Free Radical Biology and Medicine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Molecular pathophysiology and genetic mutations in congenital sideroblastic anemia

Tohru Fujiwara, Hideo Harigae*

Department of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai 980-8575, Japan

ARTICLE INFO

Keywords:
Congenital sideroblastic anemia
Heme
Iron
Mitochondria
ALAS2

ABSTRACT

Sideroblastic anemia is a heterogeneous congenital and acquired disorder characterized by anemia and the presence of ring sideroblasts in the bone marrow. Congenital sideroblastic anemia (CSA) is a rare disease caused by mutations in genes involved in the heme biosynthesis, iron–sulfur [Fe–S] cluster biosynthesis, and mitochondrial protein synthesis. The most prevalent form of CSA is X-linked sideroblastic anemia, caused by mutations in the erythroid-specific δ-aminolevulinate synthase (*ALAS2*), which is the first enzyme of the heme biosynthesis pathway in erythroid cells. To date, a remarkable number of genetically undefined CSA cases remain, but a recent application of the next-generation sequencing technology has recognized novel causative genes for CSA. However, in most instances, the detailed molecular mechanisms of how defects of each gene result in the abnormal mitochondrial iron accumulation remain unclear. This review aims to cover the current understanding of the molecular pathophysiology of CSA.

1. Introduction

Sideroblastic anemia comprises a group of disorders that share characteristics of the presence of bone marrow ring sideroblasts, reflecting the excess mitochondrial iron deposition [1–5]. In adults, these syndromes are often found to be related to myelodysplastic syndrome (MDS), of which a vast majority of cases might result from a mutation in the RNA-splicing machinery component splicing factor 3b, subunit 1 (*SF3B1*) [6]. In addition, sideroblastic anemia occurs after exposure to certain drugs, alcohol, or zinc or with copper deficiency [1,7].

Typically, congenital forms of sideroblastic anemia (congenital sideroblastic anemia, CSA) are rare and constitute a diverse class of inherited disorders. Most CSA cases are clinically related to ineffective erythropoiesis and secondary iron overload. Often, anemia is the only manifestation of CSA and might be manifested prenatally, at birth, in childhood, and even adulthood, depending on the mutation pattern of the individual causative gene and, perhaps, other factors [4]. In some CSA cases, nonhematopoietic manifestations, such as neuromuscular and metabolic disorders, are also accompanied by hematological disorders. Based on the pathophysiology of the mitochondrial iron–heme metabolism, causative genes for CSA can be categorized into the following three subtypes: heme biosynthesis, iron–sulfur (Fe–S) cluster biosynthesis, and mitochondrial protein synthesis [1–5]. Recently, the application of the next-generation sequencing technology has recognized several causative genes for CSA. However, in most cases, the detailed molecular mechanisms of how defects of each gene results in the abnormal mitochondrial iron accumulation remain unclear. Hence, this review summarizes the recent progress in the molecular genetics, pathophysiology, and clinical features of CSA.

2. Genetic features and pathophysiology of CSA

As mentioned earlier, CSA is caused by the mutation in genes involved in heme biosynthesis, Fe–S cluster biosynthesis, and mitochondrial protein synthesis (Table 1) [1–5]. In addition, the presence of nonhematopoietic complications differentiates syndromic CSAs from nonsyndromic CSAs, depending on the kind of genes mutated (Table 1). Later, we will describe the suggested roles of these genes in the pathophysiology of sideroblastic anemia.

2.1. CSA caused by defects in heme biosynthesis

The mammalian heme biosynthetic pathway comprises eight enzymes (Fig. 1) [8–12]. The first and rate-limiting step of this pathway is the condensation of glycine and succinyl-CoA to form a 5-aminolevulinic acid (ALA) in the mitochondrial matrix; this reaction is catalyzed by δ-aminolevulinate synthase (ALAS). ALAS comprises two isozymes encoded by the housekeeping and erythroid-specific *ALAS* genes,

* Corresponding author.
E-mail address: harigae@med.tohoku.ac.jp (H. Harigae).

https://doi.org/10.1016/j.freeradbiomed.2018.08.008
Received 17 July 2018; Received in revised form 2 August 2018; Accepted 4 August 2018

Table 1
Genetic features of congenital sideroblastic anemias (CSAs).

	Inheritance	Causative gene	CSA class	Associated abnormalities
Heme biosynthesis				
XLSA	X-linked	*ALAS2*	Non-syndromic	Iron overload
SA/SLC25A38	AR	*SLC25A38*	Non-syndromic	Iron overload
SA/STEAP3	AD?	*STEAP3*	Syndromic	Hypogonadism
[Fe-S] cluster biosynthesis				
XLSA/A	X-linked	*ABCB7*	Syndromic	Ataxia
SA/GLRX5	AR	*GLRX5*	Non-syndromic	Iron Overload
SA/HSPA9	AR/Dominant?[a]	*HSPA9*	Non-syndromic	
Mitochondrial protein synthesis				
PMPS	Maternal[b]	*mtDNA*	Syndromic	Metabolic acidosis, Exocrine insufficiency, Renal failure
MLASA1/PUS1	AR	*PUS1*	Syndromic	Lactic acidosis, Myopathy, Cardiomyopathy
MLASA2/YARS2	AR	*YARS2*	Syndromic	Lactic acidosis, Myopathy, Cardiomyopathy
SIFD/TRNT1	AR	*TRNT1*	Syndromic	Fever, Iron overload, Deafness, Cardiomyopathy, CNS derangement
TRMA	AR	*SLC19A2*	Syndromic	Diabetes, Deafness, Psychiatric symptoms, Heart malformations
SA/NDUFB11	X-linked	*NDUFB11*	Syndromic	Lactic acidosis

Abbreviations: XLSA, X-linked sideroblastic anemia; AR, Autosomal recessive; AD, Autosomal dominant; XLSA/A, X-linked sideroblastic anemia with ataxia; PMPS, Pearson Marrow Pancreas Syndrome; TRMA, Thiamine-responsive megaloblastic anemia; MLASA, Mitochondrial myopathy and sideroblastic anemia; SIFD, syndromic form of congenital sideroblastic anemia associated with B-cell immunodeficiency, periodic fevers,and developmental delay; CNS, Central nervous system.

[a] A majority of cases are inherited with pseudo-dominant pattern.

[b] Sporadic cases are also observed.

