Iron transport: From enterocyte to mitochondria

Demir taşınımı: Enterositten mitokondriye

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Abstract

Transport of iron to tissues is of vital importance. Remarkable advances have been made concerning the mechanisms involving iron metabolism after its absorption. Studies assessing cellular and mitochondrial iron metabolism have resulted in interesting findings. This review highlights the recent advances in the mechanisms involving transport and delivery of iron to tissues, cellular and mitochondrial iron metabolism, iron-related molecules, and mitochondrial disorders. *(Turk J Hematol 2010; 27: 137-46)*
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Özet

Demirin dokulara taşınması yaşamsal önem taşır. Demirin emiliminden sonraki metabolizmasıyla ilgili büyük ilerlemeler kaydedilmiştir. Selüler ve mitokondriyal demir metabolizmasıyla ilgili araştırmalar ilginç sonuçlar vermektedir. Bu derleme demirin dokulara taşınması, verilmesi, hücresel ve mitokondriyal demir metabolizması, demirle ilişkili moleküller ve mitokondriyal hastalıklarla ilgili yeni gelişmeleri vurgulamaktadır. *(Turk J Hematol 2010; 27: 137-46)*
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A current theme in the field of hematology as well as in other disciplines is the molecular mechanisms concerning iron metabolism. Mechanisms involving absorption of iron by enterocytes has been reviewed elsewhere [1-3]. In this review, delivery of iron to tissues, mechanisms concerning cellular and mitochondrial iron metabolism, iron-related molecules, and mitochondrial disorders are presented (Table 1).

Transport of iron in plasma

Binding to transferrin (Tf) is the first step in iron transport. Tf has high affinity for ferric iron; therefore, the oxidation of ferrous (Fe^{+2}) to the ferric (Fe^{+3}) form by *hephaestin* is an essential step for transport. Hephaestin is a ceruloplasmin homolog, and colocalizes with ferroportin at the basolateral membrane of enterocytes. The role of hephaestin in iron absorption was first shown in the *sla* (sex-

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Table 1. Genetic and functional identification of some iron-related proteins

	OMIM#	Gene	Locus	Function	Reference
Hephaestin	300167	HEPH	Xq11-q12	Oxidizes Fe^{+2} to Fe^{+3}	4, 5
Hepcidin	606464	HAMP	19q13	Induces internalization, ubiquitination and degradation of ferroportin via Janus kinase 2	6, 7
Transferrin	190000	TF	3q21	Transports iron from enterocyte to cells	8
Transferrin receptor 1 (CD71)	190010	TFR1	3q29	Transports Tf through the cell membrane	13
Transferrin receptor 2	604720	TFR2	7q22	Transports Tf through the cell membrane especially in hepatocytes	14
STEAP3	609671	STEAP3 (TSAP6)	2q14.2	Converts Fe^{+3} to Fe^{+2} in endosomes	22
IRP1	100880	IRP1 (ACO1)	9p22-p13	Bifunctional protein, acts as aconitase or iron response protein due to cellular iron levels	28
IRP2	147582	IRP2 (IREB2)	15q25.1	Binds to IRE in low-iron conditions	29
MRCKα	603412	MRCKA	1q41-q42	Regulates Tf-vesicle movement through actin/myosin filament assembly in response to decreased intracellular iron level	30
CDC14	603504	CDC14A	1p21	Operates in cell cycle, acts as a tumor suppressor	31
Poly r(C)-Binding Protein 1	601209	PCBP1	2p13-p12	Delivers iron from endosomes to ferritin	35
Mitoferrin	610387	MFRN	8p21	An iron importer on the inner membrane of the mitochondria	38
Frataxin	606829	FXN	9q13	Bifunctional mitochondrial protein that acts as a chaperone or stores iron due to cellular iron levels	40
Mitochondrial ferritin	608847	FTMT	5q21.3	Stores iron in mitochondrial matrix	41
ATP-Binding Cassette, Subfamily B, Member 7 (ABCB7)	300135	ABCB7	Xq13.1-q13.3	Transports ISCs to cytoplasm, a putative role in heme synthesis is also suggested	43, 44
ATP-Binding Cassette, Subfamily B, Member 6 (ABCB6)	605452	ABCB6	2q36	Transports porphyrin to mitochondria and functionally linked to heme biosynthesis	45, 46
Glutaredoxin	609588	GLRX5	14q32	Required for ISC assembly	60
BCS1L	603647	BCS1L	2q33	A chaperone that facilitates insertion of Rieske Fe/S protein into mitochondrial respiratory chain complex III	66

