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Congenital Sideroblastic Anemias: Iron and Heme Lost in Mitochondrial Translation

Mark D. Fleming[1]

[1]*Children's Hospital Boston, Boston, MA*

The congenital sideroblastic anemias (CSAs) are an uncommon, diverse class of inherited hematopoietic disorders characterized by pathological deposition of iron in the mitochondria of erythroid precursors. In recent years, the genetic causes of several clinically distinctive forms of CSA have been elucidated, which has revealed common themes in their pathogenesis. In particular, most, if not all, can be attributed to disordered mitochondrial heme synthesis, iron-sulfur cluster biogenesis, or pathways related to mitochondrial protein synthesis. This review summarizes the clinical features, molecular genetics, and pathophysiology of each of the CSAs in the context of these pathways.

Introduction

The congenital sideroblastic anemias (CSAs) are an uncommon class of inherited anemias characterized by the morphological hallmark of pathological iron deposition in the mitochondria of erythroid precursors in the BM (reviewed by Bottomley[1] and by Camaschella[2] and summarized in Table 1). Most are also clinically associated with reticulocytopenia, ineffective erythropoiesis, and secondary iron overload. Whereas anemia is often the only manifestation of the disease, several CSAs are syndromically associated with other clinical features, particular neuromuscular and metabolic phenotypes, which, in some instances, cause the most overt symptoms. The anemia may be recognized prenatally, at birth, in childhood, in young adulthood, and even in older adulthood depending upon the particular disease entity, the individual disease-causing mutation, and other factors. Likewise, patients may be transfusion dependent or not at all anemic, some having only minor variations in RBC parameters. RBCs may be microcytic, normocytic, or macrocytic, the latter of which may be megaloblastic or nonmegaloblastic. Therefore, the only consistent feature that the CSAs share is the BM ringed sideroblast. Nonetheless, as described below, evolving knowledge of the molecular basis of each of the clinical phenotypes has led to subgroups sharing a common pathophysiology often associated with similar clinical features.

Mitochondrial genetics and metabolism: a CSA-centric view

Each erythroblast contains approximately 200 mitochondria originally derived from the maternal oocyte (ie, maternally inherited). Each mitochondrion contains 5-10 copies of a 16 569-bp circular genome that encodes 13 mRNAs for components of the mitochondrial respiratory complexes, which are translated by mitochondrial ribosomes, 22 mitochondrial transfer RNAs (mt-tRNAs), and 2 mitochondrial ribosomal RNAs (mt-rRNAs). In this way, the mitochondrial genome disproportionally encodes factors essential for not only oxidative phosphorylation, but also the components required to translate mitochondrially encoded mRNAs into protein. In addition, the nuclear genome encodes > 1000 genes that are transcribed in the nucleus, translated in the cytoplasm, and transported as polypeptides into the mitochondrion.[3] Among these are all of the Krebs cycle enzymes, 4 of the heme synthesis enzymes, additional components of the respiratory complexes, all of the transporters required to shuttle substrates and products in and of the organelle, and all of the structural proteins of the mitochondrial ribosome.

Mitochondria serve several functions critical to cellular iron metabolism. Of particular relevance to the erythroblast is the role of the mitochondrion in the biosynthesis of heme (iron-protoporphyrin IX, Fe-PPIX; shown in Figure 1 and reviewed by Ponka[4,5]). Heme synthesis begins in the mitochondrial matrix with the decarboxylative condensation of glycine with succinyl-coenzyme A (CoA) to form 5-aminolevulinic acid (ALA). This reaction is catalyzed in developing erythroid cells by an erythroid-specific isoform of the pyridoxal phosphate-dependent enzyme aminolevulinic acid synthase 2 (ALAS2). ALA must then be transported to the cytoplasm, possibly by the activity of the mitochondrial carrier family transporter solute carrier 25A38 (SLC25A38),[6] whereupon cytosolic enzymes catalyze the assembly of the porphyrin precursor coproporphyrinogen III, which is further modified to PPIX in the mitochondrial intermembrane space. PPIX must be transported across the inner mitochondrial membrane, where ferrochelatase (FECH) catalyzes the chelation of ferrous iron (Fe^{2+}) transported into the mitochondrion by mitoferrin 1 (MFRN1),[7] into the porphyrin ring to form heme. In the case of cytoplasmic proteins such as α- and β-globin, heme must be transported into the cytoplasm for assembly with apoproteins to form mature heme proteins.

