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Function of ALAS2 Gene in Congenital Sideroblastic Anemia

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Abstract: *Congenital sideroblastic anemia (CSA) is an inherited disease characterized by the presence of sideroblasts in bone marrow. Microcytic hypochromic anemia and systemic iron overload are the common syndromes. X-linked slderoblastic anemia (XLSA) is the most common, and its pathogenesis is related to the mutation of ALAS2 gene. The abnormal structure and processing of ALAS2 enzyme due to mutation of ALAS2 gene, or the decrease of ALAS2 gene expression caused by mutation of GATA1 site, or the disorder of iron metabolism are all related to the pathogenesis of XLSA. At present, the diagnosis and treatment of CSA depend on the combination of clinical characteristics and gene sequencing, but a considerable number of CSA mutation genes are still unclear. Through the next generation sequencing technology (NGS) and cell or animal model experiments, we will further deepen our understanding of CSA.*

Keywords: *Congenital sideroblastic anemia; ALAS2 gene; GATA1; iron metabolism.*

Abbreviations *Congenital sideroblastic anemia (CSA), X-linked slderoblastic anemia (XLSA), next generation sequencing technology (NGS), 5-aminolevulinic acid (ALA),ALA synthases (ALAS),succinylcoenzyme A (CoA), pyridoxal 5-phosphate (PLP), X-linked protoporphyria (XLPP),BTB and CNC homology 1 (Bach 1),SLC25A37 (mitoferrin-1),ferrochelatase (FECH), iron-sulfur cluster (ISC), mitochondrial ferritin (FTMT), iron regulatory proteins (IRP),iron responsive element (IRE), hypoxia inducible factor 2α (HIF-2α), transferrin receptor 1 (Tfr1),5'untranslated region (UTR),growth differentiation factor 15 (GDF15),twisted gastrulation (TWSG1), deferiprone (DFP), deferasirox (DFX), desferrioxamine (DFO), ATP specific CoA synthase (A-SCS) allogeneic.*

1. INTRODUCTION

According to its pathogenic mode, sideroblastic anemia is divided into congenital and acquired. CSA consists of syndromic CSA, non-syndromic CSA and undefined CSA (Table 1). Its pathogenesis mainly involves multiple gene mutations in the process of heme biosynthesis, iron-sulfur cluster biogenesis and mitochondrial solute transport or metabolism (Figur1) [1]. XLSA is the most common CSA, accounting for about 40% [2], which clinical features are X-linked inheritance, microcytic hypochromic anemia and iron overload [3]. To date, others include mutations in SLC25A38, GLRX5, HSPA9, HSCB, ABCB7, SLC19A2, mtDNA, PUS1, TRNT1, YARS2, LARS2, MT-ATP6 and NDUFB11 [45].

The ALAS2 gene is located on the X chromosome p11.21.ALAS2 gene mutation leads to the abnormal synthesis ofALAS2 enzyme, which catalyzes the rate-limiting pathway of erythrocyte heme biosynthesis, causing mitochondrial iron overload and anemia further, leading to XLSA eventually. More than 80 different mutations in the ALAS2 gene have been reported [6], most of which are missense or nonsense mutations located in exon 5- 11. In recent years, mutations in the enhancer [7] and intron 1 [89] have also been revealed. All in all, the abnormal protein structure and processing of ALAS2, the decrease of ALAS2 gene expression, and the deficiency of substrate or cofactor affinity in the catalytic reaction are all associated with the pathogenesis of XLSA. In addition, mutations in the genes of the heme biosynthetic pathway can lead to the production of porphyria and CSA, because of the impairment of mitochondrial respiratory chain activity, oxygen delivery, iron metabolism and drug metabolism [10].

This article focus on the basics of ALAS2, GATA1, iron, and their potential links to XLSA.We believe that this review will contribute to the study of ALAS2 gene and CSA disease. The effect of ALAS2 enzyme on catalytic reaction and XLSA

Table1. *List of virulence genes for congenital sideroblastic anemia*

Disease	Gene	Protein	Chromosome	Inheritance	Type of anemia	Severity of anemia	Associated Abnormalities
Non syndromic							
XLSA	ALAS2	ALAS2	Xp11.21	XR	Microcytic Macrocytic ♀	Mild to severe	Iron overload
SLC25A38 deficiency	SLC25A38	Mt transporter SLC25A38	3p22.1	AR	Microcytic	severe	Iron overload
Glutaredoxin 5 deficiency	GLRX5	Glutaredoxin 5	14q32.13	AR	Microcytic	Mild to severe	Iron overload
HSPA9 deficiency	HSPA9	HSPA9	5q31.2	AR	Normocytic or Microcytic	Mild to severe	Iron overload
HSCB deficiency	HSCB	HSCB	22q12.1	AR	Normocytic	Moderate	None
Syndromic							
XLSA/A	ABCB7	mt transporter ABCB7	Xq13.3	XR	Microcytic	Mild to moderate	Ataxia
PMPS	mt DNA	Mitochondrial tRNAs, rRNAs and protin	Mitochondria	Maternal/sporadic	Macrocytic	Severe	Metabolic acidosis, exocrine pancreatic insufficiency hepatic/renal failure
TRMA	SLC19A2	Thiamine transporter	1q24.2	AR	Macrocytic	Severe	Diabetes, Psychiatric symptoms, Deafness, Heart malformations
MLASA1	PUS1	Pseudouridine synthase 1	12q24.33	AR	Normocytic or Macrocytic	Mild to severe	Lactic acidosis, Myopathy Cardiomyopathy
MLASA2	YARS2	Mt tyrosyl tRNA synthetase	12p11.21	AR	Normocytic or Macrocytic	Mild to severe	Lactic acidosis, Myopathy Cardiomyopathy
LARS2 deficiency	LARS2	Mt leucine tRNA synthase	3p21.31	AR	Macrocytic	Severe	Lactic acidosis , cardiomyopathy, hepatopathy , seizures
SIFD	TRNT1	TRNT1	3p26.2	AR	Microcytic	Severe	Iron overload, cardiomyopathy, CNS derangements, deafness
ATP6 SA	MT-ATP6	ATP synthase	Mitochondria	SP/M	Normocytic or Macrocytic	Moderate to Severe	Lactic acidosis , myopathy, Neurological abnormalities
NDUFB11 deficiency	NDUFB11	supernumerary subunits of respiratory chain complex I	Xp11.3	XR	Normocytic	Moderate	Lactic acidosis