linked anemia) mouse with moderate to severe microcytic hypochromic anemia [4,5]. Enterocytes of the *sla* mouse are able to absorb iron from lumen; however, there is a block in transport to blood due to defective hephaestin. Another molecule that has a very important influence on iron absorption at this step is *hepcidin*. If total body iron is high, hepatic synthesis of hepcidin increases. Binding of hepcidin to ferroportin in its exterior segment causes upregulation of Janus kinase 2 [6,7]. Activation of Janus kinase 2 results in internalization, ubiquitination and degradation of ferroportin, which results in decreased iron transfer to blood.

Transferrin is the major protein that binds and delivers iron to tissues. It is member of a family of homologous transport proteins that includes lactoferrin, ovotransferrin, and melanotransferrin. All of these proteins share the same structure. Due to a suggested ancestral gene duplication, N- and C- terminals (lobes) of these proteins show approximately 40% sequence identity. These terminals are separated by a short spacer sequence. Each of these two lobes (N- and C-) is divided into two equally sized domains: N1, N2 and C1, C2. Clefts between these domains are designed for transport of Fe^{+3}; therefore, each Tf molecule can transport 2 iron molecules [8,9]. Side chains of 1 asparagine, 2 tyrosines and 1 histidine provide 3 negative charges within the cleft matching 3 positive charges of Fe^{+3}. Also, binding of CO_3^{2-} ion is critical for the binding and release of Fe^{+3}. Ferric iron and CO_3^{2-} ion could not bind to Tf in the absence of the other. Conformation of the binding site is suitable with ferric iron through a delicate balance. Binding constant for Fe^{+3} is approximately 10^{20} whereas it is only 10^3 for Fe^{+2} [10]. Binding and release of iron result in conformational changes in the Tf molecule enabling rapid exchange [11].

Transferrin receptors

Transferrin receptor (TfR), the binding site of Tf on the cell, has two types; namely TfR1 and TfR2.

TfR1 has an extremely important role in embryogenesis; knockout embryos do not survive due to defective erythropoiesis and neurological development [12,13]. However, other tissues are not affected in the absence of TfR1, which indicates additional mechanism(s) for iron transport in these cells. TfR1 is expressed in all tissues except mature erythrocytes. On the other hand, TfR2 is mainly expressed in the liver, normal erythroid precursors and some leukemic cells [14,15]. Although protein structures of TfR1 and TfR2 have a high degree of homology, their functions and regulation are not the same [16].

Expression of TfR1 is tightly regulated by cellular iron levels through HFE (hereditary hemochromatosis) protein. In normal conditions, HFE protein decreases the affinity of TfR1 for Tf by a magnitude of 10 to 50 [17]. Mutations in the HFE gene may alter the iron absorption and cause hemochromatosis phenotype. Nonetheless, cellular iron levels have no effect on the regulation of TfR2. Furthermore, TfR2 has no interaction with the HFE protein in contrast to TfR1. High levels of TfR2-mRNA expression in erythroid precursors and erythroleukemia cells point out that TfR2 expression is both cell-cycle and differentiation dependent [15]. Similar to TfR1, TfR2 has high affinity for diferric Tf. TfR2-mediated endocytosis of the Tf/iron complex is also the same as with TfR1 [18]. On the other hand, affinity of TfR2 for Tf is approximately 25-30-fold less than that of TfR1. Mutations in TfR2 cause a rare type of hereditary hemochromatosis (HH), namely HFE 3. A recent study in zebrafish embryos showed that TfR2 was required for hepcidin expression [19]. In summary, TfR2 senses the body iron status by sensing the Tf saturation. Consequently, it modulates hepcidin expression adjusted to the actual body iron level. Therefore, loss of TfR2 or its function results in failure to sense body iron status by hepatocytes, which causes increased hepcidin and iron deposition, especially in the liver [20].