Mitochondria also play a role in the biogenesis of iron-sulfur (Fe-S) clusters (shown in Figure 2 and reviewed by Ye[8] and by Lill[9]), which are enzymatic cofactors and structural components of several mitochondrial proteins, including mitochondrial aconitase, succinate dehydrogenase (respiratory complex II), and FECH, as well several cytoplasmic enzymes, including iron regulatory binding protein 1 (IRBP1). IRBP1 and its functional ortholog, IRBP2, are posttranscriptional regulators of several other proteins relevant to iron metabolism, including ALAS2, transferrin receptor 1 (TFRC1), divalent metal transporter 1 (DMT1 or SLC11A2), and the iron-storage proteins L- and H-ferritin (FTL and FTH). Cellular iron deficiency leads to loss of the Fe-S cluster, converting IRBP1 from a cytoplasmic aconitase into an iron-responsive element (IRE) sequence-specific RNA-binding protein. Conversely, heme and iron regulate IRBP2 by promoting its degradation.[10] IRBP1 and IRBP2 stabilize mRNAs when IREs are located in the 3′ untranslated region (3′UTR) and repress translation when IREs are located in the 5′UTR of target mRNAs. As discussed below, cross-talk between

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Table 1. Clinical and genetic features of the molecularly defined CSAs

	XLSA	SLC25A38	XLSA/A	GLRX5†	PMPS	MLASA/PUS1	MLASA/YARS2	TRMA
Inheritance	X-linked	autosomal recessive	X-linked	autosomal recessive	Maternal*	Autosomal recessive	Autosomal recessive	Autosomal recessive
Chromosome	Xp11.21	3p22.1	Xq13	14q32.2	Mitochondrial	14q24.33	12p11.21	1q23.3
Gene	*ALAS2*	*SLC25A38*	*ABCB7*	*GLRX5*	Variable	*PUS1*	*YARS2*	*SLC19A2*
Mutation type	MS, NS‡	MS, NS, SPL	MS	SPL	DEL	MS, NS	MS	NS, MS
Sex	M>F	M=F	M	M†	M ≅ F	M = F	M = F	M = F
Carrier phenotype	+	–	+	?	N/A	–	–	–/+
MCV	↓↓§	↓↓	↓/NML	↓↓	↑	NML/↑	NML/↑	↑
Iron overload	+/++	++	–	++	–/+	–/+	–/+	–
Vitamin response	pyridoxine	–	–	–	–	–	–	Thiamine
Transfusion	–/+	++	–	+	+	+/–	+/–	–
Associated phenotypes	–	–	ataxia	–	Exocrine pancreas insufficiency pancytopenia, lactic acidosis, myopathy	Myopathy, lactic acidosis, craniofacial abnormalities, intellectual disability	Myopathy, lactic acidosis	Diabetes mellitus, deafness

+ indicates present; –, absent; ↑, increased; ↓, decreased; N/A, not applicable; MS, missense; NS, nonsense; SPL, splicing; DEL, deletion; and NML, normal.

*Essentially all cases of PMPS are sporadic, but rare maternally inherited cases are reported.

†A GLRX5 mutation has been described in only one patient.

‡Nonsense mutations have been described only in females.

§MCV is typically normal or increased in female carriers.

mitochondrial heme and Fe-S cluster synthesis and cytoplasmic protein expression, is one mechanism by which disruption of mitochondrial iron metabolism may result in the sideroblastic phenotype.

Mitochondrial Fe-S biogenesis involves the assembly of elemental sulfur, derived from cysteine, with iron on a protein scaffold (iron sulfur cluster U, ISCU) through the enzymatic activities of other iron sulfur assembly proteins, including ISCA1/2 and NIFS and the participation of the HSP70 homolog cochaperone HSPA9-HSCB complex and mitochondrial glutaredoxin 5 (GLRX5). Newly formed Fe-S clusters may then be available for incorporation into mitochondrially synthesized apoproteins. Although it is unclear what it actually transports, the mitochondrial ATP-binding cassette (ABC) transporter ABCB7 is required to supply a component necessary for the assembly of the Fe-S cluster into cytoplasmic Fe-S proteins, including IRBP1.

