 2 proteins. Fe: plasma iron. Two main mechanisms are indicated in turquoise. Mechanism #1: a defect in bone marrow iron uptake can be due to lack of plasma transferrin-iron reaching bone marrow (as in matriptase 2 deficiency, DMT1 deficiency, atransferrinemia, aceruloplasminemia, and at a lesser degree ferroportin disease). Mechanism #2: deregulation of intracellular iron metabolism in the bone marrow (targeting the mitochondria as in congenital sideroblastic anemias).

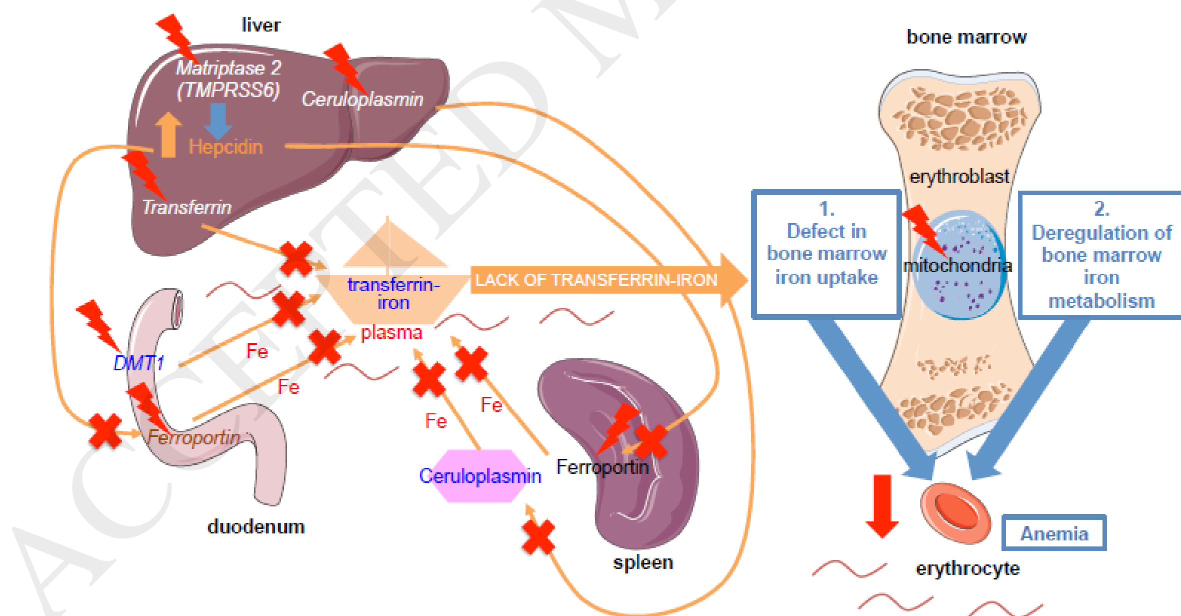

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Table 1: Anemia due to genetic defects iron metabolism genes