Transfer of iron into cells

After binding of diferric Tf to TfR, the Tf/TfR complex on the *clathrin*-coated cell membrane is internalized through a receptor-mediated endocytosis. Clathrin coat is needed for invagination of the cell membrane. Newly formed vesicles lose the clathrin coat and fuse with endosomes [21]. After formation of endosomes, the acidification process by means of an ATPase proton pump (pH 5.5-6) takes place in order to dissociate iron from Tf. A protein called *STEAP3* (Six-Transmembrane Epithelial Antigen of Prostate 3), has been shown to convert Fe^{+3} to Fe^{+2} in erythroid precursor cells [22]. An animal model revealed that STEAP3 is highly expressed in hematopoietic tissues, colocalizes with the Tf cycle endosome, and facilitates Tf-bound iron uptake. Moreover, mutations in the STEAP3 gene were found to be responsible for the iron deficiency anemia in the mouse mutant *nm1054*. Homologs of this protein, STEAP1, 2 and 4, are expressed in hepatocytes as well as other tissues [23]. Free ferrous iron released from Tf in the endosome enters into cytoplasm via DMT1 on the endosomal membrane. Then, the endosome containing Tf and TfR fuses back to the plasma membrane. This recycling process enables Tf and TfR to be used in new cycles.

Hepatic iron uptake

Hepatic iron transport has different aspects compared to the other cells [24]. Hepatocytes can acquire both transferrin-bound iron (TBI) and non-transferrin-bound iron (NTBI) from plasma (Table 2).

Table 2. Hepatocyte uptake of iron-related proteins

Form of iron	Hepatic uptake mechanism	Entry into hepatocyte
TBI	TfR1- or TfR2-mediated endocytosis Direct uptake of iron after releasing from Tf on hepatocyte surface	Through DMT1 on endosomal membrane Through DMT1, ZIP14 or calcium channels on hepatocyte membrane
NTBI	Direct uptake of iron after releasing from carrier molecule (mostly citrate) on hepatocyte surface	Through DMT1, ZIP14 or calcium channels on hepatocyte membrane
Ferritin Lactoferrin Heme-hemopexin complex Hemoglobin-haptoglobin complex	Specific receptor-mediated uptake	Through endocytosis

Table 3. Some iron-related molecules containing IREs on their mRNA

Molecule	IRE localization on mRNA	Function
TfR1	3'UTR	Iron uptake
DMT1	3'UTR	Iron uptake
Ferroportin	5'UTR	Iron export
H- and L-ferritin	5'UTR	Iron storage
ALAS1 and ALAS2	5'UTR	Iron utilization
MRCKα	3'UTR	Tf vesicle movement
CDC14A	3'UTR	Tumor suppressor

TBI uptake may occur through TfR-dependent or -independent mechanisms. As discussed above, both TfR1 and TfR2 operate in the uptake of TBI. In the process of TfR-independent delivery of Tf-bound iron, Tf releases iron at the hepatocyte surface. Following this step, a ferrireductase activity converts Fe^{+3} to Fe^{+2}, which also takes place at the surface [25]. Afterwards, Fe^{+2} enters the cell via membrane transporters. The most common form of NTBI is iron citrate, usually increased in iron overload disorders [26]. Iron citrate releases iron at the hepatocyte surface like TBI. Iron delivered by NTBI also enters hepatocytes via membrane transporters, especially DMT1 [27]. Other molecules transporting iron through the hepatocyte membrane are *ZIP14* and calcium channels.

There are some other iron-related proteins or complexes transported to hepatocytes by specific membrane receptors. These include ferritin, lactoferrin, heme-hemopexin complex, and hemoglobin-haptoglobin complex. The fate of these molecules in hepatocytes following endocytosis may be different: iron and some proteins (i.e. ferritin) may reside in the hepatocyte, some proteins may go into degradation (i.e. ferritin, heme, hemoglobin, haptoglobin, lactoferrin), whereas others may recycle back to the plasma (i.e. hemopexin, receptors of these proteins) or to bile (i.e. ferritin, hemoglobin) [24].

