Blueprint Genetics

Central Hypoventilation and Apnea Panel

Test code: PU0401

Is a 15 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical diagnosis or suspicion of central hypoventilation.

About Central Hypoventilation and Apnea

Idiopathic congenital central hypoventilation syndrome is characterized by abnormal control of respiration in the absence of neuromuscular, lung or cardiac disease, or an identifiable brainstem lesion. Patients breath normally while awake but hypoventilate with normal respiratory rates and shallow breathing during sleep. More severely affected patients hypoventilate both while they are awake and asleep. These patients typically present in the first hours of life with cyanosis and increased carbon dioxide during sleep. A deficiency in autonomic control of respiration results in inadequate or negligible ventilatory and arousal responses to hypercapnia and hypoxemia. Congenital central hypoventilation syndrome has been associated with several disorders classified as neurocristopathies, namely, aberrant phenotypes arising from a defect of migration or differentiation of neural crest cells. These include neuroblastoma, ganglioneuroma and most frequently Hirschsprung disease (HSCR) which appears in 16% of CCHS patients. The association of CCHS and HSCR is referred to as Haddad syndrome. The estimated prevalence of apnea of prematurity is 8.5/100,000 and neuroblastoma is 11/100,000 and the birth prevalence of Hirschsprung disease is 12.1/100,000.

Availability

4 weeks

Gene Set Description

Genes in the Central Hypoventilation and Apnea Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
CHAT	Myasthenic syndrome, congenital	AR	24	73
CHRNA1	Myasthenic syndrome, congenital	AD/AR	28	35
CHRNB1	Myasthenic syndrome	AD/AR	11	11
CHRND	Myasthenic syndrome	AD/AR	18	26
CHRNE	Myasthenic syndrome	AD/AR	48	134
COLQ	Myasthenic syndrome, congenital	AR	23	67
EDN3	Hirschsprung disease, Central hypoventilation syndrome, congenital, Waardenburg syndrome	AD/AR	7	21
GLRA1	Hyperekplexia	AD/AR	39	69
MECP2	Angelman-like syndrome, Autism, Rett syndrome, Encephalopathy, Mental retardation	XL	506	1039
PHOX2B	Central hypoventilation syndrome, congenital, Neuroblastoma, susceptiblity to, Neuroblastoma with Hirschsprung disease	AD	11	86
RAPSN	Myasthenic syndrome, congenital	AR	26	58

RET	Hirschsprung disease, Central hypoventilation syndrome, congenital, Pheochromocytoma, Medullary thyroid carcinoma, Multiple endocrine neoplasia	AD/AR	122	407
SCN4A	Hyperkalemic periodic paralysis, Myotonia, potassium-aggravated, Paramyotonia congenita, Myasthenic syndrome, congenital, Normokalemic potassium-sensitive periodic paralysis	AD/AR	57	126
SLC6A5	Hyperekplexia	AR	15	33
ZEB2*	Mowat-Wilson syndrome	AD	154	287

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Some, or all, of the gene is duplicated in the genome. Read more.

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The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#). Due to possible limitations these genes may not be available as single gene tests.

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
CHRNE	Chr17:4804936	c.501-16G>A	NM_000080.3	
CHRNE	Chr17:4806452	c.-94G>A	NM_000080.3	
CHRNE	Chr17:4806453	c.-95G>A	NM_000080.3	
CHRNE	Chr17:4806454	c.-96C>T	NM_000080.3	rs748144899
EDN3	Chr20:57875743	c.-125G>A	NM_000114.2	
EDN3	Chr20:57875849	c.-19C>A	NM_000114.2	rs375594972
RAPSN	Chr11:47469717	c.193-15C>A	NM_005055.4	
RAPSN	Chr11:47470715	c.-199C>G	NM_005055.4	
RAPSN	Chr11:47470726	c.-210A>G	NM_005055.4	rs786200905