An important precursor for the synthesis of heme, 5-aminolevulinic acid (ALA), is produced by the pyridoxal 5-phosphate (PLP)-dependent enzymatic reaction of glycine with

succinylcoenzyme A (CoA) (Figure1). This reaction is catalyzed by ALA synthases (ALAS), which has two isozymes.One is non-specifc ALAS (ALAS-N or ALAS1): expressed ubiquitously, and another is erythroid-specifc ALAS (ALAS-E or ALAS2): expressed in erythroid precursors only [1]. Different genes encode these two isozymes separately, and transcriptional regulation is completely different [1]. In this reaction, PLP acts as a cofactor to bind to lysine residue of exon 9 and enhances the activity of ALAS2 enzyme[2]. As we all know, in the subsequent process of heme synthesis, ALA is exported to the cytosol and generates heme eventually after a series of reactions. (Figure 1). Therefore, the efficient synthesis of heme depends on the efficient and suitable supply of ALAS2 protein, glycine and CoA.

Human ALAsynthases is encoded by ALAS2 gene. The mutation of ALAS2 gene leads to a structural change of protein, which not only affects catalytic response, but also is related to XLSA [1112]. The carboxyl C-terminal of ALAS2 protein is conserved, which inhibits enzyme activity and protein stability in mitochondria [13]. Five mutations in this area are classified as functional loss mutations, which are the reasons of XLSA [1314].On the contrary, some mutations can cause gain-function of ALAS2, which lead to oversynthesis of PPIX to X-linked protoporphyria (XLPP) [12]. It has beenrevealedthat five new mutations at the C-terminal truncated exon 11 of the ALAS2 gene and identified a key region in which the opening of the active site loop or the increase of molecular flexibility results in the functional gain of ALAS2 and eventually enhances theactivity of enzyme [515]. In summary, the C-terminal of ALAS2 enzyme can act as an internal modifier and participate in functional regulation. We can boldly speculate whether these sites can be targeted for treatment in patients with XLSA.

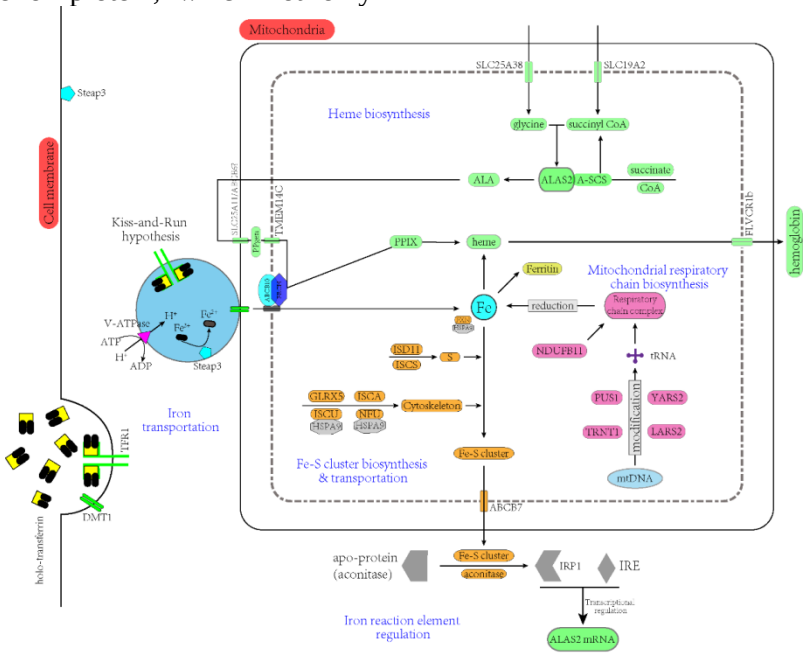

Figure1. *The figure contains iron transportation: mechanism and pathway of iron entry into mitochondria. Iron reaction element regulation: IRP and IRE regulate the transcription of ALAS2 gene. Heme synthesis, iron sulfur cluster synthesis & transportation and mitochondrial respiratory chain biosynthesis diagram and gene involved.*

2. GATA1 Loci of ALAS2 Gene and XLSA

The human GATA1 gene is located on the X chromosome (Xp21-11), and it is the X-linked [9]. GATA1, functioning as an important transcription factor, activates the expression of erythrocyte specific genes such as β- and γ-globin during the development of erythroid progenitor cells [16]. On the other hand, it can inhibit the expression of the genes that are not conducive to erythrocyte development and differentiation such as Cdk6 related to cell cycle and Myc related to cell proliferation [17]. To achieve the above functions, GATA-1 can interact with different transcription factors such as FOG-1, TAL-1/SCL, CBP/p300 and other auxiliary factors [18-20].

According to the literature, GATA1 is mainly activated by three functional domains: N terminal

domain, N Finger domain and C Finger domain, which play a key regulatory role in the development of the erythroid cells [2122]. It is thought to be an important cis-regulatory element of ALAS2 gene during erythropoiesis in vivo, and GATA1 mutation may be one of the causes of XLSA [21]. Zhang et al. produced female hybrid mice without GATA locusand hybridized the female hybrid micewith wild male mice, which showed that only the offspring with genotype (Alas2-13 / Y) died of severe anemia during embryonic stage[9]. It was proved that the deletion of GATA site significantly decreased the level of ALAS2 mRNA and protein and the erythroid differentiation stagnated in the immature erythrocyte progenitor cell stage [9].In addition, the expression of ALAS2 significantly decreased in erythrocytes with GATA1 promoter destroyed in vitro [23] and XLSA patients with int-1-GATA mutation [8]. It is suggested that ALAS2 may be an important downstream target of GATA1, and the deletion of intron GATA1 site leads to the decrease of ALAS2 gene expression, the inhibition of heme synthesis and the arrest of erythroid differentiation.