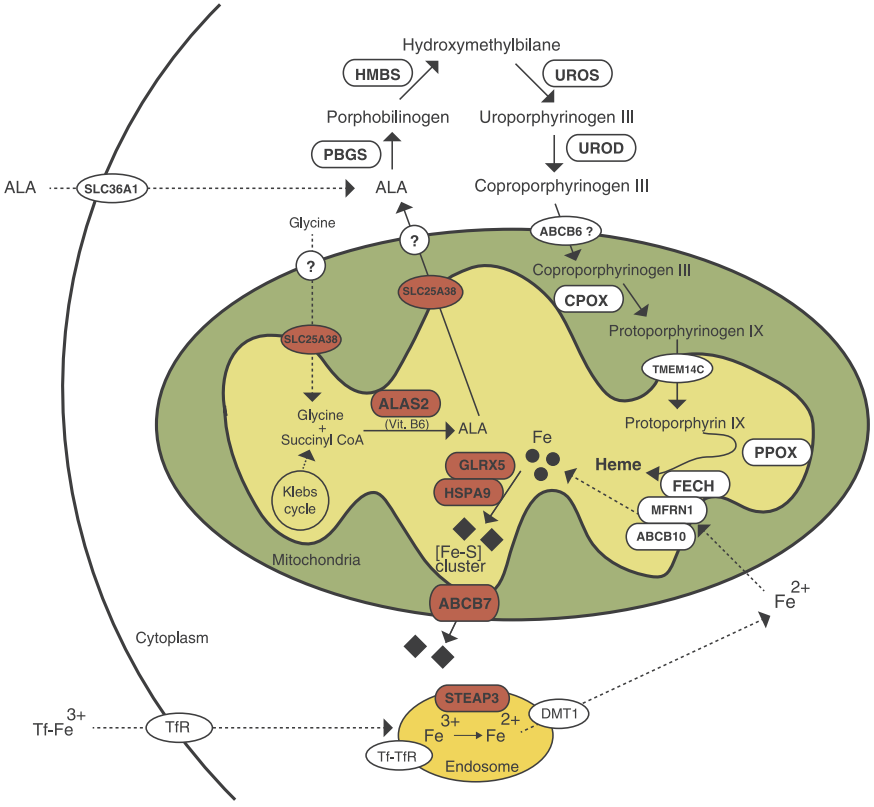

Fig. 1. The heme biosynthetic pathway in erythroid cells. A schematic representation of the heme biosynthetic pathway in erythroid cells. Heme synthesis starts with the condensation of glycine and succinyl-CoA to form ALA. Then, ALA is transported outside the mitochondria and catalyzed to form coproporphyrinogen III. CPOX converts coproporphyrinogen III to protoporphyrinogen IX, which is subsequently oxidized to protoporphyrin IX by PPOX. Finally, ferrous iron is incorporated into protoporphyrinogen IX to form heme, a reaction catalyzed by FECH. FECH is localized in the inner mitochondrial membrane in association with MFRN1 and ABCB10. In addition, both SLC25A38 and ABCB10 have been proposed as mitochondrial ALA exporters on the inner mitochondrial membrane. ABCB6 and TMEM14C have been proposed as putative coproporphyrinogen III and protoporphyrinogen IX importer, respectively. FLVCR1b is a mitochondrial heme exporter. Furthermore, mitochondrial iron is used to generate the Fe–S cluster by the Fe–S cluster assembly complex, including HSPA9 and GLRX5, which is subsequently exported by ABCB7. Responsive genes for CSA are highlighted in red. Abbreviations: ALAS2, erythroid-specific δ-aminolevulinate synthase; ALA, δ-aminolevulinic acid; PBGS, porphobilinogen synthase; HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen synthase; UROD, uroporphyrinogen decarboxylase; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen IX oxidase; FECH, ferrochelatase; MFRN1, mitoferrin 1; Vit. B6, vitamin B6; SLC25A38, solute carrier family 25 member 38; ABCB10, ATP-binding cassette subfamily B member 10; TMEM14C, transmembrane protein 14C; Tf, transferrin; TfR, transferrin receptor; STEAP3, six-transmembrane epithelial antigen of the prostate 3; DMT1, divalent metal transporter 1; HSPA9, heat shock protein family A (Hsp70) member 9; GLRX5, glutaredoxin 5; ABCB7, adenosine triphosphate (ATP) binding cassette B7.

termed *ALAS1* and *ALAS2*, respectively. *ALAS2* needs pyridoxal 5′-phosphate (PLP, vitamin B6) as a cofactor for its enzymatic activity [13]. However, the transcription factor GATA-1 is considered to induce the erythroid-specific expression of the *ALAS2* gene [14]; an iron-responsive element (IRE) located at the 5′-untranslated region (UTR) of *ALAS2* is responsible for regulating the *ALAS2* expression at the post-transcriptional level by the function of iron-responsive proteins (IRPs; explained later) [1,5]. ALA is exported to the cytosol by the solute carrier family 25, member 38 (SLC25A38), where two molecules of ALA are condensed into the monopyrrole porphobilinogen (PBG), which is generated by PBG synthase (PBGS). Then, four PBG molecules are joined by hydroxymethylbilane synthase (HMBS) to form the first cyclic tetrapyrrole HMB, which is, then, converted to uroporphyrinogen III by uroporphyrinogen synthase (UROS). Subsequently, uroporphyrinogen III is decarboxylated by uroporphyrinogen decarboxylase (UROD) to form coproporphyrinogen III. Coproporphyrin III enters the mitochondria, where it is oxidatively decarboxylated by coproporphyrinogen oxidase (CPOX) to form protoporphyrinogen IX. Then, protoporphyrinogen IX is oxidized to protoporphyrin IX by protoporphyrinogen oxidase (PPOX). Finally, ferrous iron is inserted into protoporphyrin IX by ferrochelatase (FECH) to form heme; FECH is another rate-limiting enzyme of the heme biosynthetic pathway that

exists as a homodimer in which each subunit contains a Fe–S cluster that is necessary for the enzymatic activity [15].

In addition, heme biosynthesis relies on the intracellular availability of iron. In erythroid cells, iron is acquired by transferrin receptor–mediated endocytosis of circulating transferrin–iron(III) (Fe^{3+}) complexes. Once internalized, transferrin-bound Fe^{3+} are released, reduced to Fe^{2+} by six-transmembrane epithelial antigen of the prostate 3 (STEAP3) [16], exit the endosome through divalent metal transporter 1 (DMT1), enter the mitochondria, and, eventually, used to generate heme (Fig. 1).

2.1.1. X-linked sideroblastic anemia due to ALAS2 defect

The most prevalent form of CSA is X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene *ALAS2*, located at Xp11.21 [17]. Before the determination of the *ALAS2* gene as a causative gene for CSA, the disease entity was known as pyridoxine-responsive anemia because of the characteristic improvement of anemia by the PLP administration [18].

Clinically, patients with XLSA are typically males who present at various ages, usually aged < 40 years [4]; however, late-onset XLSA cases have also been reported [19,20]. Anemia is a hypochromic, microcytic disease with a varying degree and also accompanied by the systemic iron overload, even in XLSA cases without requiring red blood cell transfusion. In nearly half of the cases, anemia is responsive to PLP [21,22]. In addition, mutations in the *ALAS2* gene in patients with XLSA are heterogeneous and usually missense mutations of conserved amino acids that result in the loss-of-function [2–5,22]. To date, > 80 different mutations in *ALAS2* have been reported in patients with XLSA [2,4,22]. Reportedly, missense mutations in *ALAS2* are frequently observed in exons 5–11, encompassing exon 9, which contains the lysine responsible for the PLP binding [23]. Conversely, mutations in the *ALAS2* regulatory region, such as the promoter [24] and intron 1 [25,26], have also been reported, which result in the decreased *ALAS2* expression. Overall, a reduction in the *ALAS2* expression level, as well as defects in catalysis, substrate, or cofactor affinity, and protein processing of *ALAS2* have been implicated in the XLSA pathogenesis, and supplementation with PLP might contribute to the mitigation of these impairments.

Although patients with XLSA are predominantly males, as described above, cases of several female patients with the heterozygous *ALAS2* mutation have also been reported [27–36]. Regarding the underlying molecular mechanisms, several studies have hypothesized the role of the skewed X chromosome inactivation in female heterozygous carriers, in which wild-type *ALAS2*-expressing hematopoietic cells were preferentially inactivated [27,30,31,33]. However, some other studies have reported no evidence of the skewed X chromosome inactivation in heterozygous female carrier presenting as macrocytic sideroblastic anemia [34,36]. In the latter case, the rare clinical phenotype of macrocytic sideroblastic anemia is attributed to the severe loss-of-function mutation in *ALAS2*, resulting in the intramedullary apoptosis of *ALAS2*-mutated erythroid precursors and enabling only wild-type erythroid cells to reach the circulation. Supporting the hypothesis, these cases were refractory by the PLP treatment, and the complete skewing toward the expression of wild-type ALAS2 mRNA was validated in peripheral reticulocytes [34–36]. As MDS cases typically exhibit macrocytic anemia [37], it is imperative to consider the possibility of the presence of the heterozygous *ALAS2* mutation in the diagnosis of MDS with ring sideroblasts [38], especially in females with a family history of anemia and only minor dysplastic morphological changes.

Nearly half of the XLSA cases are unresponsive to PLP [21], and the oral ALA supplementation was inadequate to improve anemia in XLSA, necessitating the exploration of novel therapeutic strategies [39]. However, evidence regarding the molecular characteristics of ring sideroblasts is scarce because of the lack of biological models. To date, several attempts, such as *Alas2*-knockout mice [40], *Alas2*-knockout embryonic stem (ES) cells [41], CRISPR/Cas9-based targeted disruption of the GATA-1 binding motif at intron 1 of murine *Alas2 in vitro* [42] and *in vivo* [43], have not illustrated the emergence of ring sideroblasts that could be applied for further molecular analyses. We recently succeeded in establishing human ring sideroblasts from induced pluripotent cells (iPS) of XLSA [44], as well as CRISPR/Cas9-based targeted disruption of *ALAS2* intron 1 enhancer based on human proerythroblast cell line [45]. While it has been assumed that abnormal iron accumulation in the mitochondria may induce apoptotic death of erythroblasts by inducing oxidative stress, our results imply that ring sideroblasts exhibited accumulation of anti-apoptotic enzymes, such as *HSP70* (heat shock protein 70), and concomitantly reduced level of reactive oxygen species, indicating that ring sideroblast formation might be a consequence of cytoprotective reaction [45]. Further analyses of the XLSA models would facilitate in elucidating its molecular etiology as well as establish novel therapeutic strategies.

