THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) is characterized by severe neutropenia at birth [1]. Bone marrow exhibits arrest of neutrophil maturation at the promyelocyte or myelocyte stage of development [1]. By age 6 months, 90% of patients with SCN develop bacterial infections such as skin or deep tissue abscesses, oral ulcers and pneumonia [1]. Despite improvements in therapy there remains a 12% risk of death due to sepsis by age 15 years [1]. Patients with SCN also have an increased risk of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), with a hazard rate of 2% per year [1].

Our Severe Congenital Neutropenia Sequencing Panel includes sequence analysis of all 8 genes listed below. Our Severe Congenital Neutropenia Deletion/Duplication Panel includes deletion/duplication analysis of 7 genes listed in bold below.

Severe Congenital Neutropenia Sequencing Panel				
CSF3R	CXCR4	**ELANE (ELA2)**	**G6PC3**	**GFI1**
HAX1	**VPS45**	**WAS**		

Gene	Inheritance	Clinical Features
CSF3R	Recessive	Biallelic loss-of-function mutations in *CSF3R* have been described in patients with SCN [2]. Plo *et al.* (2009) identified a heterozygous activating mutation in *CSF3R* in a family with dominantly inherited chronic neutropenia [3]. One affected family member also developed MDS.
CXCR4	Dominant	Heterozygous mutations in the *CXCR4* gene WHIM syndrome is an immunodeficiency disease characterized by neutropenia, hypogammaglobulinemia, and extensive human papillomavirus (HPV) infection [4, 5].
ELANE (ELA2)	Dominant	Heterozygous mutations in the *ELANE* gene are responsible for the majority of cases of SCN [6]. *ELANE* can also be associated with cyclic neutropenia [6]. To clear phenotype-genotype correlations exist, and there is significant overlap between predicted severity of the mutation and the clinical phenotype [6].
G6PC3	Recessive	Biallelic mutations in *G6PC3* have been associated with SCN type 4 [7]. Patients with *G6PC3* deficiency commonly present with congenital anomalies including cardiac anomalies, urogenital malformations and venous angietasia [7]. Alangari *et al.* (2013) described a consanguineous family where affected individuals presented with either SCN or cyclic neutropenia [7].
GFI1	Dominant	Dominant-negative mutations in *GFI1* have been associated with SCN [8]. *GFI1* mutations have also been identified in patients with nonimmune chronic idiopathic neutropenia of adults [9].
HAX1	Recessive	Biallelic mutations in *HAX1* account for 15% of cases of SCN [8]. A proportion of patients with *HAX1*-associated SCN also develop neurological disease such as cognitive impairment, developmental delay, and epilepsy [8].
VPS45	Recessive	Stepensky *et al.* (2013) identified homozygous mutations in *VPS45* in patients with SCN [10]. Affected individuals developed neutropenia, thrombasthenia, myelofibrosis and progressive bone marrow failure [10].
WAS	X-linked	Activating mutations in the X-linked *WAS* gene are associated with SCN and lmyphopenia [8]. Loss of function mutations in *WAS* have been associated with Wiskott-Aldrich syndrome, associated with immunodeficiency, eczema, microthromobocytopenia, and susceptibility to malignant lymphoma [8].

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and

rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Severe Congenital Neutropenia Sequencing Panel (sequence analysis of 8 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube. NOTE: blood samples are not accepted if patient has a history of MDS or leukemia. Please send 2 T-25 flasks of cultured skin fibroblasts instead.
Cost:	$2000
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the Severe Congenital Neutropenia Sequencing panel

Severe Congenital Neutropenia Deletion/Duplication Panel (deletion/duplication analysis of 7 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube. NOTE: blood samples are not accepted if patient has a history of MDS or leukemia. Please send 2 T-25 flasks of cultured skin fibroblasts instead.
Cost:	$1545
CPT codes:	81406
Turn-around time:	4-6 weeks

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire Fanconi Anemia Sequencing Panel. All abnormal results are reported by telephone or email.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Wilson, D.B., et al., *Inherited bone marrow failure syndromes in adolescents and young adults.* Ann Med, 2014: p. 1-11.
2. Triot, A., et al., *Inherited biallelic CSF3R mutations in severe congenital neutropenia.* Blood, 2014.
3. Plo, I., et al., *An activating mutation in the CSF3R gene induces a hereditary chronic neutrophilia.* J Exp Med, 2009. **206**(8): p. 1701-7.
4. Hernandez, P.A., et al., *Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease.* Nat Genet, 2003. **34**(1): p. 70-4.
5. Balabanian, K., et al., *WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12.* Blood, 2005. **105**(6): p. 2449-57.
6. Germeshausen, M., et al., *The spectrum of ELANE mutations and their implications in severe congenital and cyclic neutropenia.* Hum Mutat, 2013. **34**(6): p. 905-14.
7. Alangari, A.A., et al., *A novel homozygous mutation in G6PC3 presenting as cyclic neutropenia and severe congenital neutropenia in the same family.* J Clin Immunol, 2013. **33**(8): p. 1403-6.
8. Boztug, K. and C. Klein, *Genetics and pathophysiology of severe congenital neutropenia syndromes unrelated to neutrophil elastase.* Hematol Oncol Clin North Am, 2013. **27**(1): p. 43-60, vii.
9. Person, R.E., et al., *Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2.* Nat Genet, 2003. **34**(3): p. 308-12.
10. Stepensky, P., et al., *The Thr224Asn mutation in the VPS45 gene is associated with the congenital neutropenia and primary myelofibrosis of infancy.* Blood, 2013. **121**(25): p. 5078-87.

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