So how does the GATA1 locus regulate the expression of ALAS2? The study of Zhang et al. suggested that in order to activate the translation of ALAS2 to promote the expression of ALAS2 gene in erythroid cells, the intron GATA1, as an anchor, connects the GATA site in intron 8 to the proximal promoter, and forms a long range enhanced cycle that regulated by an enhancer protein complex centered on GATA1, including TAL1, LDB1, LMO2 and Pol Ⅱ [9]. And compared to int-8-GATA, int-1-GATA sites play a more important role in ALAS2 transcription [9].

From the experimental results, we can infer that the phenotypic differences between deletion and mutation at int-1-GATA sites may be determined by the amount of residual ALAS2 expression giving rise to deletion or mutation. The level of ALAS2 expression indicates that other factors, for example, epigenetic modification, may also be involved in the regulation of ALAS2 expression [9]. Therefore, it is believed that int-1-GATA loci are expected to be a valuable diagnostic screening site for patients with unknown mutations in XLSA. A deeper study should be carried out to demonstrate that CSA patients reduce ALAS2 expression by breaking the enhanced cycle, and if so, where is it happens? How is it adjusted? What targeted measures can be taken?

Consequently, it is believed that the further research based on the ALAS2 gene, combined with the NGS and the study of molecular mechanism of modern disease model, which will further promote the development of therapeutic targets and targeted drugs for XLSA, which is helpful to reasonable and efficient therapy.

Heme can also affect the expression of ALAS2 through GATA1. Tanimura et al. found that the regulatory factor GATA1 requires heme to establish and maintain erythrocyte transcriptome [24]. GATA1 induces the biosynthesis of heme, globin chains and ALAS2, while heme inhibits BTB and CNC homology 1 (Bach1) [24]. Bach1 is a transcriptional inhibitor, which can promote heme degradation through proteasome when it binds to heme [25]. So when heme is deficient, it can induce bach1 accumulation, which can inhibit the transcription of the target gene, and vice versa [24]. As a result, the change of heme can also be indirectly involved in the regulation of ALAS2 through the cycle. Besides, it has been reported that excessive heme can inhibit the translation of ALAS2 mRNA in immature erythrocytes [26] or inhibit the precursor of ALAS2 into mitochondria [27], resulting in feedback inhibition of its synthesis.

3. The Relationship between Iron Metabolism and Disease

Iron is an important material for heme synthesis. In the cytoplasm, iron is stored in the form of ferritin and used as a repair group of some proteins. Richardson et al. proposed the mechanism of ‘kiss-and-run’, involving the iron from endosome into mitochondrial, which indicates that brief contact between the endosome and the mitochondria can directly transfer the ferrous iron ions into the mitochondria [28]. It has been reported that in the above iron transport process, the complex formed by cytosolic iron in mitochondrial SLC25A37 (mitoferrin-1), ABCB10 and ferrochelatase (FECH) is very important during the process of erythrocyte differentiation [1]. Of course, iron in the mitochondria is used for the synthesis of heme or iron-sulfur cluster (ISC) and transferred to the cytoplasm subsequently. It is confirmed that during ISC synthesis, ABCB7 molecules transfer unknown molecules, the glutathione-conjugated 2Fe-2S clusteror sulfur-containing compound, from mitochondria to cytoplasm [29]. This is important for the synthesis of ISC and the maintenance of iron homeostasis, as reduced precursor or heme

release will lead to mitochondrial iron loading [30]. The gene mutation in iron transport or the abnormal of transport process involved can bring about the load of the mitochondrial iron, which is related to the formation of the iron-grain and young cells.

Mitochondrial iron deposition is a marker of CSA; however, the mechanism is largely unclear. The iron load in the XLSA patient is not always related to the severity of the anemia. Previous cell models have shown that the mutation of the enhancer of ALAS2 gene can lead to the formation of ring sideroblasts, and that the expression of mitochondrial ferritin (FTMT) may be a necessary condition for the formation of annular fibroblasts [31]. Besides, FTMT encodes ferritin heavy chain like protein in mitochondria, which highly expresses in erythrocytes of CSA patients and has iron oxidase activity [32]. So this does make us to wonder whether stopping the generation of FTMT can prevent the formation of CSA.

During erythroid development, iron regulatory proteins (IRP) regulate iron metabolism of cells which function by binding to iron responsive element (IRE), including two iron regulatory proteins 1 and 2 (IRP1 and IRP2) [33]. IRP2 regulates erythropoiesis and iron absorption only as RNA binding protein by controlling the translation of hypoxia inducible factor 2α (HIF-2α) mRNA. IRP1, as RNA binding protein or cellular solute aconitase, regulates heme biosynthesis and iron uptake by regulating the expression of transferrin receptor 1 (Tfr1) and ALAS2 mRNA. Under normal circumstances, IRE is not combined with IRP, and ALAS2 mRNA translation can occur normally. In the case of excess iron, iron converts the protein into a non-RNA binding form and inactivates IRP1, while IRP2 is ubiquitized and degraded by the proteasome [33]. Under the condition of iron deficiency, IRP1 binds to the stem-ring structure of IRE in ALAS2 mRNA 5'untranslated region (UTR), which inhibits the translation of ALAS2 and ferritin or protects mRNA from degradation by inducing the translation of TFR1 or DMT1 [33] (Figure 1). So IRE/IRP system plays an important role in regulating the production of the erythrocyte, absorption of the dietary iron, metabolism of the liver iron and the iron balance in vivo. XLSA patients are often associated with systemic iron load, the cause of which is unknown. However, recent reports have indicated that three cytokines expressed by iron modulin: growth differentiation factor 15 (GDF15) [34], erythroferron [35] and twisted gastrulation (TWSG1) [36]are all related to body iron overload. The mitochondrial iron overload in XLSA patients is related to iron homeostasis and regulation above.

