Congenital Neutropenia Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 35 genes known to cause congenital neutropenia, including cyclic neutropenia, non-syndromic neutropenia, and syndromic neutropenia with non-hematological manifestations. This panel can be ordered as:

- **Next-generation sequencing (NGS) only**
- **NGS with reflex to array comparative genomic hybridization (aCGH) deletion/duplication analysis if clinically significant variants explaining the patient's phenotype are not detected by sequencing**
- **NGS with concurrent aCGH deletion/duplication analysis (both testing methodologies performed simultaneously) OR**
- **Deletion/duplication analysis by aCGH only**

Additional Description

Congenital neutropenia is a heterogeneous group of disorders characterized by reduction in the absolute neutrophil count (ANC) of variable severity with or without extra-hematologic (syndromic) manifestations. When severe, it can be associated with recurrent infections, fever, and inflammation of the skin and mucous membranes. Some of the disorders in this group, particularly those that lead to severe congenital neutropenia (SCN), have a predisposition to myelodysplastic syndrome and acute myeloid leukemia (AML).

Diagnosis is based on clinical findings and serial measurement of the ANC showing persistently decreased neutrophil counts or variable neutrophil counts with severe neutropenia presenting with a predictable periodicity (cyclic neutropenia). Diagnosis of a specific congenital neutropenia disorder may be difficult to establish based solely on functional studies or clinical history, and many of the known causes of congenital neutropenia do not have a distinct clinical or laboratory phenotype. Accurate diagnosis provides information about phenotype and prognosis, guides medical management decisions, assists with the identification of affected family members and allows for accurate genetic recurrence risk assessment.

Variants in many genes are known to cause syndromic or non-syndromic congenital neutropenia, which may be inherited in an autosomal dominant, autosomal recessive or X-linked manner. Some of the most common and more thoroughly characterized causes of congenital neutropenia are *ELANE*-related neutropenia (autosomal dominant inheritance) and *HAX1*-related neutropenia (autosomal recessive inheritance). Cyclic neutropenia is typically inherited in an autosomal dominant manner caused by heterozygous pathogenic variants in *ELANE*. Additional genes in this panel are associated with isolated neutropenia or congenital syndromes that have neutropenia as a common finding among other non-hematologic features.

The NGS panel evaluates for single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease. However, large deletions and duplications, also referred to as copy number variations (CNVs), are a known cause of genetic disorders, but can escape detection by next-generation sequence analysis. Additional testing with aCGH deletion/duplication analysis is available for all genes on this panel to evaluate for large deletions and duplications encompassing one or more exons, or affecting an entire gene.