CSAs due to primary heme synthesis defects (Figure 1)

XLSA due to ALAS2 deficiency

In many ways, deficiency of ALAS2 encoded on the X chromosome is the prototype for all CSAs, because it is by far the most common type of CSA, constituting ~ 40% of cases.[11] Before ringed sideroblasts were found to be associated with the disease, the disorder was known as pyridoxine-responsive anemia due to the characteristic response of the anemia to pharmacological doses (>50 mg/d) of pyridoxine (vitamin B6).[12] Patients with X-linked sideroblastic anemia (XLSA) are typically, but not exclusively, males who present at a wide variety of ages, but usually before the age of 40, with a hypochromic, microcytic anemia of variable severity. Transfusion dependence is, however, unusual. The peripheral blood smear suggests the differential diagnosis of severe iron deficiency, β-thalassemia, or a hemoglobinopathy, which can often be excluded by iron studies, reticulocyte counts, and a hemoglobin electrophoresis. In fact, in the absence of transfusions, most patients present with evidence of iron overload, including an increased transferrin saturation and hyperferritinemia, and may even develop cirrhosis due to iron overload; the latter can be averted by iron-chelation therapy or, in those who can tolerate it, judicious phlebotomy.[13] Morphological cues to the diagnosis of CSA on the peripheral blood smear include a dimorphic RBC population in which there are populations of nearly normal RBCs and very hypochromic microcytes, often with unusual shapes, such as teardrops, that leads to an increased RBC distribution width (RDW). Numerous Pappenheimer bodies may be seen in erythrocytes, particularly after splenectomy, and their reactivity for iron in a coarsely granular pattern will confirm that these are siderocytes—the mature, enucleated erythrocyte counterpart of the ringed sideroblast. In affected individuals, an iron-stained BM aspiration will reveal numerous ringed sideroblasts, particularly in the latest nucleated erythroid precursors. The presence of RBC dimorphism and an increased RDW can be seen in nonanemic female carriers and is a characteristic that may help to distinguish XLSA from other microcytic anemias. Women may also present with clinically significant disease in childhood or in later life due to congenital or acquired skewing of X-chromosome inactivation, respectively.

More than 60 different mutations in ALAS2 have been reported in patients with XLSA.[1,14] Except for several nonsense mutations in clinically affected female carriers, all disease-associated alleles are single nucleotide changes encoding missense mutations, indicating

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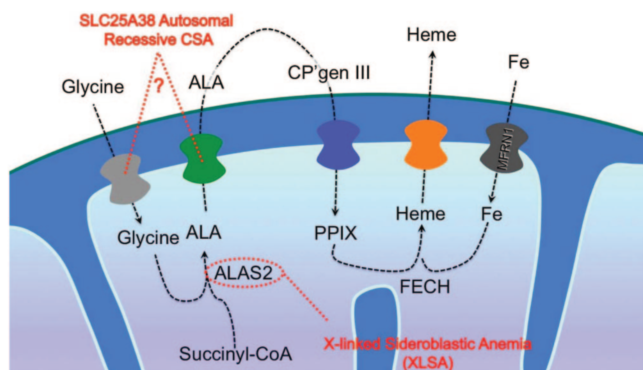

Figure 1. Heme synthesis and CSA. The intra-and extramitochondrial step in heme biosynthesis, as well as the inferred transport steps for heme precursors across the mitochondrial inner membrane, are depicted in relation to 2 heme-related causes of CSA: XLSA and SLC25A38 deficiency. 5-ALA is synthesized from succinyl-CoA and glycine by ALAS2. SLC25A38 likely imports glycine into mitochondria and might also export ALA to the cytosol. In the cytosol, ALA molecules are assembled into coproporphyrinogen III (CP'gen III) that is further modified to protoporphyrin IX (PPIX) in the mitochondrial intermembrane space and then transported into the mitochondria by an unknown transporter. Iron transported into mitochondria by MFRN1 is combined with PPIX to form heme by the enzyme FECH.

that at least some residual ALAS2 activity is required for viability. Mutations occur most commonly, but not exclusively, in exons 5 and 9, which encode residues important for enzymatic activity and interactions at the homodimer interface.[15] Defects in catalysis, substrate, or cofactor affinity, enzyme stability, and protein processing have all been implicated in the pathogenesis of the ALAS2 deficiency, and pyridoxine supplementation may contribute to mitigating several of these impairments.