Iron overload is a key problem, which can significantly increase morbidity and mortality. In recent years, more and more reports indicate that systemic iron overload can lead to complications such as hepatocellular carcinoma [37], atherosclerosis [38], heart damage [39] and kidney injury [40]. For the treatment of iron overload in CSA patients, the venotomy can be considered if suffering mild to moderate anemia (Hb≥90 g/ L).While patients with more severe anemia who cannot tolerate blood-letting therapy and even need regular blood transfusion to maintain heme levelsrequire iron chelation therapy [6]. Recent studies have shown that for iron overload patients, the combination of oral deferiprone (DFP) and deferasirox (DFX) can better remove iron from the body. Intramuscular injection of desferrioxamine (DFO) is also possible, but the compliance rate is lower because it cannot be taken orally [41]. Splenectomy is sometimes performed in CSA patients with severe anemia and splenomegaly to reduce hemolysis and anemia, but recent studies do not recommend splenectomy in CSA patients to prevent fatal thromboembolic complications [42]. The abnormal of iron transport and/or regulation may be associated with iron overload, the incidence and high mortality of CSA.

4. Role of ALAS2 in CSA

Clinically, XLSA is common in men under 40 years of age [43], and there are also reports of delayed cases [44]. Those patients are characterized by varied degrees of microcytic hypochromic anemia, and most of them show moderate levels.

Studies have been reported that women with heterozygotes suffer from macrocytic sideroblasticanemia [34546]. We can speculate that the inactivation of inclined X chromosome in female heterozygote carriers may be the mechanism of female XLSA, and inactivation increases with age. The hematopoietic cells expressing mutant ALAS2 were inactivated at the progenitor cell stage, which causes the compensatory stimulation of erythrocytes expressing wild type ALAS2, and macrocytic anemiahappens finally [45].

Previously, because of pyridoxine supplementation improving symptoms, CSA was also defined as pyridoxine responsive anemia. It is reported that about half of XLSA patients respond to pyridoxine [6], and there were also differences in clinical efficacy with the mutation site of ALAS2 [47]. Whether the mutation site affects the binding region of pyridoxine, the dosage of pyridoxine and the overload of iron in mitochondria can affect the response of the disease to pyridoxine [48].The mutation of ALAS2 gene identified in patients and families of pyridoxin-reactive XLSA patients proved that the mutations reduced the activity of ALAS2 enzyme in vitro[4449]. So there is no doubt that the other half of XLSA patients did not respond to drug doses of pyridoxine. It has been certificated that some of the XLSA patients with ALAS2 mutation were pyridoxine resistant, but had normal enzyme activity in vitro [14]. Moreover, studies have shown that ATP specific CoA synthase (A-SCS) complexes with ALAS2 proteins are important for providing CoA in the catalytic reaction of heme rate-limiting steps, and if the complex is not formed, ALAS2 enzyme activity will decrease, causing XLSA [150].

Besides, mutations in the ALAS2 promoter and the first intron GATA binding site of ALAS2are also defined as the cause of pyridoxine refractory XLSA [78]. So when the clinical symptoms and signs are unable to make definite diagnosis of XLSA, the detection of each gene mutation site is helpful. At the same time, we can judge whether the patient has a response to pyridoxine treatment based on the results of the test. Therefore, pyridoxine therapy remains the first choice until molecular confirmation. Some experiments have supported that ALA can stimulate the increase of heme level in a dose-dependent manner. As a result, XLSA, which is not reactive to pyridoxine, can be treated by oral ALA [51]. But other reports suggest that ALA alone is not enough to improve anemia in patients with XLSA [52], so novel treatments are needed. There was a report that hematopoietic stem cell transplant (HSCT) was tried to treat a girl with CSA. Her blood transfusion dependence was completely reversed and she remained healthy five years after the transplant [53].

Recently, with the advent of the Location Cloning and the Human Genome Project, the development of NGS and Solid-State Genotyping Techniques, more than two thirds of CSA has been attributed to mutations in the pathogenicity gene and related loci (table 1) [5]. This conduces to avoid misdiagnosis and provides the possibility for further gene therapy.At present, Yu et al. Successfully diagnosed a 12-month-old XLSA child by large-scale parallel sequencing [54]. (As Shown in Table 1): Gene mutations in non-syndromic CSA include those affecting heme biosynthesis (e.g. ALAS2 and SLC25A38 [55] and affecting Fe-S cluster biosynthesis (e.g. GLRX5 [56], HSPA9 [57], HSCB [58]). The gene mutation of syndromic CSA was related to mitochondrial protein biosynthesis (e.g. mtDNA [59], PUS1 [60], TRNT1 [61], YARS2 [6263] and LARS2 [64]) and mitochondrial respiratory chain (e.g. MT-ATP6 [65], NDUFB11 [66].

However, in syndromic CSA, ABCB7 [67] was associatedwith iron-sulfur cluster biosynthesis and SLC19A2 [68] to thiamine uptake [14-669]. Patients with mutations in the heme biosynthesis pathway had relatively mild symptoms.Many of them were followed up for a long time, did not require special treatment, and could obtain long-term survival [4].

Some of the patients had different therapeutic effects on ALA, pyridoxine, glycine or thiamine supplementation [5152]. Similarly patients with mutations in the Fe-S cluster pathway were more likely to have mild symptoms, and so far, no specific treatment had been reported. However, unlike the former, gene mutations in mitochondrial respiratory chain synthesis pathway not only affect the respiratory chain of erythrocytes, but also affect the respiratory chain of other tissues.

As a consequence, this group of patients tend to develop symptoms early and generally fail to survive for a long time, and most of them die during juveniles. Fortunately, sometimes early allogeneic hematopoietic stem cell transplan-tation can relieve symptoms [53].

Therefore, under the present situation, more discoveries in the aspect of gene mutation patterns and functional explanations can help us to strengthen the study and understanding of the potential mechanism of CSA.Finally, the association among ring sideroblasts, β-thalassemia and hemoglobin disease was found, which needs further investigation.