2.1.2. CSA due to defects in SLC25A38

The second most prevalent nonsyndromic form of CSA is caused by the mutation in the *SLC25A38* gene, which encodes an erythroid-specific protein of the inner mitochondrial membrane [22]. Assumedly, *SLC25A38* is involved in the mitochondrial import of glycine, which is essential for the ALA synthesis (Fig. 1) [46]. Hence, the molecular basis contributing to the ring sideroblast formation is similar to that of XLSA. Patients typically present at birth or early childhood as severe, transfusion-dependent microcytic anemia and iron overload (high serum ferritin and transferrin saturation), which clinically resembles thalassemia major [22,47]. However, the patterns of mutation vary, including nonsense, frameshift, splice acceptor site, and missense mutations, and the mode of inheritance is autosomal recessive [46–50]. The present treatment comprises chronic blood transfusion coupled with iron chelation. In addition, hematopoietic stem cell transplantation is suggested as the only curative treatment [47], although clinicians should be cautious to decide its indication. Recently, based on a zebrafish *slc25a38* CSA model, the combination of glycine and folate administration was shown to exhibit promising results [51]; however, its efficacy has not been validated in humans yet [52].

2.1.3. CSA due to other heme biosynthesis defects

As described above, STEAP3 encodes ferrireductase that is responsible for the reduction of Fe^{3+} to Fe^{2+} in the endosomes of erythroblasts [16]. A study reported the human *STEAP3* mutation in 3 affected siblings from nonconsanguineous parents, who exhibited severe hypochromic anemia, ring sideroblasts in the bone marrow, and gonadal dysfunction [53]. While a heterozygous mutation was inherited from the father, no mutation was observed in the mother. Perhaps, *STEAP3* is an expressed quantitative trait locus (e-QTL), and the nonmutated allele was expressed at lower levels in affected children than that in their father. Concomitantly, mice lacking *Steap3* has also demonstrated hypochromic anemia [54]. The presence of microcytic anemia in both human and mice by reduced ferrireductase activity suggests that heme biosynthesis may be compromised by the reduction of ferrous iron (Fe^{2+}), although it cannot be ruled out the possibility that the reduced Fe-S cluster biosynthesis may also contribute to the pathophysiology. Nevertheless, this disease has been described in only one family, warranting further analyses associated with the molecular mechanisms of the ring sideroblast formation.

2.2. CSA caused by defects of the Fe–S cluster biosynthesis

The Fe–S clusters are prevalent inorganic cofactors comprising iron and sulfur, which are used in fundamental biological processes such as energy production, DNA maintenance, gene expression regulation, and protein translation [55,56]. Frataxin facilitates the delivery of iron transported from MFRN1 (mitoferrin-1, encoded by SLC25A37) to the Fe–S cluster assembly complex, including the scaffold protein ISCU (Fe–S cluster assembly enzyme), the mitochondrial HSP70 homolog

HSPA9 [heat shock protein family A (Hsp70) member 9] and its co-chaperone HSC20 (HscB mitochondrial Fe–S cluster cochaperone), and GLRX5 (Glutaredoxin 5). While the synthesized Fe–S cluster is provided to mitochondrial apoproteins, such as FECH [15], it is also transported from the mitochondria to the cytosol by the mitochondrial transporter ABCB7 [adenosine triphosphate (ATP)–binding cassette B7] (Fig. 1) [57].

Among cytosolic Fe–S proteins, IRPs function as an aconitase that require the Fe–S cluster for its enzymatic activity, whereas in the absence of the Fe–S cluster, they bind to IREs [58]. IRPs comprise two independent proteins, IRP1 and IRP2, the latter of which predominantly regulates the iron homeostasis *in vivo* [59]. As described above, the IRP–IRE system plays a vital role in the post-transcriptional regulation of *ALAS2*. Under iron deficiency conditions, the *ALAS2* translation is suppressed by the binding of IRP1/IRP2 to the IRE; however, the IRPs detach from the IRE under conditions of iron sufficiency, resulting in the increased ALAS2 translation [60]. In addition, heme binds to IRP2 to induce its ubiquitination and proteasomal degradation [61]; thus, the increased heme concentration along with erythroid differentiation induces the IRP2 degradation, presumably contributing to the enhanced ALAS2 translation.

2.2.1. CSA due to defects in ABCB7

XLSA with ataxia (XLSA/A) is a rare form of sideroblastic anemia inherited in an X-linked manner similar to XLSA. Based on the findings of the molecular analysis, XLSA/A is caused by mutations in the *ABCB7* gene, which is located at Xp13.3 and encodes a mitochondrial transporter of the Fe–S cluster. To date, XLSA/A has been reported in 17 patients in 4 pedigrees, of which 5 were heterozygous females [22,47]. Male hemizygous patients with XLSA/A present with mild anemia, motor delay, and evidence of spinocerebellar dysfunction, including early-onset ataxia associated with severe cerebellar hypoplasia [62,63]. Conversely, none of the female heterozygous cases exhibited neurological symptoms [47]. Of note, the systemic iron overload has not been reported in this disease. While mutations are missense and anticipated to be loss-of-function [62], nonsense mutations have not been reported, presumably, because the complete loss of *ABCB7* would be incompatible with life, as it occurs in *Abcb7*-deficient mice [64]. During the impaired ABCB7 activity, iron remains trapped in the mitochondria and the levels of Fe–S cluster-dependent enzyme activities are declined in affected cells, resulting in the compromised *ALAS2* translation by binding of IRPs to the IRE located in the 5′- UTR of *ALAS2* mRNA. Furthermore, studies have suggested that *ABCB7* may contribute to the FECH activity by direct interaction [65], which would be reflected by elevated red blood cell PPIX in patients with XLSA/A.

2.2.2. CSA due to defects in GLRX5

As described earlier, *GLRX5* encodes the mitochondrial protein essential for the Fe–S cluster biogenesis [66]. To date, only two CSA cases because of the *GLRX5* mutation have been reported; while one case harbored a homozygous *GLRX5* splicing mutation that markedly decreased mRNA and protein levels [3], the other exhibited two compound heterozygous *GLRX5* mutations [67]. Both cases presented mild anemia in the middle age, with a relatively low number of ring sideroblasts in the bone marrow and systemic iron overload [5,67]. Regarding the pathological relationship, it has been reported that, based on the zebrafish model [68] as well as fibroblasts derived from a patient [69], the loss of the Fe–S cluster by the *GLRX5* mutation blocked the *ALAS2* translation by binding of IRPs to the IRE, similar to the mechanism caused by the *ABCB7* mutation.

2.2.3. CSA due to defects in HSPA9

HSPA9 is a component of the Fe–S cluster assembly complex, involved in its biogenesis [55,56]. Recently, CSA caused by the *HSPA9* mutation has been reported [70]. As the *HSPA9* gene is located on chromosome 5q, the disease is estimated to be inherited as an autosomal recessive trait. However, most cases validated a common coding single-nucleotide polymorphism associated with lower mRNA expression in trans with a severe loss-of-function allele, resulting in the pseudo-dominant pattern of inheritance [70]. Like CSA caused by *GLRX5* defects, the compromised *ALAS2* translation might contribute to the pathophysiology. Intriguingly, although *HSPA9* is one of the genes commonly deleted in 5q- MDS [71], ring sideroblasts are uncommon in this subtype.