Autosomal recessive sideroblastic anemia due to mutations in SLC25A38

After the discovery and routine molecular diagnosis of XLSA, it became evident that there was a subset of patients with severe hypochromic, microcytic CSA that in many ways resembled XLSA but lacked *ALAS2* mutations. However, this subgroup of patients was distinctive insofar as the anemia usually presented in the first weeks or months of life, was uniformly unresponsive to pyridoxine, and nearly always required chronic transfusional support. Furthermore, the incidence of CSA in affected pedigrees was approximately equal in males and females, and there was no apparent phenotype in obligate carriers, suggesting an autosomal recessive mode of inheritance. Guernsey et al identified mutations in the mitochondrial carrier family protein SLC25A38 in 13 of these probands, and one subsequent report has confirmed this finding in 11 additional patients.[6,16] In total, nonsense and splicing errors are approximately equal in frequency to missense mutations, the latter of which nearly always occur in highly evolutionarily conserved amino acids. Nearly 2/3 of patients have 2 copies of the same mutation, as might be predicted of a rare recessive disorder occurring at higher frequency in relatively inbred populations.

SLC25A38 is highly and selectively expressed in erythroblasts and is a member of the SLC25 class of inner mitochondrial membrane carrier proteins which share an amino terminal mitochondrial targeting signal and 6 transmembrane helices encoded by 3 mitochondrial carrier family protein structural domains. Most SLC25 proteins promote the exchange of one metabolite for another across the inner mitochondrial membrane, although some act as metabolite importers. They are subdivided into 3 major groups—keto acid, amino acid, and adenine nucleotide carriers—that are defined by conserved amino acids that are thought to provide contact points that determine substrate specificity.[17] On this basis, SLC25A38 is predicted to be an amino acid carrier. Indeed, several of the described disease-causing missense mutations occur in conserved amino acids at these locations.

The phenotypic similarity between patients with *SLC25A38* and *ALAS2* mutations raised the possibility that the autosomal recessive form of the disorder might equally result from a heme biosynthetic defect. To assess this possibility, Guernsey et al examined the phenotype of the yeast *Saccharomyces cerevisiae*, with a germline deletion in the putative SLC25A38 ortholog YDL119c.[6] They found that these yeast had a defect in mitochondrial respiration and in a cell-surface, heme-dependent ferrireductase, suggesting that SLC25A38 could be involved in heme biosynthesis. Because the structural features indicated that SLC25A38 was most likely an amino acid transporter, the investigators tested the ability of the amino acids glycine and ALA, a precursor and the product of the ALAS2 reaction, respectively, to complement the heme deficiency phenotype, and found that either could do so when added to the medium in large amounts. Furthermore, biochemical analyses documented a severe deficiency in cellular ALA in the yeast deletion strain, suggesting that loss of YDL119c impairs ALA biosynthesis in vivo. These data in yeast strongly suggest that the SLC25A38 is involved in making glycine available for heme synthesis in the mitochondria. Because most SLC25 family members function as importers or exchangers, this would suggest at a minimum that SLC25A38 is responsible for glycine import into mitochondria, and also that it might exchange glycine for ALA across the mitochondrial inner membrane. More importantly, this finding immediately suggests that dietary supplementation with glycine or ALA might ameliorate SLC25A38 anemia and reduce or eliminate the need for chronic transfusions.

Other heme synthesis defects

Some patients with erythropoietic protoporphyria typically due to mutations in FECH have occasional ringed sideroblasts.[18] Nevertheless, to the extent that it has been investigated, it is unclear why ringed sideroblasts are not prevalent in other heme biosynthesis disorders. What is clear is that, whatever the mechanism is, it ensures that the stoichiometric import of iron and porphyrin synthesis fails,[5] resulting in mitochondrial iron overload. It may be that iron uptake into mitochondria is not a regulated process and that in the setting of porphyrin deficiency its relative excess causes its accumulation.

CSA due to primary Fe-S biogenesis defects (Figure 2)

XLSA/A

X-linked sideroblastic anemia with ataxia (XLSA/A) is a rare syndromic form of CSA; only 4 families with this disorder have been reported. XLSA/A patients present with motor delay and evidence of spinocerebellar dysfunction, including an early onset ataxia associated with severe cerebellar hypoplasia.[19-22] The anemia of XLSA/A is typically mild and may be overlooked or not substantially characterized until long after the neurological presentation. Affected males often have slightly decreased or low

