Congenital Sideroblastic Anemia

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

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

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 hepatospenomegaly. 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:
Using genomic DNA obtained from a blood specimen, the coding regions and splice junctions of the eight genes are enriched using a proprietary targeted capture system developed by GeneDx. These targeted regions are sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with paired-end reads. Bi-directional sequence is assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequencing is used to obtain sequence for regions where fewer than 15 reads were achieved by NextGen sequencing. The presence of any disease-associated sequence variant is confirmed by conventional dideoxy sequence analysis or other methods. Concurrent deletion/duplication testing is performed for the genes in the panel using exon-level oligo array CGH (ExonArrayDx). Data analysis is performed using gene-specific filtering. Probe sequences and locations are based on human genome build GRCh37/UCSC hg19. Confirmation of copy number changes is performed by MLPA, qPCR, or repeat array CGH analysis. Sequence and array CGH alterations are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. 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.

Test 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 37% of all the patients with CSAs. [1,2,19-22]

Autosomal recessive pyridoxine-refractory sideroblastic anemia (SLC25A38, GLRX5): The largest cohort study to date suggests that approximately 17% 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. No gross deletions or insertions 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.[6,8,22,23]

Mitochondrial myopathy and sideroblastic anemia 1 (PUS1): Currently only three unrelated families have been reported with mitochondrial myopathy and sideroblastic anemia 1. A homozygous missense variant in PUS1 that appears to affect the catalytic domain of the protein has been identified in two families with MLASA1, and a homozygous nonsense variant was identified in two brothers born to distantly related parents. [9,24]

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.[25-27]

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, all of which are missense or nonsense variants.[1,13,14,28]

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 ~60%.

References:

1. OMIM, Online Mendelian Inheritance in Man, (TM). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), http://www.ncbi.nlm.nih.gov/omim/
2. Bergmann AK et al. Systematic molecular genetic analysis of congenital sideroblastic anemia: evidence for genetic heterogeneity and identification of novel mutations. Pediatric Blood & Cancer. 2010 Feb 54(2):273-8. (PMID:19731322)
3. Ohba R et al. Clinical and genetic characteristics of congenital sideroblastic anemia: comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS). Annals Of Hematology. 2013 Jan 92(1):1-9. (PMID:22983749)
4. Bekri S, D'Hooghe M, Vermeersch P. X-Linked Sideroblastic Anemia and Ataxia. 2006 Mar 1 [Updated 2014 Apr 3]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1321/
5. Aivado M et al. X-linked sideroblastic anemia associated with a novel ALAS2 mutation and unfortunate skewed X-chromosome inactivation patterns. Blood Cells, Molecules & Diseases. 37(1):40-5. (PMID: 16735131)
6. Guernsey DL et al. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. Nature Genetics. 2009 Jun 41(6):651-3. (PMID: 19412178)
7. Rouault TA and Tong WH. Iron-sulfur cluster biogenesis and human disease. Trends In Genetics : Tig. 2008 Aug 24(8):398-407. (PMID:18606475)
8. Ye H et al. Glutaredoxin 5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts. The Journal Of Clinical Investigation. 2010 May 120(5):1749-61. (PMID:20364084)
9. Bykhovskaya Y et al. Missense mutation in pseudouridine synthase 1 (PUS1) causes mitochondrial myopathy and sideroblastic anemia (MLASA). American Journal Of Human Genetics. 2004 Jun 74(6):1303-8. (PMID: 15108122)
10. Bergmann AK et al. Thiamine-responsive megaloblastic anemia: identification of novel compound heterozygotes and mutation update. The Journal Of Pediatrics. 2009 Dec 155(6):888-892. (PMID: 19643445)
11. Chakraborty PK et al. Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD). Blood. 2014 Oct 30 124(18):2867-71. (PMID: 25193871)
12. Wiseman DH et al. A novel syndrome of congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD). Blood. 2013 Jul 4 122(1):112-23. (PMID: 23553769)
13. Riley LG et al. Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia--MLASA syndrome. American Journal Of Human Genetics. 2010 87(1):52-9. (PMID: 20598274)
14. Riley LG et al. Phenotypic variability and identification of novel YARS2 mutations in YARS2 mitochondrial myopathy, lactic acidosis and sideroblastic anaemia. Orphanet Journal Of Rare Diseases. 2013 8:193. (PMID: 24344687)
15. DiMauro S, Hirano M. Mitochondrial DNA Deletion Syndromes. 2003 Dec 17 [Updated 2011 May 3]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1203/
16. Bekri S et al. Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron-sulfur protein maturation. Blood. 2000 96(9):3256-64. (PMID: 11050011)
17. D'Hooghe M et al. X-linked sideroblastic anemia and ataxia: a new family with identification of a fourth ABCB7 gene mutation. European Journal Of Paediatric Neurology : Ejpn : Official Journal Of The European Paediatric Neurology Society. 2012 16(6):730-5. (PMID: 22398176)
18. Maguire A et al. X-linked cerebellar ataxia and sideroblastic anaemia associated with a missense mutation in the ABC7 gene predicting V411L. British Journal Of Haematology. 2001 Dec 115(4):910-7. (PMID: 11843825)
19. Bishop DF et al. X-linked sideroblastic anemia due to carboxyl-terminal ALAS2 mutations that cause loss of binding to the β-subunit of succinyl-CoA synthetase (SUCLA2). The Journal Of Biological Chemistry. 2012 287(34):28943-55. (PMID:22740690)
20. Campagna DR et al. X-linked sideroblastic anemia due to ALAS2 intron 1 enhancer element GATA-binding site mutations. American Journal Of Hematology. 2014 Mar 89(3):315-9. (PMID:24166784)
21. Kaneko K et al. 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. 2014 Feb 99(2):252-61. (PMID:23935018)
22. Stenson et al. (2014) The Human Gene Mutation Database (HGMD®) Human genetics 133(1):1-9 (PMID: 24077912)
23. Kannengiesser C et al. Missense SLC25A38 variations play an important role in autosomal recessive inherited sideroblastic anemia. Haematologica. 2011 Jun 96(6):808-13. (PMID:21393332)
24. Fernandez-Vizarra E et al. Nonsense mutation in pseudouridylate synthase 1 (PUS1) in two brothers affected by myopathy, lactic acidosis and sideroblastic anaemia (MLASA). Journal Of Medical Genetics. 2007 Mar 44(3):173-80. (PMID: 17056637)
25. Oishi K, Diaz GA. Thiamine-Responsive Megaloblastic Anemia Syndrome. 2003 Oct 24 [Updated 2014 Nov 20]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1282/
26. Labay V et al. Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. Nature Genetics. 1999 Jul 22(3):300-4. (PMID: 10391221)
27. Scharfe C et al. A novel mutation in the thiamine responsive megaloblastic anaemia gene SLC19A2 in a patient with deficiency of respiratory chain complex I. Journal Of Medical Genetics. 2000 Sep 37(9):669-73. (PMID: 10978358)

28. Shahni R et al. A distinct mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA) phenotype associates with YARS2 mutations. American Journal Of Medical Genetics. Part A. 2013 161(9):2334-8. (PMID:23918765)