5. Construction of Ring Sideroblasts Biologic Model

In order to further study CSA, researchersused various methods to construct a model of sideroblasts. The function of GATA1 site and its

regulation on ALAS2 gene were investigated in vitro and in vivo based on CRISPR/Cas9 targeted destruction of GATA1 binding motif in mouse ALAS2 intron 1 [924]. Besides, experiments about ALAS2 gene knockout embryonic stem cells and mice showed abnormal iron homeostasis.Whereas iron did not exist in mitochondria but dispersed in cytoplasm, and the sideroblasts model was not successfully obtained at last [7071].

Uptodate, Hatta et al. Encoded seven factors (OCT3/4, SOX2, KLF4, L-MYC, LINL28, GLIS1 and shp53) in bone marrow mesenchymal stem cells using non-integrating episomal vector (It has been confirmed that there is an ALAS2 mutation among them.) [72]; Kaneko et al.introduced mutations into ALAS2 intron 1 enhancer in HUDEP2 cells based on CRISPR/Cas9 editing [31]; Saito et al.

Introduced mutations in ALAS2 GATA-1 motifs in HiDEP cells based on CRISPR/Cas9 editing and co-cultured with OP9 cells in the presence of ferrous citrate [73]. The biologic model of ring sideroblasts has been builtthrough the methods above, which lays a good foundation for the further study of ALAS2 gene and congenital sideroblastic anemia, providing convenience for further molecular mechanism research and clinical trials in the future.

6. Conclusion

In summary, ALAS2 gene plays a critical important role in CSA. ALAS2 gene detection is of great significance in the diagnosis and treatment of CSA, and is benefit for screening and genetic counseling. Cell models have been established to provide more possibilities for further research. It is believed that mounting number of patients will benefit from it. Nowadays, we are more aware of the mutant genes of CSA, but it is limited to understand the exact molecular mechanism of CSA. It is hoped that in the next few years we can clarify its precise mechanism as soon as possible to help future treatment.

References

[1] Furuyama K., and Kaneko K., Iron metabolism in erythroid cells and patients with congenital sideroblastic anemia, Int. J. Hematol. 107(1), 44 (2018).

[2] Cazzola M., and Malcovati L., Diagnosis and treatment of sideroblastic anemias: from defective heme synthesis to abnormal RNA splicing, Hematology Am. Soc. Hematol. Educ. Program. 2015, 19 (2015).

[3] Katsurada T., Kawabata H., Kawabata D., Kawahara M., Nakabo Y., Takaori-Kondo A., and Yoshida Y., A Japanese family with X-linked sideroblastic anemia affecting females and manifesting as macrocytic anemia, Int. J. Hematol. 103(6), 713 (2016).

[4] Long Z., Li H., Du Y., and Han B., Congenital sideroblastic anemia: Advances in gene mutations and pathophysiology, Gene. 668, 182 (2018).

[5] Ducamp S., and Fleming MD., The molecular genetics of sideroblastic anemia, Blood. 133(1), 59 (2019).

[6] Bottomley SS., and Fleming MD., Sideroblastic anemia: diagnosis and management, Hematol. Oncol. Clin. North Am. 28(4), 653 (2014).

[7] Kaneko K., Furuyama K., Fujiwara T., Kobayashi R., Ishida H., Harigae H., and Shibahara S., 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), 252 (2014).

[8] Campagna DR., de Bie CI., Schmitz-Abe K., Sweeney M., Sendamarai AK., Schmidt PJ., Heeney MM., Yntema HG., Kannengiesser C., Grandchamp B., Niemeyer CM., Knoers NVAM., Swart S., Marron G., van Wijk R., Raymakers RA., May A., Markianos K., Bottomley SS., Swinkels DW., and Fleming MD., X-linked sideroblastic anemia due to ALAS2 intron 1 enhancer element GATA-binding site mutations, Am. J. Hematol. 89(3), 315 (2014).

[9] Zhang YC., Zhang JL., An WB., Wan Y., Ma SH., Yin J., Li XC., Gao J., Yuan WP., Guo Y., Engel JD., Shi LH., Cheng T., and Zhu XF., Intron 1 GATA site enhances ALAS2 expression indispensably during erythroid differentiation, Nucleic Acids Res. 45(2), 657 (2017).

[10] Dailey HA., and Meissner PN., Erythroid heme biosynthesis and its disorders, Cold Spring Harb. Perspect. Med. 3(4), a011676 (2013).

[11] Stojanovski BM., and Ferreira GC., Murine erythroid 5-aminolevulinate synthase: Adenosyl-binding site Lys221 modulates substrate binding and catalysis, Febs Open Bio. 5, 824 (2015).

[12] SD W., S D., L G., B G., C B., MN B., GH E., SA H., AV A., M P., AV C., PN M., RJ H., JT M., Y M., G M-V., JC D., and H P., C-terminal deletions in the ALAS2 gene lead to gain of function and cause X-linked dominant protoporphyria without anemia or iron overload., Am. J. Hum. Genet. 83(3), 408 (2008).

[13] Kadirvel S., Furuyama K., Harigae H., Kaneko K., Tamai Y., Ishida Y., and Shibahara S., The carboxyl-terminal region of erythroid-specific 5-aminolevulinate synthase acts as an intrinsic modifier for its catalytic activity and protein stability, Exp. Hematol. 40(6), 477 (2012).

[14] Bishop DF., Tchaikovskii V., Hoffbrand AV., Fraser ME., and Margolis S., X-linked sideroblastic anemia due to carboxyl-terminal ALAS2 mutations that cause loss of binding to the beta-subunit of succinyl-CoA synthetase (SUCLA2), The Journal of biological chemistry. 287(34), 28943 (2012).

[15] Tchaikovskii V., Desnick RJ., and Bishop DF., Molecular expression, characterization and mechanism of ALAS2 gain-of-function mutants, Mol. Med. 25(1), 4 (2019).

[16] Kim YW., Kim S., Kim CG., and Kim A., The distinctive roles of erythroid specific activator GATA-1 and NF-E2 in transcription of the human fetal gamma-globin genes, Nucleic Acids Res. 39(16), 6944 (2011).