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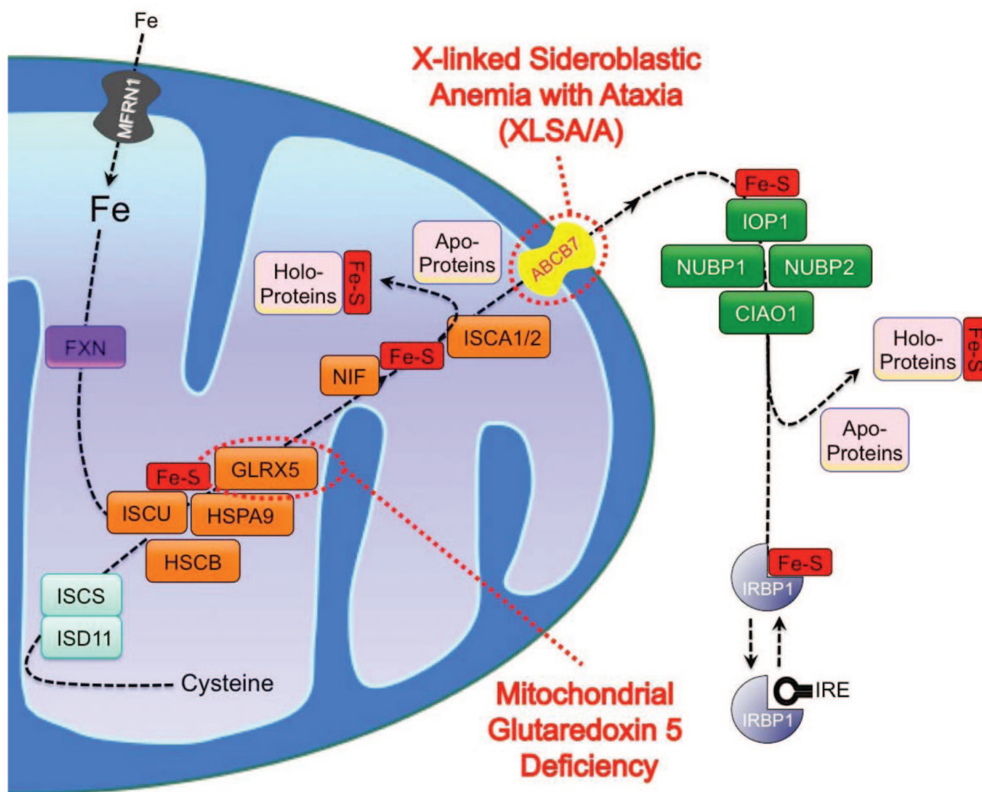

Figure 2. Fe-S cluster biogenesis and CSA. Intra- and extramitochondrial pathways of Fe-S cluster synthesis and assembly into apoproteins are shown in relation to 2 CSA phenotypes: XLSA/A and GLRX5 deficiency. Frataxin (FXN) is thought to facilitate the delivery of iron, transported into mitochondria by MFRN1, to an Fe-S cluster assembly complex that involves the scaffold protein ISCU, the mitochondrial HSP70 homolog HSPA9 and its cochaperone HSCB, and glutaredoxin 5 (GLRX5). Elemental sulfur for this process is derived from cysteine by the cysteine desulfurase complex that includes the ISCS and ISD11 proteins. Subsequent to the assembly of the cluster, NIF and ISCA1/2 and other proteins are required to deliver the nascent Fe-S cluster to mitochondrial apoproteins. An unknown component required for cytosolic Fe-S cluster assembly of mitochondria is transported by ABCB7 to the multiprotein cytosolic Fe-S cluster assembly machinery that involves IOP1, NUBP1, NUBP2, and CIAO1. Among the cytosolic Fe-S proteins is IRBP1, which exists in a form containing an Fe-S cluster that functions as a cytosolic aconitase and in a clusterless form that binds IRE RNA stem-loop structures, thereby controlling translation or degradation of several mRNAs, including ALAS2.

normal hemoglobin with a similar degree of microcytosis and an increased RDW, usually with erythrocyte dimorphism. Pappenheimer bodies may be seen in the peripheral blood. BM examination shows ringed sideroblasts. Obligate female carriers of the disorder nearly always have erythrocyte dimorphism or an increased RDW and may have Pappenheimer bodies in the peripheral blood. Ringed sideroblasts are also present in the BM of carrier females.[23]