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Congenital Neutropenia Panel: gene, clinical phenotype and inheritance pattern.		
Gene	Clinical Phenotype	Inheritance
AK2	**Reticular dysgenesis (Adenylate kinase-2 deficiency):** severe combined immunodeficiency characterized by severe neutropenia, lymphopenia and sensorineural hearing loss.	Autosomal Recessive
AP3B1	**Hermansky-Pudlak syndrome type 2 (HPS2):** oculocutaneous albinism, mild bleeding due to platelet storage pool disorder, neutropenia and pulmonary fibrosis.	Autosomal Recessive
AP3D1	**Hermansky-Pudlak syndrome type 10 (HPS10):** oculocutaneous albinism, mild bleeding due to platelet storage pool disorder, neutropenia, seizures, and developmental delay.	Autosomal Recessive
BTK	**X-linked agammaglobulinemia**: primary B cell immunodeficiency characterized by severe recurrent bacterial infections, small/absent tonsils and adenoids, near total absence of all isotypes of immunoglobulins, and in some cases, neutropenia.	X-linked
CLPB	**Autosomal Recessive CLPB deficiency (3-methylglutaconic aciduria type 7):** syndromic metabolic disorder characterized by elevated urinary 3-methylglutaconic acid and otherwise highly variable phenotype, including varying degrees of neurological involvement, neutropenia and cataracts.	Autosomal Recessive
	Autosomal dominant CLPB deficiency: highly variable disorder typically characterized by severe congenital neutropenia with or without mild-to-severe syndromic manifestations, including elevated urinary 3-methylglutaconic acid and/or varying degrees of neurological involvement.	Autosomal Dominant
*CSF3R**	***CSF3R*-related congenital neutropenia (severe congenital neutropenia 7):** severe neutropenia that does not respond to granulocyte-colony stimulating factor (GCSF).	Autosomal Recessive
CXCR2	***CXCR2*-related disorder:** chronic and congenital neutropenia, recurrent infections, and myelokathexis.	Autosomal Recessive
*CXCR4**	**WHIM syndrome:** neutropenia, lymphopenia, warts, hypogammaglobulinemia, infections and myelokathexis.	Autosomal Dominant
EFL1	**Shwachman-Diamond syndrome:** highly variable syndromic disorder which can include exocrine pancreatic dysfunction; bony metaphyseal dysostosis; varying degrees of marrow dysfunction with cytopenias, including neutropenia, which can often be the presenting feature; and an increased risk of myeloid neoplasms.	Autosomal Recessive
ELANE	***ELANE*-related neutropenia:** severe congenital neutropenia or cyclic neutropenia with an increased risk of myeloid neoplasms.	Autosomal Dominant
G6PC3	***G6PC3* deficiency:** severe congenital neutropenia with or without syndromic hematologic and extra-hematologic associations, such as intermittent thrombocytopenia, increased superficial venous marking, cardiovascular defects and/or urogenital anomalies.	Autosomal Recessive
GATA1	***GATA1*-related cytopenia:** thrombocytopenia and/or anemia with moderate bleeding due to platelet alpha granule deficiency; mild beta-thalassemia, neutropenia and congenital erythropoietic porphyria, which can be seen with hemizygous variants; and mild-to-moderate symptoms with heterozygous variants.	X-linked
GATA2	**GATA2 deficiency:** significant intra- and interfamilial phenotypic variability with features including a severe deficiency of B lymphocytes, NK cells, and monocytes; mild chronic neutropenia; recurring life-threatening opportunistic infections; viral warts; hearing loss; lymphedema; pulmonary disease; and progression to myeloid neoplasms.	Autosomal Dominant
GFI1	***GFI1*-related neutropenia:** non-syndromic severe neutropenia.	Autosomal Dominant
GINS1	***GINS1*-related immunodeficiency:** intrauterine growth restriction, chronic neutropenia and NK cell deficiency.	Autosomal Recessive
HAX1	***HAX1*-related neutropenia:** severe neutropenia and increased risk of myeloid neoplasms, with or without extra-hematologic associations, including neurocognitive impairment of variable severity and hypogonadism.	Autosomal Recessive
JAGN1	***JAGN1*-related neutropenia:** severe congenital neutropenia with or without extra-hematologic manifestations.	Autosomal Recessive
LYST	**Chediak-Higashi syndrome:** partial oculocutaneous albinism, immunodeficiency, neutropenia and a mild bleeding tendency due to platelet delta granule deficiency.	Autosomal Recessive
RAC2	***RAC2*-related neutrophil dysfunction (neutrophil immunodeficiency syndrome):** neutrophil dysfunction characterized by bacterial infections and impaired wound healing.	Autosomal Dominant
SBDS	**Shwachman-Diamond syndrome:** syndromic disorder characterized by variable features that can include exocrine pancreatic dysfunction; bony metaphyseal dysostosis; varying degrees of marrow dysfunction with cytopenias, including neutropenia and an increased risk of myeloid neoplasms.	Autosomal Recessive
SLC37A4	**Glycogen storage disease type Ib:** metabolic disorder due to inadequate conversion of glucose-6-phosphate into glucose, characterized by growth retardation, hepatomegaly, renomegaly, hypoglycemia, and other metabolic abnormalities, neutropenia and neutrophil dysfunction .	Autosomal Recessive
SMARCD2	**SMARCD2 deficiency:** specific granule deficiency, neutropenia, recurrent infections and dysplastic myelopoiesis.	Autosomal Recessive
SRP19 *SRP54* *SRP68* *SRP72* *SRPRA*	***SRP*-related neutropenia:** severe congenital neutropenia characterized by low neutrophil counts and recurrent infections, with or without features of Shwachman-Diamond syndrome.	Autosomal Recessive/ Autosomal Dominant

TAFAZZIN	**Barth syndrome:** cardiomyopathy, short stature, severe neutropenia, hypocholesterolemia, impaired cognition, mild dysmorphic features and mitochondrial dysfunction.	X-linked
TCIRG1	***TCIRG1*-related neutropenia:** non-syndromic neutropenia of variable severity. Pathogenic variants in *TCIRG1* are also associated with autosomal recessive osteopetrosis.	Autosomal Dominant
USB1	**Poikiloderma with neutropenia:** chronic neutropenia, skin and nail manifestations, sinopulmonary infections and bronchiectasis, short stature, hypogonadism, dysmorphism, and an increased risk for myeloid neoplasms.	Autosomal Recessive
VPS13B	**Cohen syndrome:** variable developmental delay, microcephaly, facial dysmorphism, joint hypermobility, retinopathy and neutropenia.	Autosomal Recessive
VPS45	***VPS45*-related neutropenia:** severe neutropenia with neutrophil dysfunction, bone marrow fibrosis and nephromegaly from renal extramedullary hematopoiesis.	Autosomal Recessive
WAS	***WAS*-related disorders:** spectrum of disorders including Wiskott-Aldrich syndrome, characterized by microthrombocytopenia, eczema, recurrent infections, thrombocytopenia and neutropenia.	X-linked
WDR1	**WDR1 deficiency:** abnormal neutrophil morphology and function, mild neutropenia, and recurrent bacterial infections.	Autosomal Recessive
WIPF1	**Wiskott-Aldrich syndrome type 2:** recurrent infections, eczema, thrombocytopenia, defective T cell proliferation and impaired natural killer cell function.	Autosomal Recessive

* Somatic variants in *CSF3R* are associated with chronic neutrophilic leukemia (CNL) and other myeloid neoplasms. Somatic variants in *CXCR4* are associated with Waldenström Macroglobulinemia (WM). Please note that this assay is not designed for detection of somatic variants. See Assay Sensitivity and Limitations below.