Molecular control of iron content in cells

The need for iron is different in different body cells. For example, cardiomyocytes, muscle cells and neurons require higher amounts of iron compared to the other cells. Therefore, uptake of iron is individually regulated in each cell regardless of total body iron need. Important molecules concerning cellular iron uptake are iron-responsive proteins (IRP1 and IRP2) and iron-responsive elements (IRE). Two IRPs have been defined to date: IRP1 and IRP2 [28,29]. In iron-depleted conditions, IRP1 and 2 bind to IREs of a number of molecules related to iron metabolism (Table 3). These molecules are assigned to iron uptake (*TfR1, DMT1*), utilization [*5-aminolevulinate synthase (ALAS1) and erythroid ALAS (ALAS2)*], storage (*H- and L-ferritin*) or export (*ferroportin*). Recent studies showed that at least two cell cycle-related molecules, *MRCK*α (myotonic dystrophy kinase-related Cdc42-binding kinase) and *CDC14A* (cell division cycle 14A), also bear IREs in their mRNA at the 3' untranslated region (UTR). It has been suggested that MRCKα, a serine/threonine kinase, regulates Tf-vesicle movement through actin/myosin filament assembly in response to decreased intracellular iron level. Supporting this suggestion, *MRCK*α-mRNA levels in various tissues strongly positively correlated with the level of *TfR*-mRNA levels [30]. The second cell cycle-related molecule, CDC14A, is a phosphatase involved in the dephosphorylation of several critical cell cycle proteins and is suggested to act as a tumor suppressor [31].

Synthesis of IRPs is regulated by IRPs to maintain optimum iron levels in each cell. Depending on the location of IRE, the IRE/IRP interaction results in stabilization or translational repression of mRNA [32]. During low-iron conditions, synthesis of both IRP1 and IRP2 increases. Interaction of IRPs with IREs in the 3' UTR of mRNA results in stabilization; therefore, increased expression of molecules such as TfR1 and DMT1. Meanwhile, interaction of IRPs with IREs in the 5' UTR of mRNA results in translational repression; the expression of molecules such as ferritin, ferroportin, and ALAS2 will be suppressed. During high-iron levels, both IRP1 and IRP2 lose their affinity for IRE. As a result, mRNAs bearing IRE in the 5' UTR stabilize and expression of molecules such as ferritin, ferroportin, and ALAS2 increases whereas synthesis of TfR1 and DMT1 decreases. IRP1 is a bifunctional protein that binds iron-sulfur clusters (ISC) in case of high intracellular iron. After binding ISC [4Fe-4S], IRP1 functions as aconitase, and loses its IRE-related functions. However, the high intracellular iron levels do not cause a conformational change but rather cause degradation of IRP2.

Erythroid cells require more iron than any other cell in the body. Therefore, these cells need specific adaptation mechanism(s) for high iron influx. A recent study revealed that terminal erythropoiesis caused a switch of regulation to a different mode in the IRP/IRE system [33]. Since committed erythro-

blasts need much more iron than any other cell, they should maintain high TfR1 expression despite high intracellular iron levels. In this context, the erythroid IRP/IRE system should sense a low-iron state despite increased cytosolic iron. Furthermore, although both *ferritin*- and *ALAS2*-mRNAs bear IRE on their 5' UTR, strong inhibition of *ferritin*-mRNA translation and efficient *ALAS2*-mRNA translation have been observed in differentiating, iron-rich erythroblasts. Another conflicting data regarding IRE/IRP regulation is of ferroportin. *Ferroportin*-mRNA also bears IRE on its 5' UTR. Therefore, during low-iron conditions, mRNA stability of ferroportin should decrease in all cells. However, this is not the case for duodenal absorptive cells and erythroid precursors. During low-iron conditions, increased absorption of iron by enterocytes and release to blood via ferroportin is a physiologically relevant way. A very recent study documented that duodenal epithelial and erythroid precursor cells utilize an alternative upstream promoter to express a ferroportin transcript, ferroportin1B, which lacks the IRE and is not repressed in iron-deficient conditions [34]. The identification of ferroportin1B may explain how high ferroportin expression is possible in duodenal epithelial and erythroid precursor cells during high intracellular iron levels. This study strongly suggests that different regulatory systems operate influx of iron into different cells.