2.3. CSA due to abnormal mitochondrial protein synthesis

The mammalian mitochondrial respiratory chain comprises several subunits, some of which are encoded by the mitochondrial DNA (mtDNA) and *NDUFB11* (NADH: ubiquinone oxidoreductase subunit B11); the latter encodes a non-catalytic component of complex I of the mitochondrial chain. In the final step of the heme biosynthesis, iron should be reduced (Fe^{2+}) when incorporated into PPIX by FECH [8]. However, respiratory chain dysfunction results in the defect of the cytochrome *c* enzymatic activity, responsible for keeping the iron in a reduced (Fe^{2+}) state [1], presumably accounting for the impaired heme biosynthesis and iron accumulation in the mitochondria. In addition, as indicated in secondary sideroblastic anemia associated with antibiotics chloramphenicol (an inhibitor of bacterial ribosomal translation, which is closely related to mammalian mitochondrial ribosomes structurally) [72], mutations in genes involved in the mitochondrial ribosomal RNA metabolism, such as *PUS1* (pseudouridylate synthase 1), *YARS2* (tyrosyl-tRNA synthase 2), *LARS2* (leucyl-tRNA synthase 2), and *TRNT1* (tRNA nucleotidyl transferase 1), might also result in the global mitochondrial impairment by suppressing the translation of multiple mtDNA-encoded proteins [1,2].

2.3.1. Pearson marrow-pancreas syndrome

Pearson marrow-pancreas syndrome (PMPS) is a rare syndrome that involves different organs and systems, typically presenting with sideroblastic anemia, accompanied by metabolic acidosis, ataxia, renal failure, and endocrine pancreas dysfunction [73,74]. PMPS is usually fatal, and patients die during infancy. In addition, anemia, often severe and macrocytic, usually detected in the first blood count, is frequently in the context of pancytopenia. Besides the emergence of ring sideroblasts, bone marrow aspiration specimens characteristically exhibit vacuolization of early erythroid and myeloid progenitors in 70–90% of cases [74]. Although PMPS is usually sporadic, individuals with PMPS might be born to mothers with milder mitochondrial phenotypes. Nearly half of all patients with PMPS can be reported to have heteroplasmy for a 4977-bp deletion in the mitochondrial genome [75], whereas PMPS cases with rearrangements or duplication of the mtDNA have also been reported [22,75]. Moreover, sideroblastic anemias because of single-nucleotide mutation in the mtDNA-encoded *ATP6* gene (m.8969G > A) have been reported [76,77]. The patients present variable phenotypes, including mitochondrial myopathy with lactic acidosis and ring sideroblasts (MLASA)-like phenotype [76], as well as CSA with only milder syndromic phenotype or a complex metabolic phenotype [77]. Regarding the molecular mechanism contributing to ring sideroblast formation, each mtDNA defect would result in a deficiency of mitochondrially encoded subunits, which might cause defective heme synthesis, mitochondrial iron accumulation, and impaired protein synthesis.

2.3.2. CSA due to defects in PUS1 or YARS2

The correlation between CSA and the defective mitochondrial protein expression can be observed most directly by the MLASA phenotype, which results from mutations in genes encoding either of the two proteins, *PUS1* and *YARS2*, also termed as MLASA1 and MLASA2, respectively [2,22]. Patients with *PUS1* mutation–caused MLASA typically present with lactic acidosis and mitochondrial myopathy related to the reduction in respiratory complexes I and IV [78,79]. *PUS1*

functions in the pseudouridine modification of tRNAs [78]. Pseudouridine affects the structure of the tRNA and strengthens base pairing. Thus, failure of the pseudouridine modification might result in aberrant translation. However, most patients with MLASA die in childhood, and only a few survive adulthood [80,81]. Conversely, *YARS2* mutation–caused MLASA has been identified in several pedigrees [82–87]. Perhaps, the decreased aminoacylation activity of mutant YARS2 enzyme might result in the decreased mitochondrial protein synthesis, leading to mitochondrial respiratory chain dysfunction [32]. Intriguingly, cytoplasmic vacuoles in normoblasts have been reported, which is a characteristic of PMPS [87].

2.3.3. CSA due to defects in LARS2

LARS2 encodes mitochondrial leucyl-tRNA synthase, which attaches leucine to its cognate tRNA [88]. To date, two cases of sideroblastic anemia caused by the *LARS2* mutation have been reported; while one reported 2 patients from different pedigrees (one exhibited homozygous mutation; p.Thr522Asn, the other exhibited compound heterozygous mutation; p.Thr629Met & c.1077delT), exhibiting premature ovarian failure and hearing loss (these symptoms compose Perrault syndrome) [89], the other (compound heterozygous mutation; p.Ala430Val & p.Thr522Asn) exhibited hydrops, lactic acidosis, and multiorgan failure [88]. Regarding the underlying molecular mechanisms, the mutation resulted in the decreased level of complex I protein, which might explain variable phenotypes.

2.3.4. CSA due to defects in SLC19A2

Thiamine-responsive megaloblastic anemia (TRMA) represents sideroblastic anemia with systemic symptoms, including diabetes and deafness [90,91]. However, the clinical manifestations can differ among cases. For example, some cases are not accompanied by diabetes, whereas others exhibit recurrent psychiatric manifestations or cardiac anomalies [92–94]. In addition, mutations in the high-affinity thiamine transporter *SLC19A2* form the basis of the disorder, which is responsive to the thiamine supplementation [90,91]. Although it remains unclear how mutations in *SLC19A2* are involved in the sideroblast formation, it is anticipated that the impairment of thiamine-dependent generation of succinyl-CoA, which is a substrate for *ALAS*, is the cause of the ring sideroblast abnormality. However, thiamine is also an essential cofactor in the *de novo* synthesis of ribose, which essential for protein synthesis. Nevertheless, the correlation between the TRMA and the mitochondrial protein synthesis must be experimentally validated in future studies.

2.3.5. CSA due to defects in TRNT1

A recent study reported a syndromic form of CSA associated with immunodeficiency, periodic fevers, and developmental delays (SIFD) [95]. SIFD is inherited with an autosomal recessive pattern and is caused by a mutation in the CCA-adding enzyme *TRNT1*, which is crucial for the tRNA maturation [95]. In addition, another recent study reported that the *TRNT1* mutation caused a spectrum of disease ranging from a childhood-onset complex disease affecting most organs, including SIFD, hydrops, cardiomyopathy, sensorineural hearing loss, and renal dysfunction, to an adult-onset retinitis pigmentosa [96–98]. Similar to the mutation in genes involved in the mitochondrial ribosomal RNA metabolism, the *TRNT1* mutation might account for the decreased mitochondrial protein synthesis, resulting in mitochondrial respiratory chain dysfunction in erythroid cells.

2.3.6. CSA due to defects in NDUFB11

Recently, two studies reported CSA because of the mutation in the X-linked gene *NDUFB11*, which encodes noncatalytic subunits of mitochondrial respiratory complex I [99,100]. Both studies reported a common mutation of *NDUFB11* (c.276_278del, pF93del), presented as sideroblastic anemia and variable symptoms, including lactic acidosis [99,100]. Conversely, it has been reported that *NDUFB11* mutations other than c.276_278del (p.F93del) do not present with anemia and are related to histiocytoid cardiomyopathy and microphthalmia with linear skin defects syndrome [99,100]. However, the targeted introduction of p.F93del into K562 erythroleukemia cells results in a proliferation defect [100]; the underlying molecular mechanism by which the p.F93del mutation induces sideroblastic anemia remains unclear.

3. Conclusions

Although CSA is a rare hematological disorder, it is imperative to detect genes mutations responsible for genetically unclassified CSA cases based on the next-generation sequencing technique. Perhaps, analyzing their function, based on model cells or animals, will further our knowledge of the mitochondrial iron and heme metabolism.

Acknowledgments

We thank the member of Department of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, for their support. This manuscript is supported by JSPS (Japan Society for the Promotion of Science) KAKENHI Grant No. 18K08314 to T.F., and 26293225/17H04668 to H.H.

Conflict of interest

All authors declare no conflict of interest.

