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.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF3R</td>
<td>Recessive</td>
<td>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.</td>
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<tr>
<td>CXCR4</td>
<td>Dominant</td>
<td>Heterozygous mutations in the CXCR4 gene WHIM syndrome is an immunodeficiency disease characterized by neutropenia, hypogammaglobulinemia, and extensive human papillomavirus (HPV) infection [4, 5].</td>
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<tr>
<td>ELANE (ELA2)</td>
<td>Dominant</td>
<td>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].</td>
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<tr>
<td>G6PC3</td>
<td>Recessive</td>
<td>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 angiabras [7]. Alangari et al. (2013) described a consanguineous family where affected individuals presented with either SCN or cyclic neutropenia [7].</td>
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<tr>
<td>GFI1</td>
<td>Dominant</td>
<td>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].</td>
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<tr>
<td>HAX1</td>
<td>Recessive</td>
<td>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].</td>
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<tr>
<td>VPS45</td>
<td>Recessive</td>
<td>Stepensky et al. (2013) identified homozygous mutations in VPS45 in patients with SCN [10]. Affected individuals developed neutropenia, thrombocytopenia, myelofibrosis and progressive bone marrow failure [10].</td>
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<tr>
<td>WAS</td>
<td>X-linked</td>
<td>Activating mutations in the X-linked WAS gene are associated with SCN and lymphopenia [8]. Loss of function mutations in WAS have been associated with Wiskott-Aldrich syndrome, associated with immunodeficiency, eczema, microthrombocytopenia, and susceptibility to malignant lymphoma [8].</td>
</tr>
</tbody>
</table>

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 to 10 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 to 10 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:

Committed to CUSTOMIZED DIAGNOSTICS, TRANSLATIONAL RESEARCH & YOUR PATIENTS’ NEEDS