[17] Rylski M., Welch JJ., Chen YY., Letting DL., Diehl JA., Chodosh LA., Blobel GA., and Weiss MJ., GATA-1-mediated proliferation arrest during erythroid maturation, Mol. Cell. Biol. 23(14), 5031 (2003).

[18] Tsang AP., Visvader JE., Turner CA., Fujiwara Y., Yu C., Weiss MJ., Crossley M., and Orkin SH., FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation, Cell. 90(1), 109 (1997).

[19] Mikkola HK., Klintman J., Yang H., Hock H., Schlaeger TM., Fujiwara Y., and Orkin SH., Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene, Nature. 421(6922), 547 (2003).

[20] Han LP., Lu J., Pan LN., Wang XL., Shao YG., Han SY., and Huang BQ., Histone acetyltransferase p300 regulates the transcription of human erythroid-specific 5-aminolevulinate synthase gene, Biochem. Biophys. Res. Commun. 348(3), 799 (2006).

[21] Bresnick EH., Katsumura KR., Lee HY., Johnson KD., and Perkins AS., Master regulatory GATA transcription factors: mechanistic principles and emerging links to hematologic malignancies, Nucleic Acids Res. 40(13), 5819 (2012).

[22] Newton A., Mackay J., and Crossley M., The N-terminal zinc finger of the erythroid transcription factor GATA-1 binds GATC motifs in DNA, J. Biol. Chem. 276(38), 35794 (2001).

[23] Suwabe N., Takahashi S., Nakano T., and Yamamoto M., GATA-1 regulates growth and differentiation of definitive erythroid lineage cells during in vitro ES cell differentiation, Blood. 92(11), 4108 (1998).

[24] Tanimura N., Miller E., Igarashi K., Yang D., Burstyn JN., Dewey CN., and Bresnick EH., Mechanism governing heme synthesis reveals a GATA factor/heme circuit that controls differentiation, Embo Reports. 17(2), 249 (2016).

[25] K O., J S., S T., O N., C N., S S., N H., M Y., S S., H F., and K I., Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1, The EMBO journal. 20(11), 2835 (2001).

[26] MP T. Regulation by heme of mitochondrial protein transport through a conserved amino acid motif. Science (New York, N.Y.). 259(5094), 522 (1993).

[27] Smith SJ., and Cox TM., Translational control of erythroid delta-aminolevulinate synthase in immature human erythroid cells by heme, Cell. Mol. Biol. (Noisy-le-grand). 43(1), 103 (1997).

[28] Hamdi A., Roshan TM., Kahawita TM., Mason AB., Sheftel AD., and Ponka P., Erythroid cell mitochondria receive endosomal iron by a "kiss-and-run" mechanism, Biochim. Biophys. Acta. 1863(12), 2859 (2016).

[29] Schaedler TA., Faust B., Shintre CA., Carpenter EP., Srinivasan V., van Veen HW., and Balk J., Structures and functions of mitochondrial ABC transporters, Biochem. Soc. Trans. 43(5), 943 (2015).

[30] Richardson DR., Lane DJ., Becker EM., Huang ML., Whitnall M., Suryo Rahmanto Y., Sheftel AD., and Ponka P., Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol, Proc. Natl. Acad. Sci. U. S. A. 107(24), 10775 (2010).

[31] Kaneko K., Kubota Y., Nomura K., Hayashimoto H., Chida T., Yoshino N., Wayama M., Ogasawara K., Nakamura Y., Tooyama I., and Furuyama K., Establishment of a cell model of X-linked sideroblastic anemia using genome editing, Exp. Hematol. 65, 57 (2018).

[32] Levi S., Corsi B., Bosisio M., Invernizzi R., Volz A., Sanford D., Arosio P., and Drysdale J., A human mitochondrial ferritin encoded by an intronless gene, J. Biol. Chem. 276(27), 24437 (2001).

[33] Wilkinson N., and Pantopoulos K., The IRP/IRE system in vivo: insights from mouse models, Front. Pharmacol. 5, 176 (2014).

[34] Tanno T., Bhanu NV., Oneal PA., Goh SH., Staker P., Lee YT., Moroney JW., Reed CH., Luban NLC., Wang RH., Eling TE., Childs R., Ganz T., Leitman SF., Fucharoen S., and Miller JL., High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin, Nat. Med. 13(9), 1096 (2007).

[35] Kautz L., Jung G., Valore EV., Rivella S., Nemeth E., and Ganz T., Identification of erythroferrone as an erythroid regulator of iron metabolism, Nat. Genet. 46(7), 678 (2014).

[36] Tanno T., Porayette P., Sripichai O., Noh SJ., Byrnes C., Bhupatiraju A., Lee YT., Goodnough JB., Harandi O., Ganz T., Paulson

RF., and Miller JL., Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells, Blood. 114(1), 181 (2009).

[37] Molina-Sanchez P., and Lujambio A., Iron overload and liver cancer, J. Exp. Med. 216(4), 723 (2019).

[38] Vinchi F., Porto G., Simmelbauer A., Altamura S., Passos ST., Garbowski M., Silva AMN., Spaich S., Seide SE., Sparla R., Hentze MW., and Muckenthaler MU., Atherosclerosis is aggravated by iron overload and ameliorated by dietary and pharmacological iron restriction, Eur. Heart J. (2019).

[39] Rozwadowska K., Raczak G., Sikorska K., Fijalkowski M., Kozlowski D., and Danilowicz-Szymanowicz L., Influence of hereditary hemochromatosis on left ventricular wall thickness: does iron overload exacerbate cardiac hypertrophy?, Folia Morphol. (2019).

[40] Ige AO., Ongele FA., Adele BO., Emediong IE., Odetola AO., and Adewoye EO., Pathophysiology of iron overload-induced renal injury and dysfunction: Roles of renal oxidative stress and systemic inflammatory mediators, Pathophysiology. (2019).