References

[1] P. Ponka, J.T. Prchal, Hereditary Hematology, 8th edition, pp. 865 – 881.
[2] T. Fujiwara, H. Harigae, Pathophysiology and genetic mutations in congenital sideroblastic anemia, Pediatr. Int. 55 (6) (2013) 675–679.
[3] C. Camaschella, Hereditary sideroblastic anemias: pathophysiology, diagnosis, and treatment, Semin. Hematol. 46 (2009) 371–377.
[4] M.D. Fleming, Congenital sideroblastic anemias: iron and heme lost in mitochondrial translation, Hematol. Am. Soc. Hematol. Educ. Program 2011 (2011) 525–531.
[5] K. Furuyama, K. Kaneko, Iron metabolism in erythroid cells and patients with congenital sideroblastic anemia, Int. J. Hematol. 107 (1) (2018) 44–54.
[6] K. Yoshida, M. Sanada, Y. Shiraishi, D. Nowak, Y. Nagata, R. Yamamoto, Y. Sato, A. Sato-Otsubo, A. Kon, M. Nagasaki, G. Chalkidis, Y. Suzuki, M. Shiosaka, R. Kawahata, T. Yamaguchi, M. Otsu, N. Obara, M. Sakata-Yanagimoto, K. Ishiyama, H. Mori, F. Nolte, W.K. Hofmann, S. Miyawaki, S. Sugano, C. Haferlach, H.P. Koeffler, L.Y. Shih, T. Haferlach, S. Chiba, H. Nakauchi, S. Miyano, S. Ogawa, Frequent pathway mutations of splicing machinery in myelodysplasia, Nature 478 (7367) (2011) 64–69.
[7] D.M. Williams, Copper deficiency in humans, Semin. Hematol. 20 (1983) 118–128.
[8] K. Furuyama, K. Kaneko, P.D. Vargas, Heme as a magnificent molecule with multiple missions: heme determines its own fate and governs cellular homeostasis, Tohoku J. Exp. Med. 213 (1) (2007) 1–16.
[9] S. Sassa, Modern diagnosis and management of the porphyrias, Br. J. Haematol. 135 (3) (2006) 281–292.
[10] D. Chiabrando, S. Mercurio, E. Tolosano, Heme and erythropoiesis: more than a structural role, Haematologica 99 (6) (2014) 973–983.
[11] H.A. Dailey, P.N. Meissner, Erythroid heme biosynthesis and its disorders, Cold Spring Harb. Perspect. Med. 3 (2013) a011676.
[12] T. Fujiwara, H. Harigae, Biology of heme in mammalian erythroid cells and related disorders, Biomed. Res. Int. 2015 (2015) 278536.
[13] T.C. Cox, S.S. Bottomley, J.S. Wiley, M.J. Bawden, C.S. Matthews, B.K. May, X-linked pyridoxine-responsive sideroblastic anemia due to a Thr388-to-Ser substitution in erythroid 5-aminolevulinate synthase, N. Engl. J. Med. 330 (10) (1994) 675–679.
[14] T. Fujiwara, H. O'Geen, S. Keles, K. Blahnik, A.K. Linnemann, Y.A. Kang, K. Choi, P.J. Farnham, E.H. Bresnick, Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy, Mol. Cell 36 (4) (2009) 667–681.
[15] C.K. Wu, H.A. Dailey, J.P. Rose, A. Burden, V.M. Sellers, B.C. Wang, The 2.0 A structure of human ferrochelatase, the terminal enzyme of heme biosynthesis, Nat. Struct. Biol. 8 (2) (2001) 156–160.
[16] R.S. Ohgami, D.R. Campagna, E.L. Greer, B. Antiochos, A. McDonald, J. Chen, J.J. Sharp, Y. Fujiwara, J.E. Barker, M.D. Fleming, Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells, Nat. Genet. 37 (11) (2005) 1264–1269.
[17] H. Harigae, N. Suwabe, P.H. Weinstock, M. Nagai, H. Fujita, M. Yamamoto, S. Sassa, Deficient heme and globin synthesis in embryonic stem cells lacking the erythroid-specific delta-aminolevulinate synthase gene, Blood 91 (3) (1998) 798–805.
[18] J.W. Harris, R.M. Whittington, R. Weisman Jr, D.L. Horrigan, Pyridoxine responsive