XLSA/A is due to mutations in *ABCB7*,[19] the human ortholog of the yeast mitochondrial ABC transporter *ATM1*, which when deleted results in mitochondrial dysfunction and iron deposition.[24] The ABC family of transporters is a large family of multiple transmembrane proteins that share the ABC domain, which binds and hydrolyzes ATP to facilitate transport of diverse small molecules across membranes. A selective deficiency of cytosolic, but not mitochondrial, Fe-S proteins in the yeast ATM1 mutant suggested that ABCB7 may transport a component required for cytosolic Fe-S cluster assembly of the mitochondria.[25] The 3 reported XLSA/A mutations, all missense alleles, occur in close proximity to one another in a region thought to be involved in binding of the transported substrate. In yeast phenotypic complementation assays, these mutations have mild phenotypic effects, suggesting that they are mild partial loss-of-function mutations in vivo, and that a more severe allele would be lethal in humans.[22] Indeed, *Abcb7* is an essential gene in mice.[26] Tissue-specific deletion in hepatocytes confirmed that mammalian Abcb7 contributes to cytoplasmic Fe-S cluster protein maturation. This lack of Fe-S clusters leads to the constitutive activation of IRBP1 in its RNA-binding form,[26,27] which, as described below, might account for the pathogenesis of ringed sideroblasts in the BM of XLSA/A patients.

Autosomal recessive sideroblastic anemia due to a mutation in GLRX5

A single patient of consanguineous southern Italian descent has been described with a hypochromic, microcytic sideroblastic anemia associated with iron overload due to a homozygous mutation in the splice donor site of intron 1 of the mitochondrial glutaredoxin 5 (GLRX5), which is involved in Fe-S cluster biogenesis.[28] The patient was known to have an otherwise uncomplicated mild anemia when he presented in the fifth decade with type II diabetes mellitus, skin bronzing, elevated liver enzymes, and hepatosplenomegaly associated with iron overload, which, over the subsequent 2 decades, evolved to hepatic cirrhosis and hypogonadism due to severe iron overload. Interestingly, transfusion exacerbated the anemia,

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whereas chelation with deferoxamine led to an improvement in erythroid parameters.

GLRX5 had previously been shown by Wingert et al to be mutated in the zebrafish hypochromic microcytic anemia mutant *shiraz*.[29] They hypothesized that the pathogenesis of the anemia in the *shiraz* mutant could be attributable to dysregulation of the IRE-IRBP dependent posttranscriptional control of ALAS2 protein expression; specifically that IRBP1, as in the ABCB7 mutant, was maintained in the Fe-S deficient, IRE-binding state that represses ALAS2 translation. Indeed, these investigators found that expression of zebrafish *alas2* mRNAs lacking a functional IRE, and thus independent of IRBP1-dependent control, complemented the anemia. Likewise, knock-down of zebrafish irbp1 with a gene-specific antisense morpholino suppressed the phenotype equally well. The disease-causing nature of the mutation, alterations in Fe-S biogenesis, and effects on the IRE-IRBP system and ALAS2 expression were subsequently substantially confirmed in fibroblasts derived from this patient or in erythroblasts in which GLRX5 expression had been reduced by siRNAs.[30] These data are the most compelling results collected so far indicating that alterations in mitochondrial Fe-S biogenesis can secondarily affect heme synthesis in the erythroblast.

CSA due to abnormal mitochondrial protein synthesis (Figure 3)

PMPS

As the name implies, Pearson marrow-pancreas syndrome (PMPS) is a syndromic disorder characterized by BM and exocrine pancreas dysfunction.[31] However, PMPS patients often present as infants with a variety of other signs and symptoms, including failure to thrive, lactic acidosis, malabsorption, myopathy, and liver dysfunction. Supportive care for clinical symptoms and organ dysfunction is the only indicated therapy at this time. Erythropoiesis in PMPS is macrocytic and nonmegaloblastic. In addition to ringed sideroblasts, BM aspiration specimens characteristically demonstrate vacuolization of early erythroid and myeloid progenitors. PMPS is the only CSA that commonly presents with pancytopenia.

PMPS can be considered within the spectrum of mitochondrial cytopathies[32,33] that also includes Kearns-Sayre syndrome (KSS) and progressive external ophthalmoplegia, which are associated with mitochondrial heteroplasmy (cells harboring a mixture of mitochondria with normal and mutant genomes). Nevertheless, while maternally inherited and congenital in presentation, PMPS rarely segregates in pedigrees. In unusual circumstances, individuals with PMPS may be born to mothers with milder mitochondrial phenotypes. The clinical severity of the hematological and other manifestations of the disease can be correlated with the relative frequency of abnormal mitochondrial DNA in each affected or unaffected tissue, which may even change over time.[32] Rare individuals not succumbing to PMPS have evolved to have a KSS-like mitochondrial myopathy.