[41] Lin CH., Chen X., Wu CC., Wu KH., Song TS., Weng TF., Hsieh YW., and Peng CT., Therapeutic mechanism of combined oral chelation therapy to maximize efficacy of iron removal in transfusion-dependent thalassemia major - a pilot study, Expert Rev. Hematol. 1 (2019).

[42] Le Rouzic MA., Fouquet C., Leblanc T., Touati M., Fouyssac F., Vermylen C., Jakel N., Guichard JF., Maloum K., Toutain F., Lutz P., Perel Y., Manceau H., Kannengiesser C., and Vannier JP., Non syndromic childhood onset congenital sideroblastic anemia: A report of 13 patients identified with an ALAS2 or SLC25A38 mutation, Blood Cells Mol. Dis. 66, 11 (2017).

[43] Fleming MD. Congenital sideroblastic anemias: iron and heme lost in mitochondrial translation, Hematology Am. Soc. Hematol. Educ. Program. 2011, 525 (2011).

[44] Furuyama K., Harigae H., Kinoshita C., Shimada T., Miyaoka K., Kanda C., Maruyama Y., Shibahara S., and Sassa S., Late-onset X-linked sideroblastic anemia following hemodialysis, Blood. 101(11), 4623 (2003).

[45] Sankaran VG., Ulirsch JC., Tchaikovskii V., Ludwig LS., Wakabayashi A., Kadirvel S., Lindsley RC., Bejar R., Shi J., Lovitch SB., Bishop DF., and Steensma DP., X-linked macrocytic dyserythropoietic anemia in females with an ALAS2 mutation, J. Clin. Invest. 125(4), 1665 (2015).

[46] Fujiwara T., Fukuhara N., Ichikawa S., Kobayashi M., Okitsu Y., Onishi Y., Furuyama K., and Harigae H., 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), 1955 (2017).

[47] Liu G., Guo S., Kang H., Zhang F., Hu Y., Wang L., Li M., Ru Y., Camaschella C., Han B., and Nie G., Mutation spectrum in Chinese patients affected by congenital sideroblastic anemia and a search for a genotype-phenotype relationship, Haematologica. 98(12), e158 (2013).

[48] Mendez M., Moreno-Carralero MI., Morado-Arias M., Fernandez-Jimenez MC., de la Iglesia Inigo S., and Moran-Jimenez MJ., Sideroblastic anemia: functional study of two novel missense mutations in ALAS2, Mol Genet Genomic Med. 4(3), 273 (2016).

[49] Ducamp S., Kannengiesser C., Touati M., Garcon L., Guerci-Bresler A., Guichard JF., Vermylen C., Dochir J., Poirel HA., Fouyssac F., Mansuy L., Leroux G., Tertian G., Girot R., Heimpel H., Matthes T., Talbi N., Deybach JC., Beaumont C., Puy H., and Grandchamp B., 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), 590 (2011).

[50] Furuyama K., and Sassa S., Interaction between succinyl CoA synthetase and the heme-biosynthetic enzyme ALAS-E is disrupted in sideroblastic anemia, J. Clin. Invest. 105(6), 757 (2000).

[51] Fujiwara T., Okamoto K., Niikuni R., Takahashi K., Okitsu Y., Fukuhara N., Onishi Y., Ishizawa K., Ichinohasama R., Nakamura Y., Nakajima M., Tanaka T., and Harigae H., Effect of 5-aminolevulinic acid on erythropoiesis: a preclinical in vitro characterization for the treatment of congenital sideroblastic anemia, Biochem. Biophys. Res. Commun. 454(1), 102 (2014).

[52] Ishida H., Imamura T., Morimoto A., Fujiwara T., and Harigae H., Five-aminolevulinic acid: New approach for congenital sideroblastic anemia, Pediatr. Int. 60(5), 496 (2018).

[53] Kim MH., Shah S., Bottomley SS., and Shah NC., Reduced-toxicity allogeneic hematopoietic stem cell transplantation in congenital sideroblastic anemia, Clin Case Rep. 6(9), 1841 (2018).

[54] Yu HJ., Lee YJ., Shim JW., Kim DS., Shim JY., Park MS., Woo HY., Park H., Jung HL., and Kwon MJ., Mutation Analysis of X-linked Sideroblastic Anemia in a 12-Month-Old Boy by Massively Parallel Sequencing, Ann. Lab. Med. 38(4), 389 (2018).

[55] Kannengiesser C., Sanchez M., Sweeney M., Hetet G., Kerr B., Moran E., Fuster Soler JL.,

Maloum K., Matthes T., Oudot C., Lascaux A., Pondarre C., Sevilla Navarro J., Vidyatilake S., Beaumont C., Grandchamp B., and May A., Missense SLC25A38 variations play an important role in autosomal recessive inherited sideroblastic anemia, Haematologica. 96(6), 808 (2011).

[56] Liu G., Wang Y., Anderson GJ., Camaschella C., Chang Y., and Nie G., Functional Analysis of GLRX5 Mutants Reveals Distinct Functionalities of GLRX5 Protein, J. Cell. Biochem. 117(1), 207 (2016).

[57] Schmitz-Abe K., Ciesielski SJ., Schmidt PJ., Campagna DR., Rahimov F., Schilke BA., Cuijpers M., Rieneck K., Lausen B., Linenberger ML., Sendamarai AK., Guo C., Hofmann I., Newburger PE., Matthews D., Shimamura A., Snijders PJ., Towne MC., Niemeyer CM., Watson HG., Dziegiel MH., Heeney MM., May A., Bottomley SS., Swinkels DW., Markianos K., Craig EA., and Fleming MD., Congenital sideroblastic anemia due to mutations in the mitochondrial HSP70 homologue HSPA9, Blood. 126(25), 2734 (2015).

[58] Crispin A., Schmidt P., Campagna D., Cao C., Lichtenstein D., Sendamarai A., Guo C., Chen C., Hildick-Smith GJ., Huston NC., Boudreaux J., Bottomley SS., Heeney MM., Paw BH., Fleming MD., and Ducamp S., Hscb, a Mitochondrial Iron-Sulfur Cluster Assembly Co-Chaperone, Is a Novel Candidate Gene for Congenital Sideroblastic Anemia, Blood. 130(Suppl 1), 79 (2017).