	Gene	Age of presentation	Frequency	Anemia phenotype	Iron metabolism defect / other remarks	Therapeutic approaches
DEFECTS IN SYSTEMIC PLAYERS OF IRON METABOLISM						
DMT1	*SLC11A2 (DMT1)*	Birth-young adulthood	Rare (a few cases)	Microcytic anemia	- Visceral iron overload - Mild ferritin elevation	-Iron chelation -Erythropoietin
Atransferrinemia-anemia	*TF*	Birth-young adulthood	Rare (a dozen of reported cases)	Microcytic anemia	- Severe hepatic, pancreatic, and cardiac iron overload - Presence of plasma non-transferrin bound iron	-Human plasma -Apotransferrin
STEAP3-related anemia	*STEAP3 (TSAP6)*	Childhood-young adulthood	Described in 1 family (3 cases)	Hypochromic and (mildly) microcytic anemia	- Tendency to iron overload	-Transfusions -Iron chelation
Aceruloplasminemia-related anemia	*CP*	Adulthood	Rare	Microcytic anemia	- Visceral iron overload mainly in the liver and the basal ganglia - Absence of spleen iron overload - Low plasma iron and transferrin saturation - Hyperferritinemia - Neurological symptom - Very low plasma ceruloplasmin levels	-Iron chelation -Human plasma
Ferroportin-related anemia	*SLC40A1*	Adulthood but occasionally younger subjects	Relatively frequent (type 4 hemochromatosis)	Anemia can essentially occur after venesections	- Iron overload in spleen and liver macrophages	-Bloodletting interruption
"MONOSYNDROMIC" CONGENITAL SIDEROBLASTIC ANEMIAS (CSA) : MAINLY HEMATOLOGICAL PRESENTATION						
X-linked sideroblastic anemia	*ALAS2*	Neonates to adults	One of the most frequent hereditary CSA	Microcytic, hypochromic anaemia	- Ring sideroblasts in the bone marrow - Mitochondrial iron accumulation - Systemic iron overload - Alteration of heme pathway	-Pyridoxine -Iron chelation -Venesections
SLC25A38-CSA	*SLC25A38*	Few months to adolescence	One of the most frequent hereditary CSAs	Severe microcytic anemia	- Mitochondrial iron accumulation - Iron overload - Alteration of heme pathway	-Iron chelation -Stem cell transplantation
Glutaredoxin 5-CSA	*GLRX5*	Middle-aged	1 case reported	Severe microcytic-hypochromic anemia	- Low number of ring sideroblasts, associated with pronounced body iron overload - Mitochondrial iron accumulation - Fe/S cluster machinery alteration	-Iron chelation
Ferrochelatase	*FECH*	Childhood	Rare	Microcytic anemia +	- Mitochondrial iron accumulation	-Avoid light exposure

ACCEPTED MANUSCRIPT

erythropoietic protoporphyria				erythropoietic protoporphyria	- Ring sideroblasts - Alteration of heme pathway, - May lead to photosensitivity and hepatic failure	-Hepatic transplantation (if liver failure)
“POLYSYNDROMIC“ CONGENITAL SIDEROBLASTIC ANEMIAS (CSA)						
X-linked sideroblastic anemia with ataxia (XLSA/A)	*ABCB7*	Childhood	Rare (4 reported families)	Mild hypochromic microcytic anemia and ataxia	- Mitochondrial iron accumulation - Ring sideroblasts - Alteration of Fe/S cluster machinery - Ataxia	- Inefficient pyridoxine supplementation
CSA with myopathy and lactic acidosis (MLASA)	*PUS, YARS2 or MTATP6*	Early childhood	Rare (17 reported patients	Anemia	- Mitochondrial iron accumulation - Ring sideroblasts	-Transfusions
CSA with marrow-pancreas syndrome (Pearson syndrome)	*Mitochondrial DNA rearrangments*	Childhood	Rare	Anemia	- Mitochondrial iron accumulation - Visceral damage : pancreas (insulin-dependent diabetes), liver (hepatic dysfunction), kidney (renal tubulopathy), neurological system (muscle weakness, hypotony, development delay). Lactic acidosis is frequent.	-Transfusions
CSA with B cell immunodeficiency	*TRNT1*	Early childhood	Rare (10 reported families)	Anemia	- Mitochondrial iron accumulation	-Transfusions -Iron Chelation -Immunoglobulins -Anti-TNF (perspective)
Thiamine-responsive megaloblastic anemia	*SLC19A2*	Early childhood	Rare (41 reported families)	Anemia	- Mitochondrial iron accumulation - Diabetes and deafness	-Thiamine supplementation
Miscellaneous	*HSPA9, NDUFB11….*	Early childhood	Rare (a few reported cases)	Anemia	- Mitochondrial iron accumulation	
ANEMIA WITH IRON DEFICIENCY OF GENETIC ORIGIN						
Iron refractory iron deficiency anemia (IRIDA)	*TMPRSS6 (matriptase 2)*	Childhood	Rare	Severe hypochromic and microcytic anemia	-Low plasma iron -Low iron concentration and transferrin saturation -High hepcidin production	-Parenteral iron -Hepcidin inhibitors (perspective)