Mitochondrial iron metabolism

After entering from the endosome to cytoplasm, iron can be stored within ferritin or it can be used for cellular reactions. A newly discovered protein, *PCBP1* [Poly r(C)-Binding Protein 1], has been reported to transfer iron from the endosome to ferritin [35]. Although the exact mechanism of iron transport to mitochondria is not well defined, iron can also enter the mitochondria. A recent report defined a new way, namely "kiss and run" [36]. It was suggested that delivery of iron to mitochondria happens directly from iron-containing endosomes bypassing the cytoplasm. Molecules, including iron, cross the mitochondrial outer membrane via a large diameter voltage-dependent anion channel called *porin* [37]. Transport of iron through the inner mitochondrial membrane occurs with the aid of *mitoferrin*, a special carrier. Mutation in the mitoferrin gene has been described in a zebrafish mutant that caused profound hypochromic anemia and erythroid maturation arrest owing to defects in mitochondrial iron uptake [38]. After its transport into mitochondria, iron can be stored or used for vital reactions. Two important iron-related reactions that happen within mitochondria are heme synthesis and ISC biogenesis [39]. Iron in the mitochondrial matrix is in potentially redox-active ferrous (Fe^{+2}) form. Therefore, tight control of iron influx and maintenance in bound-form is mandatory to achieve low toxic iron levels in the mitochondrial matrix. Two newly described proteins store iron within the matrix: *frataxin* (FXN) and *mitochondrial ferritin*. FXN is a bifunctional protein like IRP1 [40]. It works as a Fe^{+2} chaperone for metabolic actions when mitochondrial iron is limited. When iron is in

Table 4. Some disorders related to mitochondrial iron metabolism

Disease	OMIM#	Inheritance	Related gene locus	Related molecular defect	Clinical features
Friedreich ataxia	229300	Autosomal recessive	9q13, 9p23-p11	Expansion of GAA triplet repeats in intron 1 (97%) or point mutations in FXN gene	Progressive ataxia, cardiomyopathy, skeletal deformities, and impaired glucose tolerance or diabetes mellitus
X-linked sideroblastic anemia	300751	X- linked recessive	Xp11.21	Mutation in ALAS2 gene	Hypochromic microcytic anemia, variable response to pyridoxine treatment
Pyridoxine-unresponsive sideroblastic anemia due to GLRX5 mutation	205950	Autosomal recessive	14q32, 3p22.1	Mutation in GLRX5 gene	Severe microcytic anemia, jaundice, hepatosplenomegaly, iron overload, ringed sideroblasts, and cirrhosis
X-linked sideroblastic anemia with ataxia	301310	X- linked recessive	Xq13.1-q13.3	Mutation in mitochondrial ABCB7 gene	Hypochromic microcytic anemia, early onset nonprogressive ataxia
Myopathy associated with ISCU1 defect	255125	Autosomal recessive	12q24.1	Mutation in ISCU1 gene	Early fatigue, dyspnea, and palpitations followed by hard and tender muscles and muscle cramps
GRACILE syndrome	603358	Autosomal recessive	2q33	Mutation in BCS1L gene	Growth retardation, amino aciduria, cholestasis, iron overload, lactic acidosis, and early death

excess, it works as a storage compartment forming ferrihydrite. The mitochondrial ferritin precursor protein is coded by an unusual intronless gene and targeted to mitochondria. Within mitochondria, this precursor is processed to a smaller protein, mitochondrial ferritin. It is remarkably similar to H-ferritin that assembles into typical ferritin shells within mitochondria and has ferroxidase activity [41]. Immunohistochemical analysis showed that erythroblasts of subjects with impaired heme synthesis had high amounts of mitochondrial ferritin accumulation. In pathological conditions, it can be easily detected as "ringed sideroblast" [42].

One of the main functions of mitochondria is ISC biosynthesis. These clusters are cofactors of several proteins including electron transport, Krebs cycle, regulation of gene expression, and redox reactions [39]. Iron sulfur clusters have also been shown in cytoplasm within IRP1 and in the nucleus within an enzyme that is involved in base excision repair. After synthesis within mitochondria, ISCs are transported to cytoplasm through mitochondrial inner membrane channels by a specific carrier, *ABCB7 (*ATP-Binding Cassette, Subfamily B, Member 7) [43]. ABCB7 also interacts with ferrochelatase, suggesting a putative role in heme metabolism [44]. The mitochondrion is a major site for heme synthesis. Out of 8 steps in heme biosynthesis, 4 steps, including the first rate-limiting step of the pathway, take place within mitochondria. This step involves condensation of succinyl CoA and glycine to delta-aminolevulinate catalyzed by delta-ALAS. There are 2 types of ALAS: ALAS1 and ALAS2 (erythroid ALAS). Although the rate-limiting step in non-erythroid cells is the delta-aminolevulinate formation, in erythroid cells, the rate-limiting step is acquisition of iron to mitochondria. *ABCB6* (ABCB Member 6), a protein very similar to ABCB7, has been shown to localize at the outer mitochondrial membrane [45]. A recent study suggested that ABCB6 is upregulated by elevation of cellular porphyrins and has a function in porphyrin transport to mitochondria. The authors also predicted that ABCB6 is functionally linked to heme biosynthesis [46].