anemia in the human adult, Proc. Soc. Exp. Biol. Med. 91 (3) (1956) 427–432.
[19] P.D. Cotter, A. May, E.J. Fitzsimons, T. Houston, B.E. Woodcock, A.I. al-Sabah, L. Wong, D.F. Bishop, Late-onset X-linked sideroblastic anemia. Missense mutations in the erythroid delta-aminolevulinate synthase (ALAS2) gene in two pyridoxine-responsive patients initially diagnosed with acquired refractory anemia and ringed sideroblasts, J. Clin. Investig. 96 (4) (1995) 2090–2096.
[20] K. Furuyama, H. Harigae, C. Kinoshita, T. Shimada, K. Miyaoka, C. Kanda, Y. Maruyama, S. Shibahara, S. Sassa, Late-onset X-linked sideroblastic anemia following hemodialysis, Blood 101 (11) (2003) 4623–4624.
[21] R. Ohba, K. Furuyama, K. Yoshida, T. Fujiwara, N. Fukuhara, Y. Onishi, A. Manabe, E. Ito, K. Ozawa, S. Kojima, S. Ogawa, H. Harigae, Clinical and genetic characteristics of congenital sideroblastic anemia: comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS), Ann. Hematol. 92 (1) (2013) 1–9.
[22] S.S. Bottomley, M.D. Fleming, Sideroblastic anemia: diagnosis and management, Hematol. Oncol. Clin. North Am. 28 (4) (2014) 653–670.
[23] I. Astner, J.O. Schulze, J. van den Heuvel, D. Jahn, W.D. Schubert, D.W. Heinz, Crystal structure of 5-aminolevulinate synthase, the first enzyme of heme biosynthesis, and its link to XLSA in humans, EMBO J. 24 (18) (2005) 3166–3177.
[24] S. Bekri, A. May, P.D. Cotter, A.I. Al-Sabah, X. Guo, G.S. Masters, D.F. Bishop, A promoter mutation in the erythroid-specific 5-aminolevulinate synthase (ALAS2) gene causes X-linked sideroblastic anemia, Blood 102 (2) (2003) 698–704.
[25] K. Kaneko, K. Furuyama, T. Fujiwara, R. Kobayashi, H. Ishida, H. Harigae, S. Shibahara, Identification of a novel erythroid-specific enhancer for the ALAS2 gene and its loss-of-function mutation which is associated with congenital sideroblastic anemia, Haematologica 99 (2) (2014) 252–261.
[26] D.R. Campagna, C.I. de Bie, K. Schmitz-Abe, M. Sweeney, A.K. Sendamarai, P.J. Schmidt, M.M. Heeney, H.G. Yntema, C. Kannengiesser, B. Grandchamp, C.M. Niemeyer, N.V. Knoers, S. Swart, G. Marron, R. van Wijk, R.A. Raymakers, A. May, K. Markianos, S.S. Bottomley, D.W. Swinkels, M.D. Fleming, X-linked sideroblastic anemia due to ALAS2 intron 1 enhancer element GATA-binding site mutations, Am. J. Hematol. 89 (3) (2014) 315–319.
[27] L. Garçon, C. Kannengiesser, A double red cells population in a woman with a microcytic anemia, Blood 123 (6) (2014) 808.
[28] N. Rollón, M.C. Fernández-Jiménez, M.I. Moreno-Carralero, M.J. Murga-Fernández, M.J. Morán-Jiménez, Microcytic anemia in a pregnant woman: beyond iron deficiency, Int. J. Hematol. 101 (5) (2015) 514–519.
[29] A.E. Donker, R.A. Raymakers, H.K. Nieuwenhuis, M.J. Coenen, M.C. Janssen, M.A. MacKenzie, P.P. Brons, D.W. Swinkels, X-linked sideroblastic anaemia due to $ALAS_2$ mutations in the Netherlands: a disease in disguise, Neth. J. Med. 72 (4) (2014) 210–217.
[30] S. Ducamp, C. Kannengiesser, M. Touati, L. Garçon, A. Guerci-Bresler, J.F. Guichard, C. Vermylen, J. Dochir, H.A. Poirel, F. Fouyssac, L. Mansuy, G. Leroux, G. Tertian, R. Girot, H. Heimpel, T. Matthes, N. Talbi, J.C. Deybach, C. Beaumont, H. Puy, B. Grandchamp, Sideroblastic anemia: molecular analysis of the ALAS2 gene in a series of 29 probands and functional studies of 10 missense mutations, Hum. Mutat. 32 (6) (2011) 590–597.
[31] M. Cazzola, A. May, G. Bergamaschi, P. Cerani, V. Rosti, D.F. Bishop, Familial-skewed X-chromosome inactivation as a predisposing factor for late-onset X-linked sideroblastic anemia in carrier females, Blood 96 (13) (2000) 4363–4365.
[32] C. Rose, I. Callebaut, L. Pascal, C. Oudin, M. Fournier, L. Gouya, A. Lambilliotte, C. Kannengiesser, Lethal ALAS2 mutation in males X-linked sideroblastic anaemia, Br. J. Haematol. 178 (4) (2017) 648–651.
[33] M. Aivado, N. Gattermann, A. Rong, A.A. Giagounidis, W.C. Prall, A. Czibere, B. Hildebrandt, R. Haas, S.S. Bottomley, X-linked sideroblastic anemia associated with a novel ALAS2 mutation and unfortunate skewed X-chromosome inactivation patterns, Blood Cells Mol. Dis. 37 (1) (2006) 40–45.
[34] V.G. Sankaran, J.C. Ulirsch, V. Tchaikovskii, L.S. Ludwig, A. Wakabayashi, S. Kadirvel, R.C. Lindsley, R. Bejar, J. Shi, S.B. Lovitch, D.F. Bishop, D.P. Steensma, X-linked macrocytic dyserythropoietic anemia in females with an ALAS2 mutation, J. Clin. Investig. 125 (4) (2015) 1665–1669.
[35] T. Katsurada, H. Kawabata, D. Kawabata, M. Kawahara, Y. Nakabo, A. Takaori-Kondo, Y. Yoshida, A. Japanese, family with X-linked sideroblastic anemia affecting females and manifesting as macrocytic anemia, Int. J. Hematol. 103 (6) (2016) 713–717.
[36] T. Fujiwara, N. Fukuhara, S. Ichikawa, M. Kobayashi, Y. Okitsu, Y. Onishi, K. Furuyama, H. Harigae, A novel heterozygous ALAS2 mutation in a female with macrocytic sideroblastic anemia resembling myelodysplastic syndrome with ring sideroblasts: a case report and literature review, Ann. Hematol. 96 (11) (2017) 1955–1957.
[37] N. Takahashi, J. Kameoka, N. Takahashi, Y. Tamai, K. Murai, R. Honma, H. Noji, H. Yokoyama, Y. Tomiya, Y. Kato, K. Ishizawa, S. Ito, Y. Ishida, K. Sawada, H. Harigae, Causes of macrocytic anemia among 628 patients: mean corpuscular volumes of 114 and 130 fL as critical markers for categorization, Int. J. Hematol. 104 (3) (2016) 344–357.
[38] N.C. Narang, M. Kotru, K. Rao, M. Sikka, Megaloblastic anemia with ring sideroblasts is not always myelodysplastic syndrome, Turk. J. Haematol. 33 (4) (2016) 358–359.
[39] H. Ishida, T. Imamura, A. Morimoto, T. Fujiwara, H. Harigae, Five-aminolevulinic acid: new approach for congenital sideroblastic anemia, Pediatr. Int. 60 (5) (2018) 496–497.
[40] O. Nakajima, S. Takahashi, H. Harigae, K. Furuyama, N. Hayashi, S. Sassa, M. Yamamoto, Heme deficiency in erythroid lineage causes differentiation arrest and cytoplasmic iron overload, EMBO J. 18 (22) (1999) 6282–6289.