Nearly half of PMPS can be shown to have heteroplasmy for a 4977-bp deletion in the mitochondrial genome that is commonly also present in individuals with KSS.[34] This canonical deletion involves mitochondrially encoded subunits of respiratory complex I (NADH dehydrogenase), complex IV (cytochrome C oxidase), and complex V (ATP synthase), as well as several mt-tRNA genes. However, many patients are heteroplasmic for other, sometimes nonoverlapping, mitochondrial genomic deletions, suggesting that

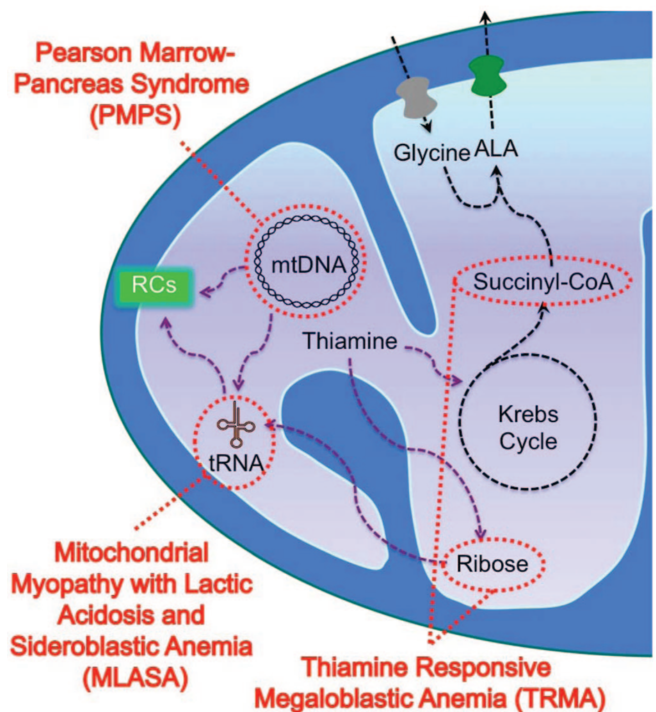

Figure 3. Mitochondrial protein synthesis and CSA. Heteroplasmic deletions in mitochondrial DNA, which may include mitochondrial tRNA genes, are associated with PMPS. MLASA is associated with mutations in PUS1 and the mitochondrial YARS2. Each of these mutations is expected to lead to defective production of multiple proteins encoded in mitochondrial DNA. Thiamine is involved in multiple mitochondrial pathways, including de novo ribose synthesis and in an enzyme that supplies metabolites to the Krebs cycle, which supplies the heme precursor succinyl-CoA. Therefore, deficiency in the cell-surface high-affinity thiamine transporter SLC19A2, which causes TRMA, might lead to defects both in mitochondrial RNA translation and in heme synthesis.

mutation of a single mitochondrial gene is not responsible for the phenotype. Rather, it appears that PMPS may be related to the simultaneous loss of multiple proteins. Although not documented in PMPS, apart from contiguous mitochondrial gene deletions, mutation of mt-tRNAs may also lead to global mitochondrial impairment by suppressing translation of multiple mitochondrial DNA-encoded proteins. Furthermore, the dose-related, toxic sideroblastic anemia associated with the antibiotic chloramphenicol may be related to its mechanism of action; chloramphenicol is an inhibitor of bacterial ribosomal translation, and mammalian mitochondrial ribosomes are more closely structurally related to bacterial ribosomes than to their mammalian cytosolic counterparts.[35]

MLASA due to mutations in PUS1 or YARS2

The association of CSA with defective mitochondrial protein expression is perhaps most directly made by the mitochondrial myopathy with lactic acidosis and ringed sideroblasts (MLASA) phenotype, which results from mutations in genes encoding either of 2 proteins, pseudouridine synthase (PUS1) or mitochondrial tyrosyl-tRNA synthetase (YARS2).