[59] Khasawneh R., Alsokhni H., Alzghoul B., Momani A., Abualsheikh N., Kamal N., and Qatawneh M., A Novel Mitochondrial DNA Deletion in Patient with Pearson Syndrome, Med Arch. 72(2), 148 (2018).

[60] Kasapkara CS., Tumer L., Zanetti N., Ezgu F., Lamantea E., and Zeviani M., A Myopathy, Lactic Acidosis, Sideroblastic Anemia (MLASA) Case Due to a Novel PUS1 Mutation, Turk J Haematol. 34(4), 376 (2017).

[61] Chakraborty PK., Schmitz-Abe K., Kennedy EK., Mamady H., Naas T., Durie D., Campagna DR., Lau A., Sendamarai AK., Wiseman DH., May A., Jolles S., Connor P., Powell C., Heeney MM., Giardina PJ., Klaassen RJ., Kannengiesser C., Thuret I., Thompson AA., Marques L., Hughes S., Bonney DK., Bottomley SS., Wynn RF., Laxer RM., Minniti CP., Moppett J., Bordon V., Geraghty M., Joyce PB., Markianos K., Rudner AD., Holcik M., and Fleming MD., Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD), Blood. 124(18), 2867 (2014).

[62] Riley LG., Cooper S., Hickey P., Rudinger-Thirion J., McKenzie M., Compton A., Lim SC., Thorburn D., Ryan MT., Giege R., Bahlo M., and Christodoulou J., Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia--MLASA syndrome, Am. J. Hum. Genet. 87(1), 52 (2010).

[63] Sommerville EW., Ng YS., Alston CL., Dallabona C., Gilberti M., He L., Knowles C., Chin SL., Schaefer AM., Falkous G., Murdoch D., Longman C., de Visser M., Bindoff LA., Rawles JM., Dean JCS., Petty RK., Farrugia ME., Haack TB., Prokisch H., McFarland R., Turnbull DM., Donnini C., Taylor RW., and Gorman GS., Clinical Features, Molecular Heterogeneity, and Prognostic Implications in YARS2-Related Mitochondrial Myopathy, JAMA Neurol. 74(6), 686 (2017).

[64] Riley LG., Rudinger-Thirion J., Schmitz-Abe K., Thorburn DR., Davis RL., Teo J., Arbuckle S., Cooper ST., Campagna DR., Frugier M., Markianos K., Sue CM., Fleming MD., and Christodoulou J., LARS2 Variants Associated with Hydrops, Lactic Acidosis, Sideroblastic Anemia, and Multisystem Failure, JIMD Rep. 28, 49 (2016).

[65] Berhe S., Heeney MM., Campagna DR., Thompson JF., White EJ., Ross T., Peake RWA., Hanrahan JD., Rodriguez V., Renaud DL., Patnaik MS., Chang E., Bottomley SS., and Fleming MD., Recurrent heteroplasmy for the MT-ATP6 p.Ser148Asn (m.8969G>A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity, Haematologica. 103(12), e561 (2018).

[66] Torraco A., Bianchi M., Verrigni D., Gelmetti V., Riley L., Niceta M., Martinelli D., Montanari A., Guo Y., Rizza T., Diodato D., Di Nottia M., Lucarelli B., Sorrentino F., Piemonte F., Francisci S., Tartaglia M., Valente EM., Dionisi-Vici C., Christodoulou J., Bertini E., and Carrozzo R., A novel mutation in NDUFB11 unveils a new clinical phenotype associated with lactic acidosis and sideroblastic anemia, Clin. Genet. 91(3), 441 (2017).

[67] D'Hooghe M., Selleslag D., Mortier G., Van Coster R., Vermeersch P., Billiet J., and Bekri S., X-linked sideroblastic anemia and ataxia: a new family with identification of a fourth ABCB7 gene mutation, Eur. J. Paediatr. Neurol. 16(6), 730 (2012).

[68] Mozzillo E., Melis D., Falco M., Fattorusso V., Taurisano R., Flanagan SE., Ellard S., and Franzese A., Thiamine responsive megaloblastic anemia: a novel SLC19A2 compound heterozygous mutation in two siblings, Pediatr. Diabetes. 14(5), 384 (2013).

[69] An W., Zhang J., Chang L., Zhang Y., Wan Y., Ren Y., Niu D., Wu J., Zhu X., and Guo Y., Mutation analysis of Chinese sporadic congenital sideroblastic anemia by targeted

capture sequencing, J. Hematol. Oncol. 8, 55 (2015).

[70] Harigae H., Nakajima O., Suwabe N., Yokoyama H., Furuyama K., Sasaki T., Kaku M., Yamamoto M., and Sassa S., Aberrant iron accumulation and oxidized status of erythroid-specific delta-aminolevulinate synthase (ALA S2)-deficient definitive erythroblasts, Blood. 101(3), 1188 (2003).

[71] Nakajima O., Takahashi S., Harigae H., Furuyama K., Hayashi N., Sassa S., and Yamamoto M., Heme deficiency in erythroid lineage causes differentiation arrest and cytoplasmic iron overload, EMBO J. 18(22), 6282 (1999).

[72] Hatta S., Fujiwara T., Yamamoto T., Saito K., Kamata M., Tamai Y., Kawamata S., and Harigae H., A defined culture method enabling the establishment of ring sideroblasts from induced pluripotent cells of X-linked sideroblastic anemia, Haematologica. 103(5), e188 (2018).

[73] Saito K., Fujiwara T., Hatta S., Morita M., Ono K., Suzuki C., Fukuhara N., Onishi Y., Nakamura Y., Kawamata S., Shimizu R., Yamamoto M., and Harigae H., Generation and Molecular Characterization of Human Ring Sideroblasts: A Key Role of Ferrous Iron in Terminal Erythroid Differentiation and Ring Sideroblast Formation, Mol. Cell. Biol. (2019).

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