[41] H. Harigae, O. Nakajima, N. Suwabe, H. Yokoyama, K. Furuyama, T. Sasaki, M. Kaku, M. Yamamoto, S. Sassa, Aberrant iron accumulation and oxidized status of erythroid-specific delta-aminolevulinate synthase (ALAS2)-deficient definitive erythroblasts, Blood 101 (3) (2003) 1188–1193.
[42] N. Tanimura, E. Miller, K. Igarashi, D. Yang, J.N. Burstyn, C.N. Dewey, E.H. Bresnick, Mechanism governing heme synthesis reveals a GATA factor/heme circuit that controls differentiation, EMBO Rep. 17 (2) (2016) 249–265.
[43] Y. Zhang, J. Zhang, W. An, Y. Wan, S. Ma, J. Yin, X. Li, J. Gao, W. Yuan, Y. Guo, J.D. Engel, L. Shi, T. Cheng, X. Zhu, Intron 1 GATA site enhances ALAS2 expression indispensably during erythroid differentiation, Nucleic Acids Res. 45 (2) (2017) 657–671.
[44] S. Hatta, T. Fujiwara, T. Yamamoto, K. Saito, M. Kamata, Y. Tamai, S. Kawamata, H. Harigae, A defined culture method enabling the establishment of ring sideroblasts from induced pluripotent cells of X-linked sideroblastic anemia, Haematologica 103 (5) (2018) e188–e191.
[45] K. Saito, T. Fujiwara, S. Hatta, Y. Okitsu, N. Fukuhara, Y. Onishi, Y. Nakamura, H. Harigae, Establishment and characterization of *in vitro* model of X-linked sideroblastic anemia, Blood 130 (2017) 171.
[46] D.L. Guernsey, H. Jiang, D.R. Campagna, S.C. Evans, M. Ferguson, M.D. Kellogg, M. Lachance, M. Matsuoka, M. Nightingale, A. Rideout, L. Saint-Amant, P.J. Schmidt, A. Orr, S.S. Bottomley, M.D. Fleming, M. Ludman, S. Dyack, C.V. Fernandez, M.E. Samuels, Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia, Nat. Genet. 41 (6) (2009) 651–653.
[47] A.E. Donker, R.A. Raymakers, L.T. Vlasveld, T. van Barneveld, R. Terink, N. Dors, P.P. Brons, N.V. Knoers, D.W. Swinkels, Practice guidelines for the diagnosis and management of microcytic anemias due to genetic disorders of iron metabolism or heme synthesis, Blood 123 (25) (2014) 3873–3886.
[48] C. Kannengiesser, M. Sanchez, M. Sweeney, G. Hetet, B. Kerr, E. Moran, J.L. Fuster Soler, K. Maloum, T. Matthes, C. Oudot, A. Lascaux, C. Pondarré, J. Sevilla Navarro, S. Vidyatilake, C. Beaumont, B. Grandchamp, A. May, Missense SLC25A38 variations play an important role in autosomal recessive inherited sideroblastic anemia, Haematologica 96 (6) (2011) 808–813.
[49] W.S. Wong, H.F. Wong, C.K. Cheng, K.O. Chang, N.P. Chan, M.H. Ng, K.F. Wong, Congenital sideroblastic anaemia with a novel frameshift mutation in SLC25A38, J. Clin. Pathol. 68 (3) (2015) 249–251.
[50] M. Mehri, M. Zarin, F. Ardalani, H. Najmabadi, A. Azarkeivan, M. Neishabury, Novel mutations in mitochondrial carrier family gene SLC25A38, causing congenital sideroblastic anemia in Iranian families, identified by whole exome sequencing, Blood Cells Mol. Dis. 71 (2018) 39–44.
[51] J.P. Fernández-Murray, S.V. Prykhozhij, J.N. Dufay, S.L. Steele, D. Gaston, G.K. Nasrallah, A.J. Coombs, R.S. Liwski, C.V. Fernandez, J.N. Berman, C.R. McMaster, Glycine and folate ameliorate models of congenital sideroblastic anemia, PLoS Genet. 12 (1) (2016) e1005783.
[52] M.A. LeBlanc, A. Bettle, J.N. Berman, V.E. Price, C. Pambrun, Z. Yu, M. Tiller, C.R. McMaster, C.V. Fernandez, Study of glycine and folic acid supplementation to ameliorate transfusion dependence in congenital SLC25A38 mutated sideroblastic anemia, Pediatr. Blood Cancer 63 (7) (2016) 1307–1309.
[53] B. Grandchamp, G. Hetet, C. Kannengiesser, C. Oudin, C. Beaumont, S. Rodrigues-Ferreira, R. Amson, A. Telerman, P. Nielsen, E. Kohne, C. Balser, H. Heimpel, A novel type of congenital hypochromic anemia associated with a nonsense mutation in the STEAP3/TSAP6 gene, Blood 118 (25) (2011) 6660–6666.
[54] L. Blanc, J. Papoin, G. Debnath, M. Vidal, R. Amson, A. Telerman, X. An, N. Mohandas, Abnormal erythroid maturation leads to microcytic anemia in the TSAP6/Steap3 null mouse model, Am. J. Hematol. 90 (3) (2015) 235–241.
[55] J.J. Braymer, R. Lill, Iron-sulfur cluster biogenesis and trafficking in mitochondria, J. Biol. Chem. 292 (31) (2017) 12754–12763.
[56] T.A. Rouault, N. Maio, Biogenesis and functions of mammalian iron-sulfur proteins in the regulation of iron homeostasis and pivotal metabolic pathways, J. Biol. Chem. 292 (31) (2017) 12744–12753.
[57] G. Kispal, P. Csere, C. Prohl, R. Lill, The mitochondrial proteins Atm1p and Nfs1p are essential for biogenesis of cytosolic Fe/S proteins, EMBO J. 18 (14) (1999) 3981–3989.
[58] N. Wilkinson, K. Pantopoulos, The IRP/IRE system in vivo: insights from mouse models, Front. Pharmacol. 5 (2014) 176.
[59] E.G. Meyron-Holtz, M.C. Ghosh, T.A. Rouault, Mammalian tissue oxygen levels modulate iron-regulatory protein activities in vivo, Science 306 (5704) (2004) 2087–2090.
[60] O. Melefors, B. Goossen, H.E. Johansson, R. Stripecke, N.K. Gray, M.W. Hentze, Translational control of 5-aminolevulinate synthase mRNA by iron-responsive elements in erythroid cells, J. Biol. Chem. 268 (8) (1993) 5974–5978.
[61] K. Iwai, S.K. Drake, N.B. Wehr, A.M. Weissman, T. LaVaute, N. Minato, R.D. Klausner, R.L. Levine, T.A. Rouault, Iron-dependent oxidation, ubiquitination, and degradation of iron regulatory protein 2: implications for degradation of oxidized proteins, Proc. Natl. Acad. Sci. USA 95 (9) (1998) 4924–4928.
[62] R. Allikmets, W.H. Raskind, A. Hutchinson, N.D. Schueck, M. Dean, D.M. Koeller, Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A), Hum. Mol. Genet. 8 (5) (1999) 743–749.
[63] S. Bekri, G. Kispal, H. Lange, E. Fitzsimons, J. Tolmie, R. Lill, D.F. Bishop, Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron-sulfur protein maturation, Blood 96 (9) (2000) 3256–3264.
[64] C. Pondarre, D.R. Campagna, B. Antiochos, L. Sikorski, H. Mulhern, M.D. Fleming, Abcb7, the gene responsible for X-linked sideroblastic anemia with ataxia, is essential for hematopoiesis, Blood 109 (8) (2007) 3567–3569.
[65] A.E. Medlock, H.A. Dailey, Examination of the activity of carboxyl-terminal chimeric constructs of human and yeast ferrochelatases, Biochemistry 39 (25) (2000) 7461–7467.
[66] C. Camaschella, A. Campanella, L. De Falco, L. Boschetto, R. Merlini, L. Silvestri,