Patients with MLASA due to *PUS1* mutations typically present with lactic acidosis and mitochondrial myopathy associated with decreases in respiratory complexes I and IV.[36,37] The CSA, which can

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be highly variable even in patients with the same genotype, is normocytic to slightly macrocytic and may require chronic transfusion support. In addition to these cardinal muscle and BM phenotypes, affected individuals may have intellectual impairment and/or craniofacial abnormalities, including hypertelorism. Among 5 families with this syndrome, 3 distinct mutations in PUS1 have been reported[11,36-38]; most patients have been of Persian-Jewish descent, due to a founder effect. PUS1 is required to convert uridine to its isomer pseudouridine (ψ) that is prevalent in structural RNAs. ψ provides increased stability of RNA secondary structures, and therefore is thought to enhance the stability and/or function of these molecules. The common PUS1 mutation profoundly decreases the modification of both mitochondrial and cytoplasmic tRNAs[39]; however, the effect of PUS1 deficiency on mitochondrial protein translation has not been assessed directly.

All 3 patients described with MLASA due to YARS2 mutations described in the literature[40] and one unpublished case (M.D.F., unpublished data) share a single missense allele due to a founder mutation occurring in the Lebanese population. Based on the limited available data, these patients have a variable myopathy and CSA similar to PUS1-deficient patients, but no intellectual disability or developmental anomalies. In addition, several patients have developed a cardiomyopathy. Whereas the number of YARS2 patients is quite limited, the initial description of the phenotype by Riley et al provided unqualified evidence that the most likely mechanism for the mitochondrial disease was a global decrease in the abundance and synthesis of mitochondrial respiratory complex proteins encoded by the mitochondrial, but not nuclear, genome.[40]

At this time, the pathogenesis of ringed sideroblasts in the disorders described above, which can be broadly defined as mitochondrial protein or translation deficiencies, remains entirely obscure. In each case, it appears that heme synthesis is normal—RBCs are well hemoglobinized and normocytic or macrocytic, and not microcytic as would be expected from a heme synthesis-related CSA. One can only speculate that global mitochondrial dysfunction uncouples mitochondrial iron uptake from mitochondrial iron requirements in a yet-to-be discovered manner that is unrelated to heme synthesis.

Thiamine responsive megaloblastic anemia due to mutations in SLC19A2, a high-affinity thiamine transporter

The syndrome of thiamine-responsive megaloblastic anemia, which is also associated with diabetes and deafness, has the unusual CSA phenotype of megaloblastic erythroid maturation with occasional ring sideroblasts. Mutations in the high-affinity thiamine transporter SLC19A2 are the basis of the disorder and explain the hallmark of the disease, clinical response of the anemia, and to a lesser extent the other clinical signs, to supraphysiological doses of thiamine.[41,42] The link between thiamine-responsive megaloblastic anemia (TRMA) and mitochondrial protein synthesis has not been experimentally validated. Impairment of the thiamine-dependent generation of succinyl-CoA, which is required for heme biosynthesis, has been suggested as the cause of the ringed sideroblast abnormality, but thiamine is also an essential cofactor in the de novo synthesis of ribose, which is essential for protein production.[43]

Summary

As in other fields in hematology and medicine, developments in modern human genetics and genomics have had an immense impact on our ability to define the molecular genetic basis of the CSAs. Furthermore, model organisms ranging from yeast to zebrafish to mice have helped to define the pathophysiology of these disorders and to group them according to shared effects on heme or Fe-S biogenesis or mitochondrial protein translation. Armed with this knowledge, it is more than likely that the approximately one-third of cases of CSA that are currently molecularly unexplained will belong in one of these pathways. For example, because of the conservation of mitochondrial Fe-S biogenesis in mammals with lower eukaryotic organisms such as yeast, it is highly likely that mutations in other mitochondrial and potentially cytoplasmic Fe-S biogenesis pathway proteins, particularly those that lead to yeast mitochondrial iron overload, may be associated with CSA in humans. Likewise, many of the transporters relevant to heme biosynthesis are unknown, and could be potential candidates for CSA genes. Lastly, because the fortuitous responses of XLSA and TRMA to pyridoxine and thiamine, respectively, can now be understood in the context of their molecular and biochemical underpinnings, understanding the disease genes should allow the rational design of therapies for other molecularly defined groups.

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Correspondence

Mark D. Fleming, MD, D Phil, Department of Pathology, Children's Hospital Boston, 300 Longwood Ave, Enders 1116.1, Boston, MA 02115; Phone: (617) 919-2664; Fax: (617) 730-0168; e-mail: mark.fleming@childrens.harvard.edu.

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