Mitochondrial disorders related to iron metabolism

The current understanding of mitochondrial iron metabolism has led to elucidation of pathophysiologic mechanisms in some disorders (Table 4). These disorders usually affect erythroid cells as well as neural tissues.

Friedreich ataxia

Friedreich ataxia (FRDA) is the most common form of autosomal recessive ataxia. The disease is characterized by progressive ataxia of all limbs, skeletal and cardiac muscle myopathy, skeletal deformities, impaired glucose tolerance or diabetes mellitus, sensorineural deafness, and optic neuropathy [47]. The molecular abnormality in more than 97% of patients is GAA trinucleotide repeat expansion in intron 1 of the *FXN* gene [48]. Normal alleles of the FXN gene have 5 to 30 GAA repeat expansions; however, disease alleles have from 70 to more than 1,000 GAA triplets. Increased GAA repeats causes triplex DNA structures, called "sticky DNA", which interfere with *FXN*-mRNA transcription [49]. As in other triplet repeat disorders, the size of triplet expansion correlates inversely with the age of onset and directly with the rate of disease progression [50].

Since known mitochondrial diseases and FRDA share some clinical manifestations, mitochondrial disturbance in the etiology of FRDA has long been suspected. For example, isolated vitamin A deficiency, a disease with mitochondrial involvement, has strikingly similar signs and symptoms with FRDA [51]. After demonstration of a yeast that has mitochondrial iron accumulation due to a defective protein homolog with FTX, subsequent studies revealed defective FTX in the etiology of FRDA [52]. FTX plays a vital role in the regulation of mitochondrial iron metabolism. However, the pathophysiology of FRDA has not been completely understood to date. Oxidative stress due to FTX deficiency has usually been cited in the FRDA pathophysiology. The decrease in the ISC-containing proteins (aconitase and mitochondrial respiratory chain complexes) observed in heart biopsies of patients with FRDA supports that this finding was related to a mitochondrial damage caused by iron overload [53]. Another study on cultured fibroblasts carrying homozygous FRDA mutation showed that these fibroblasts were more sensitive to oxidative stress than were the controls [54]. Treatment with deferoxamine and apoptosis inhibitors rescued fibroblasts with FRDA mutation. A recent study that demonstrated upregulation of stress pathways in FTX-deficient cells further supports the role of oxidative stress in FRDA as well [55].

X-linked sideroblastic anemia

X-linked sideroblastic anemia (XLSA) is a rare disorder caused by mutations in the ALAS2 gene [56]. The peripheral smear of patients typically includes two distinct erythrocyte populations: normochromic normocytic erythrocytes together with hypochromic microcytic cells. Bone marrow examination reveals ringed sideroblasts due to increased iron within mitochondrial ferritin [57]. Since neuronal cells rely on ALAS1 for iron metabolism, no neurological finding is present in these patients in contrast with XLSA with ataxia. Although the inheritance is X-linked recessive, some female carriers may present with severe anemia. This finding is attributed to an additional event following inheritance of the *ALAS2* mutation, i.e. congenital skewing led to dominance of hematopoietic cells expressing the X chromosome with the mutant gene [58]. Interestingly, a case study revealed a significantly higher frequency of coinheritance of HFE mutant allele C282Y in patients with XLSA compared to the normal population [57]. Interaction of two mutant alleles may point out the severity of iron overload. Response to pyridoxine treatment is variable among XLSA patients. This study [57] also revealed that iron overload suppressed response to pyridoxine. Another factor suggested for variability in pyridoxine response is the location of the mutation on the ALAS2 gene. Analysis of the ALAS2 crystal structure in a recent study revealed that patients with mutations close to the 5' pyridoxal phosphate binding site respond to pyridoxine [59].

