

Congenital Sideroblastic Anemia

Panel Gene List: ABCB7; ALAS2; GLRX5; PUS1; SLC19A2; SLC25A38; TRNT1; YARS2

Disorders Included: Sideroblastic anemia with spinocerebellar ataxia; X-linked sideroblastic anemia; Autosomal recessive pyridoxine-refractory sideroblastic anemia; Mitochondrial myopathy and sideroblastic anemia 1 (MLASA1); Thiamine-responsive megaloblastic anemia syndrome (TRMA); Autosomal recessive pyridoxine-refractory sideroblastic anemia; Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay; Myopathy, lactic acidosis, and sideroblastic anemia 2 (MLASA2); Pearson marrow-pancreas syndrome/Sideroblastic anemia with marrow cell vacuolization and exocrine pancreas dysfunction

Clinical Features and Inheritance Pattern/Genetics:

The congenital sideroblastic anemias (CSAs) are a group of heterogeneous bone marrow disorders characterized by the accumulation of iron deposits in the mitochondria of erythroid precursor cells (sideroblasts). Iron is deposited in the mitochondria as a result of the bone marrow's failure to properly incorporate iron into hemoglobin. Sideroblasts are abnormal nucleated erythroid precursor cells characterized by visible mitochondria with iron deposits surrounding the nucleus.¹⁻³

Sideroblastic anemia with spinocerebellar ataxia (ABCB7): Sideroblastic anemia with spinocerebellar ataxia is an X-linked syndromic form of CSA caused by pathogenic variants in the ABCB7 gene. It typically presents in early childhood with mild to moderate microcytic anemia and neurological defects including motor delay, non-progressive ataxia and incoordination, and cerebellar hypoplasia/atrophy. In this form of CSA, there is no evidence of iron storage in the organs in adulthood. Females are generally unaffected clinically, but may have detectable signs of CSA on hematological studies.^{1,2,4}

X-linked sideroblastic anemia (ALAS2): X-linked sideroblastic anemia is the most common type of CSA and is caused by pathogenic variants in the ALAS2 gene. Age of onset ranges from prenatal to the ninth decade of life, and affected individuals usually present with hypochromic microcytic anemia, ringed sideroblasts in bone marrow, and eventual systemic iron overload. Clinical symptoms are the result of iron overload and reduced hemoglobin levels, and typically include paleness, fatigue, dizziness, and hepatosplenomegaly. Males are more commonly affected than females, but some females present with clinically severe anemia. It is worth noting that all affected individuals within a kindred are typically of the same gender.^{1,2,5}

Autosomal recessive pyridoxine-refractory sideroblastic anemia (*SLC25A38*, *GLRX5*):

Autosomal recessive pyridoxine-refractory sideroblastic anemia is caused by pathogenic variants in the *SLC25A38* or the *GLRX5* gene. Affected individuals typically present in early childhood with severe microcytic hypochromic anemia that is resistant to pyridoxine treatment. Systemic iron overload and hepatosplenomegaly occur relatively early.^{1,2,6-8} Mitochondrial myopathy and sideroblastic anemia 1 (*PUS1*): Mitochondrial myopathy and sideroblastic anemia 1 (MLASA1) is an autosomal recessive syndromic form of CSA caused by pathogenic variants in the *PUS1* gene. It typically presents in childhood with muscle weakness, lactic acidosis, and normocytic anemia.^{1,2,9}

Thiamine-responsive megaloblastic anemia syndrome (*SLC19A2*): Thiamine-responsive megaloblastic anemia syndrome (TRMA) is an autosomal recessive syndromic form of CSA caused by pathogenic variants in the *SLC19A2* gene. It is characterized by the unusual CSA finding of megaloblastic anemia along with diabetes mellitus and sensorineural deafness, with onset ranging from infancy to adolescence. High doses of thiamine are effective in treating the anemia and improves diabetes in some cases.^{1,2,10}

Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay (*TRNT1*): Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD) is an autosomal recessive syndromic form of CSA caused by pathogenic variants in the *TRNT1* gene. Affected individuals present in infancy with severe microcytic anemia, followed by developmental delay, neurodegeneration, and recurrent idiopathic periodic fevers in childhood. B-cell immunodeficiency can be seen upon immunological workup. Other variable features include seizures, cerebellar abnormalities, sensorineural deafness, retinitis pigmentosa, and cardiomyopathy. Death may occur within the first decade of life.^{1,11,12}