Indications for Testing

Congenital Neutropenia Panel (NGS and/or aCGH), order code 4845:

The Congenital Neutropenia Panel should be considered in patients:

- With chronic/lifelong neutropenia presenting with or without extra-hematologic manifestations
- With a family history of unspecified congenital neutropenia when an affected relative is not available for genetic testing

Single Gene Analysis (order code 4855) or Custom Blood Disorder Panel (Order Code 4850), NGS and/or aCGH:

Analysis of genes included in this panel may also be ordered as a standalone Single Gene Analysis or as a Custom Blood Disorder Panel (2-10 genes), by NGS and/or by aCGH, as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970):

Targeted variant analysis for clinical diagnosis, carrier identification, or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant(s) is known in the family. If the proband was not tested at Versiti, a control sample may be needed (please call the laboratory to discuss). If the familial variant is a large deletion or duplication, aCGH for the involved gene is required.

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: 414-937-6396 or 800-245-3117, Option 1, to be directed to our genetic counselors and clinical support team.

Informed Consent

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of and understands the indications for and implications of the genetic test. This panel is designed for clinical detection of germline genetic variants in genes with strong or definitive evidence for causality of neutropenia or neutrophil dysfunction. Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, and/or may carry individual or familial implications such as risk for syndromic manifestation, predisposition to malignancy, and/or reproductive implications (such as carrier status). If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under Forms.

Test Method

NGS: This next-generation sequencing assay analyzes the complete coding region of 35 genes, plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence. Analysis of *BTK* also includes c.240+109C>A. These targeted regions are captured by hybridization, amplified and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low-quality coverage are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic

counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results that are not affected by maternal cell contamination.

aCGH: The specific genes are analyzed for copy number variations due to deletions or duplications by high-density, gene-focused array Comparative Genomic Hybridization. Probes are approximately 60bp in length and density of coverage in exonic regions is a minimum of 4 probes per 500 bp. Genomic DNA for the samples and biological sex-matched references are denatured, labeled with fluorescent dye and hybridized; the array is washed and scanned, and analysis is performed for the specific genes requested.

Assay Sensitivity and Limitations

NGS: The analytical sensitivity of the NGS test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp) or variants that are outside the regions sequenced. Low-level mosaicism will not be detected by this sequencing methodology.

aCGH: Chromosomal rearrangements (i.e., translocations, inversions) or point mutations that may be the cause of the clinical phenotype cannot be detected via aCGH. Any exonic deletion or duplication smaller than 500bp may not be detected. Low-level mosaicism will not be detected by aCGH. Probe performance could be affected by multiple SNPs in a given region. Breakpoints occurring outside the targeted gene(s) will not be defined.

Clinical Sensitivity

The clinical sensitivity of comprehensive genetic testing (NGS and aCGH) of the 35 genes known to be associated with congenital neutropenia is highest in patients presenting with lifelong neutropenia or a clear cyclic pattern.

Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards. Sequence variants and large deletions and duplications predicted to be pathogenic, likely pathogenic, or of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

Specimen Requirements

Parental/Patient/Pediatric: 3-5 mL whole blood (EDTA tube, lavender top), 2-5 mL bone marrow (EDTA tube, lavender top), 3-4 buccal swabs, or ≥1ug of DNA at ≥50ng/uL of high-quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; backup culture of amniocytes or chorionic villi is highly recommended. Cultured: two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions, please contact the laboratory to discuss sample requirements.

SHIP

Shipping Requirements

Ship on an ice pack or at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
638 N. 18th St
Milwaukee, WI 53233

ORDER

Required Forms

Please complete all pages of the requisition form. Clinical history (including patient-reported ancestry, clinical diagnosis, family history, and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround Time

Test code: 4845

CPT codes: For recommended CPT codes, visit versiti.org/test-catalog.

Turnaround time: NGS only, aCGH only, or NGS and aCGH concurrently: 21 days

NGS reflex to aCGH: 21 days (if NGS only, aCGH not needed) or 42 days (with reflex to aCGH)

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact Versiti Client Services: 414-937-6396 or 800-245-3117, Option 1, or labinfo@versiti.org

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Congenital Neutropenia References:

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