Pyridoxine-unresponsive sideroblastic anemia due to GLRX5 mutation

In 2007, a 60-year-old southern Italian man who presented with severe microcytic anemia, jaundice, hepatosplenomegaly, iron overload, ringed sideroblasts, and cirrhosis was reported [60]. His unaffected parents were consanguineous, consistent with autosomal recessive inheritance. Interestingly, anemia of the patient worsened with transfusion but improved with iron chelation. Striking similarities of his phenotype with mutant *shiraz* zebrafish directed the authors to the *Glutaredoxin 5* (*GLRX5*) gene, which was largely deleted in *shiraz* zebrafish. They found a homozygous mutation in this gene that interferes with intron 1 splicing and causes a drastic reduction in *GLRX5*-RNA. GLRX5 deficiency causes impaired ISC synthesis that activates cytosolic IRP1 and finally leads to increased cellular iron import. As discussed above, increased IRP1 results in increased TfR and decreased ferritin and ALAS2 synthesis. As a result, decreased ALAS2 level causes anemia in spite of increased intracellular iron level. In this low-heme environment, IRP2 does not undergo proteosomal degradation. Prolonged activity of IRP2 may contribute to increased mitochondrial iron. The authors concluded that redistribution of iron into cytoplasm by iron chelation might relieve iron excess, improving heme synthesis and anemia.

X-linked sideroblastic anemia with ataxia

X-linked sideroblastic anemia with ataxia is a mitochondrial disease caused by a mutation in the nuclear genome. The disease is characterized by hypochromic microcytic anemia with ring sideroblasts and early onset, nonprogressive spinocerebellar ataxia. Missense mutations on the ABCB7 gene cause *partial loss of function* in patients with this disease [61]. Nonsense mutations have not been identified, possibly due to the lethal effect of *complete loss of function* that was shown in a knock out mouse model [62]. The defect in the ABCB7 gene causes deficiency in ISCs in cytoplasm but not in mitochondria. However, iron is accumulated within mitochondria by an unknown mechanism [43].

Myopathy associated with ISCU1 defect

The disease was first described in patients from Sweden and characterized by low physical performance. Physical activity results in early fatigue, dyspnea, and palpitations followed by hard and tender muscles and muscle cramps. Recently, a splice mutation in the ISC scaffold protein *ISCU* has been reported in these patients [63]. The loss of FTX in FRDA and GLRX5 in pyridoxine-unresponsive sideroblastic anemia causes deficiency in ISCs as in the myopathy associated with ISCU1 defect. Typically, iron accumulation in mitochondria is a common event in these three disorders. The authors suggested that this observation supported the possibility that one or more ISC proteins act as sensor for regulation of mitochondrial iron homeostasis.

GRACILE syndrome

GRACILE syndrome (Growth Retardation, Amino aciduria, Cholestasis, Iron overload, Lactic acidosis,

and Early death) has been frequently reported in neonates from Finland [64]. Necropsy findings have revealed increased liver iron content, paucity of intrahepatic bile ducts and periportal fibrosis in the oldest patient, who died at the age of 4 months. Electron microscopic studies revealed an abundance of hemosiderin granules; however, the number and structure of mitochondria were normal. Subsequently, case reports including Turkish patients together with patients from other countries have been followed [65]. Distinctive clinical findings were reported among patients from different countries: Turkish and British patients had variable neurological symptoms and findings. Recently, mutations in the *BCS1L* gene as a cause of this disorder have been identified [66]. This gene encodes a mitochondrial inner-membrane protein. It is a chaperone that is presumed to facilitate insertion of Rieske Fe/S protein into precursors of mitochondrial respiratory chain complex III. The Rieske iron-sulfur protein is a nuclear-encoded subunit of the mammalian cytochrome complex III of the mitochondrial respiratory chain [67]. Interestingly, the British and Turkish patients with GRACILE syndrome had mitochondrial complex III deficiency, whereas in the Finnish patients, complex III activity was within the normal range. Also, a distinctive mutation in the *BCS1L* gene has been found in Turkish patients.

Conflict of interest

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

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