Myopathy, lactic acidosis, and sideroblastic anemia 2 (*YARS2*): Mitochondrial myopathy and sideroblastic anemia 2 (MLASA2) is an autosomal recessive syndromic form of CSA caused by pathogenic variants in the *YARS2* gene. This form of CSA is highly variable in onset, with some affected individuals presenting with multisystemic disease in infancy, and others presenting in the second to third decade of life with mild anemia and myopathy.^{1,13,14}

Pearson marrow-pancreas syndrome/Sideroblastic anemia with marrow cell vacuolization and exocrine pancreas dysfunction (*large mitochondrial genome deletions*): Pearson marrow-pancreas syndrome caused by large contiguous-gene deletions of the mitochondrial genome. It is characterized by sideroblastic anemia with vacuolization of erythroid precursor cells and pancreatic dysfunction, and is usually fatal in infancy. Affected individuals present with severe macrocytic anemia requiring blood transfusion and exocrine pancreatic insufficiency. In most cases, gross deletions of the mitochondrial genome associated with Pearson syndrome arise de novo and are detectable in blood.^{1,15}

Test Methods:

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. The DNA is enriched for the complete coding

regions and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons at the exon-level; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request. Whole mitochondrial genome amplification using two separate pairs of primers, each amplifying the entire mitochondrial genome, followed by massively parallel sequencing is used to detect large-scale mitochondrial genome deletions. The deletions identified are confirmed using both mitochondrial genome array CGH and junction PCR followed by capillary sequencing. The level of the deletion heteroplasmy is determined by mitochondrial genome array CGH.

Clinical Sensitivity:

Sideroblastic anemia with spinocerebellar ataxia (ABCB7): Currently only four unrelated families have been reported with sideroblastic anemia with spinocerebellar ataxia, each with a distinct missense variant in ABCB7. Because of the relatively mild presentation of this form of anemia in combination with a more severe ataxia, this condition may go undiagnosed in many cases.^{1,16-18}

X-linked sideroblastic anemia (ALAS2): To date, missense variants clustering within the catalytic domain (exons 5-11) account for the majority of pathogenic variants identified in ALAS2. Nonsense, splicing variants, and small deletions/insertions have been reported but are rare. No gross deletions or insertions of one or more exons have been reported. It is estimated that pathogenic missense variants in the ALAS2 gene constituted 23-37% of all the patients with CSAs.^{1,2,19-23}

Autosomal recessive pyridoxine-refractory sideroblastic anemia (SLC25A38, GLRX5):

The largest cohort study to date suggests that approximately 18% of cases of CSA may be due to variants in this gene. Almost all reported variants are missense variants, although nonsense and splicing variants and small deletions and insertions have been reported. One gross deletion of one or more exons of SLC25A38 have been reported. Currently only two individuals have been reported with GLRX5-related autosomal recessive sideroblastic anemia; one had a homozygous splicing variant, and the other had compound heterozygous missense variants in GLRX5.^{6,8,22-24}

Mitochondrial myopathy and sideroblastic anemia 1 (PUS1): Currently only six unrelated families have been reported with mitochondrial myopathy and sideroblastic anemia 1. The majority of cases are due to homozygous missense variants in PUS1, with a common Arg116Trp variant being present in over half of the reported cases. A homozygous nonsense variant was identified in two brothers born to distantly related parents. An unrelated individual was found to be compound heterozygous for a frameshift deletion and a missense variant.^{9,25,26}

Thiamine-responsive megaloblastic anemia syndrome (SLC19A2): Nearly 100% of individuals with the phenotypic triad associated with TRMA (megaloblastic anemia, progressive sensorineural deafness, and diabetes) have two identifiable variants in the SLC19A2 gene. The majority of variants in this gene are sequence variants, but one gross deletion has been reported.²⁷⁻²⁹

Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay (TRNT1): In 13 families with sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD), Chakraborty et al. identified homozygous or compound heterozygous variants in TRNT1 in 15 affected individuals. To date, variants reported in this gene are primarily missense, splicing, and frameshift variants.^{11,22}

Myopathy, lactic acidosis, and sideroblastic anemia 2 (YARS2): At this time, very few variants in the YARS2 gene have been reported, the majority of which are missense or nonsense variants.^{1,13,14,30}

Pearson marrow-pancreas syndrome/Sideroblastic anemia with marrow cell vacuolization and exocrine pancreas dysfunction (large mitochondrial genome deletions): Approximately 90% of individuals with Pearson syndrome have a large-scale (2-10 kb) mtDNA deletion.¹⁵

Overall, without GLRX5 and TRNT1, the positive rate of this panel for individuals with CSA is expected to be ~57%.² With these two genes included, the positive rate is expected to be ~67%.²³

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