S. Levi, A. Iolascon, The human counterpart of zebrafish shiraz shows sideroblastic-like microcytic anemia and iron overload, Blood 110 (4) (2007) 1353–1358.
[67] G. Liu, S. Guo, G.J. Anderson, C. Camaschella, B. Han, G. Nie, Heterozygous missense mutations in the GLRX5 gene cause sideroblastic anemia in a Chinese patient, Blood 124 (17) (2014) 2750–2751.
[68] R.A. Wingert, J.L. Galloway, B. Barut, H. Foott, P. Fraenkel, J.L. Axe, G.J. Weber, K. Dooley, A.J. Davidson, B. Schmid, B.H. Paw, G.C. Shaw, P. Kingsley, J. Palis, H. Schubert, O. Chen, J. Kaplan, L.I. Zon, Tübingen 2000 Screen Consortium, Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis, Nature 436 (2005) 1035–1039.
[69] H. Ye, S.Y. Jeong, M.C. Ghosh, G. Kovtunovych, L. Silvestri, D. Ortillo, N. Uchida, J. Tisdale, C. Camaschella, T.A. Rouault, Glutaredoxin 5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts, J. Clin. Investig. 120 (5) (2010) 1749–1761.
[70] K. Schmitz-Abe, S.J. Ciesielski, P.J. Schmidt, D.R. Campagna, F. Rahimov, B.A. Schilke, M. Cuijpers, K. Rieneck, B. Lausen, M.L. Linenberger, A.K. Sendamarai, C. Guo, I. Hofmann, P.E. Newburger, D. Matthews, A. Shimamura, P.J. Snijders, M.C. Towne, C.M. Niemeyer, H.G. Watson, M.H. Dziegiel, M.M. Heeney, A. May, S.S. Bottomley, D.W. Swinkels, K. Markianos, E.A. Craig, M.D. Fleming, Congenital sideroblastic anemia due to mutations in the mitochondrial HSP70 homologue HSPA9, Blood 126 (25) (2015) 2734–2738.
[71] J. Boultwood, C. Fidler, A.J. Strickson, F. Watkins, S. Gama, L. Kearney, S. Tosi, A. Kasprzyk, J.F. Cheng, R.J. Jaju, J.S. Wainscoat, Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome, Blood 99 (12) (2002) 4638–4641.
[72] A.A. Yunis, Z. Salem, Drug-induced mitochondrial damage and sideroblastic change, Clin. Haematol. 9 (3) (1980) 607–619.
[73] H.A. Pearson, J.S. Lobel, S.A. Kocoshis, J.L. Naiman, J. Windmiller, A.T. Lammi, R. Hoffman, J.C. Marsh, A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction, J. Pediatr. 95 (6) (1979) 976–984.
[74] P. Farruggia, F. Di Marco, C. Dufour, Pearson syndrome, Expert Rev. Hematol. 11 (3) (2018) 239–246.
[75] A. Rötig, T. Bourgeron, D. Chretien, P. Rustin, A. Munnich, Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome, Hum. Mol. Genet. 4 (8) (1995) 1327–1330.
[76] L.C. Burrage, S. Tang, J. Wang, T.R. Donti, M. Walkiewicz, J.M. Luchak, L.C. Chen, E.S. Schmitt, Z. Niu, R. Erana, J.V. Hunter, B.H. Graham, L.J. Wong, F. Scaglia, Mitochondrial myopathy, lactic acidosis, and sideroblastic anemia (MLASA) plus associated with a novel de novo mutation (m.8969G > A) in the mitochondrial encoded ATP6 gene, Mol. Genet. Metab. 113 (3) (2014) 207–212.
[77] S. Berhe, M.M. Heeney, D.R. Campagna, J.F. Thompson, E.J. White, T. Ross, R.W. Peake, J.D. Hanrahan, V. Rodriguez, D.L. Renaud, M.S. Patnaik, E. Chang, S.S. Bottomley, M.D. Fleming, Recurrent heteroplasmy for the MT-ATP6 p.Ser148Asn (m.8969G > A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity, Haematologica (2018), https://doi.org/10.3324/haematol.2018.199109 (In press).
[78] Y. Bykhovskaya, K. Casas, E. Mengesha, A. Inbal, N. Fischel-Ghodsian, Missense mutation in pseudouridine synthase 1 (PUS1) causes mitochondrial myopathy and sideroblastic anemia (MLASA), Am. J. Hum. Genet. 74 (6) (2004) 1303–1308.
[79] E. Fernandez-Vizarra, A. Berardinelli, L. Valente, V. Tiranti, M. Zeviani, Nonsense mutation in pseudouridylate synthase 1 (PUS1) in two brothers affected by myopathy, lactic acidosis and sideroblastic anaemia (MLASA), J. Med. Genet. 44 (3) (2007) 173–180.
[80] M.D. Metodiev, Z. Assouline, P. Landrieu, D. Chretien, B. Bader-Meunier, C. Guitton, A. Munnich, A. Rötig, Unusual clinical expression and long survival of a pseudouridylate synthase (PUS1) mutation into adulthood, Eur. J. Hum. Genet. 23 (6) (2015) 880–882.
[81] M. Cao, M. Donà, M.L. Valentino, L. Valentino, C. Semplicini, A. Maresca, M. Cassina, A. Torraco, E. Galletta, V. Manfioli, G. Sorarù, V. Carelli, R. Stramare, E. Bertini, R. Carrozzo, L. Salviati, E. Pegoraro, Clinical and molecular study in a long-surviving patient with MLASA syndrome due to novel PUS1 mutations, Neurogenetics 17 (1) (2016) 65–70.
[82] L.G. Riley, S. Cooper, P. Hickey, J. Rudinger-Thirion, M. McKenzie, A. Compton, S.C. Lim, D. Thorburn, M.T. Ryan, R. Giegé, M. Bahlo, J. Christodoulou, Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia–MLASA syndrome, Am. J. Hum. Genet. 87 (1) (2010) 52–59.
[83] F. Sasarman, T. Nishimura, I. Thiffault, E.A. Shoubridge, A novel mutation in YARS2 causes myopathy with lactic acidosis and sideroblastic anemia, Hum. Mutat. 33 (8) (2012) 1201–1206.
[84] J. Nakajima, T.F. Eminoglu, G. Vatansever, M. Nakashima, Y. Tsurusaki, H. Saitsu, H. Kawashima, N. Matsumoto, N. Miyake, A novel homozygous YARS2 mutation causes severe myopathy, lactic acidosis, and sideroblastic anemia 2, J. Hum. Genet. 59 (4) (2014) 229–232.
[85] R. Shahni, Y. Wedatilake, M.A. Cleary, K.J. Lindley, K.R. Sibson, S. Rahman, A distinct mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA) phenotype associates with YARS2 mutations, Am. J. Med. Genet. A 161A (9) (2013) 2334–2338.
[86] L.G. Riley, M.J. Menezes, J. Rudinger-Thirion, R. Duff, P. de Lonlay, A. Rotig, M.C. Tchan, M. Davis, S.T. Cooper, J. Christodoulou, Phenotypic variability and identification of novel YARS2 mutations in YARS2 mitochondrial myopathy, lactic acidosis and sideroblastic anaemia, Orphanet. J. Rare Dis. 8 (2013) 193.
[87] G. Li, L.M. Hilliard, Morphologic features of normoblasts in a case of myopathy, lactic acidosis, and sideroblastic anemia, Blood 129 (8) (2017) 1057.
[88] L.G. Riley, J. Rudinger-Thirion, K. Schmitz-Abe, D.R. Thorburn, R.L. Davis, J. Teo, S. Arbuckle, S.T. Cooper, D.R. Campagna, M. Frugier, K. Markianos, C.M. Sue, M.D. Fleming, J. Christodoulou, LARS2 variants associated with hydrops, lactic acidosis, sideroblastic anemia, and multisystem failure, JIMD Rep. 28 (2016) 49–57.
[89] S.B. Pierce, K. Gersak, R. Michaelson-Cohen, T. Walsh, M.K. Lee, D. Malach, R.E. Klevit, M.C. King, E. Levy-Lahad, Mutations in LARS2, encoding mitochondrial leucyl-tRNA synthetase, lead to premature ovarian failure and hearing loss in Perrault syndrome, Am. J. Hum. Genet. 92 (4) (2013) 614–620.
[90] V. Labay, T. Raz, D. Baron, H. Mandel, H. Williams, T. Barrett, R. Szargel, L. McDonald, A. Shalata, K. Nosaka, S. Gregory, N. Cohen, Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness, Nat. Genet. 22 (3) (1999) 300–304.
[91] A.K. Bergmann, I. Sahai, J.F. Falcone, J. Fleming, A. Bagg, C. Borgna-Pignati, R. Casey, L. Fabris, E. Hexner, L. Mathews, M.L. Ribeiro, K.J. Wierenga, E.J. Neufeld, Thiamine-responsive megaloblastic anemia: identification of novel compound heterozygotes and mutation update, J. Pediatr. 155 (6) (2009) 888–892.
[92] M.C. Wood, J.A. Tsiouris, M. Velinov, Recurrent psychiatric manifestations in thiamine-responsive megaloblastic anemia syndrome due to a novel mutation c.63_71 delACCGCTC in the gene SLC19A2, Psychiatry Clin. Neurosci. 68 (6) (2014) 487.
[93] M.T. Akbari, S. Zare Karizi, R. Mirfakhraie, B. Keikhaei, Thiamine-responsive megaloblastic anemia syndrome with Ebstein anomaly: a case report, Eur. J. Pediatr. 173 (12) (2014) 1663–1665.
[94] G. Liu, F. Yang, B. Han, J. Liu, G. Nie, Identification of four SLC19A2 mutations in four Chinese thiamine responsive megaloblastic anemia patients without diabetes, Blood Cells Mol. Dis. 52 (4) (2014) 203–204.
[95] P.K. Chakraborty, K. Schmitz-Abe, E.K. Kennedy, H. Mamady, T. Naas, D. Durie, D.R. Campagna, A. Lau, A.K. Sendamarai, D.H. Wiseman, A. May, S. Jolles, P. Connor, C. Powell, M.M. Heeney, P.J. Giardina, R.J. Klaassen, C. Kannengiesser, I. Thuret, A.A. Thompson, L. Marques, S. Hughes, D.K. Bonney, S.S. Bottomley, R.F. Wynn, R.M. Laxer, C.P. Minniti, J. Moppett, V. Bordon, M. Geraghty, P.B. Joyce, K. Markianos, A.D. Rudner, M. Holcik, M.D. Fleming, Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD), Blood 124 (18) (2014) 2867–2871.
[96] A.P. DeLuca, S.S. Whitmore, J. Barnes, T.P. Sharma, T.A. Westfall, C.A. Scott, M.C. Weed, J.S. Wiley, L.A. Wiley, R.M. Johnston, M.J. Schnieders, S.R. Lentz, B.A. Tucker, R.F. Mullins, T.E. Scheetz, E.M. Stone, D.C. Slusarski, Hypomorphic mutations in TRNT1 cause retinitis pigmentosa with erythrocytic microcytosis, Hum. Mol. Genet. 25 (1) (2016) 44–56.
[97] C. Barton, S. Kausar, D. Kerr, S. Bitetti, R. Wynn, SIFD as a novel cause of severe fetal hydrops and neonatal anaemia with iron loading and marked extramedullary haemopoiesis, J. Clin. Pathol. 71 (3) (2018) 275–278.
[98] Y. Wedatilake, R. Niazi, E. Fassone, C.A. Powell, S. Pearce, V. Plagnol, J.W. Saldanha, R. Kleta, W.K. Chong, E. Footitt, P.B. Mills, J.W. Taanman, M. Minczuk, P.T. Clayton, S. Rahman, TRNT1 deficiency: clinical, biochemical and molecular genetic features, Orphanet. J. Rare Dis. 11 (1) (2016) 90.
[99] A. Torraco, M. Bianchi, D. Verrigni, V. Gelmetti, L. Riley, M. Niceta, D. Martinelli, A. Montanari, Y. Guo, T. Rizza, D. Diodato, M. Di Nottia, B. Lucarelli, F. Sorrentino, F. Piemonte, S. Francisci, M. Tartaglia, E.M. Valente, C. Dionisi-Vici, J. Christodoulou, E. Bertini, R. Carrozzo, A novel mutation in NDUFB11 unveils a new clinical phenotype associated with lactic acidosis and sideroblastic anemia, Clin. Genet. 91 (3) (2017) 441–447.
[100] D.A. Lichtenstein, A.W. Crispin, A.K. Sendamarai, D.R. Campagna, K. Schmitz-Abe, C.M. Sousa, M.D. Kafina, P.J. Schmidt, C.M. Niemeyer, J. Porter, A. May, M.M. Patnaik, M.M. Heeney, A. Kimmelman, S.S. Bottomley, B.H. Paw, K. Markianos, M.D. Fleming, A recurring mutation in the respiratory complex 1 protein NDUFB11 is responsible for a novel form of X-linked sideroblastic anemia, Blood 128 (15) (2016) 1913–1917.