RET	Chr10:43572670	c.-37G>C	NM_020975.4	rs751005619
RET	Chr10:43572680	c.-27C>G	NM_020975.4	
RET	Chr10:43582162	c.73+9385_73+9395delAGCAACTGCCA	NM_020975.4	rs368137511
RET	Chr10:43606948	c.1522+35C>T	NM_020975.4	rs377130948
RET	Chr10:43612192	c.2284+13C>T	NM_020975.4	
RET	Chr10:43612198	c.2284+19C>T	NM_020975.4	
RET	Chr10:43613947	c.2392+19T>C	NM_020975.4	rs778745375
ZEB2	Chr2:145274987	c.-69-1G>A	NM_014795.3	
ZEB2	Chr2:145274988	c.-69-2A>C	NM_014795.3	

Test Strengths

Up to 90% of the patients with congenital central hypoventilation syndrome (CCHS) are heterozygous for a *de novo* polyalanine repeat expansion mutations (PARMs) in the *PHOX2B* gene (PMID 16888290). Repeat expansions are generally difficult to detect via NGS assays and their clinical validation at large scale is impossible due to lack of publicly available control samples with abnormal repeat expansions. So far, we have been able to detect and confirm stretches of 28 alanines instead of normal amount of 20 (genotype “20/28”). However, we do not know exact sensitivity or detection limit of our assay for these alanine repeats.

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see ‘Non-coding disease causing variants covered by this panel’ in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

Genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions

- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section.

Test Performance

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

The performance metrics listed below are from an initial validation performed at our main laboratory in Finland. The performance metrics of our laboratory in Seattle, WA, are equivalent.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	99.2% (7,745/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA

2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Microdeletion/-duplication sdrs (large CNVs, n=37))		
Size range (0.1-47 Mb)	100% (25/25)	

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth	143X
Nucleotides with >20x sequencing coverage (%)	99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

	Sensitivity %	**Specificity %**
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2026 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%

Heteroplasmic (5-10%)	92.3% (12/13)	99.98%
Heteroplasmic (<5%)	88.9% (48/54)	99.93%
Insertions and deletions by sequence analysis n=40 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	99,997%
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%
Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the ‘Test limitations” section above. If the test includes the mitochondrial genome the target region gene list contains the mitochondrial genes. The sequencing data generated in our

laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases including, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen,MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with suboptimal coverage (<20X for nuclear genes and <1000X for mtDNA) if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the crucial details of the analysis process.

Clinical Interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the ACMG guideline 2015.

The final step in the analysis is orthogonal confirmation. Sequence and copy number variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing or by orthogonal methods such as qPCR/ddPCR when they do not meet our stringent NGS quality metrics for a true positive call.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in population cohorts and detailed information about related phenotypes. We also provide links to the references, abstracts and variant databases used to help ordering providers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. Our laboratory is therefore well-positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

CPT code(s) *

81302, 81304, 81404, 81405 x2, 81406 x2, 81479

* The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.

ICD Codes

Refer to the most current version of ICD-10-CM manual for a complete list of ICD-10 codes.

Sample Requirements

- Blood (min. 1ml) in an EDTA tube
- Extracted DNA, min. 2 µg in TE buffer or equivalent
- Saliva (Please see Sample Requirements for accepted saliva kits)

Label the sample tube with your patient's name, date of birth and the date of sample collection.

We do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue. In addition, if the patient is affected with a hematological malignancy, DNA extracted from a non-hematological source (e.g. skin fibroblasts) is strongly recommended.

Please note that, in rare cases, mitochondrial genome (mtDNA) variants may not be detectable in blood or saliva in which case DNA extracted from post-mitotic tissue such as skeletal muscle may be a better option.

Read more about our sample requirements here.

For Patients

Other

- American Sleep Association
- Berry-Kravis EM et al. Congenital central hypoventilation syndrome: PHOX2B mutations and phenotype. Am J Respir Crit Care Med. 2006 Nov 15;174(10):1139-44.
- Bowel Group for Kids Inc.
- CCHS Family Network
- Di Lascio S et al. Structural and functional differences in PHOX2B frameshift mutations underlie isolated or syndromic congenital central hypoventilation syndrome. Hum Mutat. 2018 Feb;39(2):219-236.
- GeneReviews - CCHS
- GeneReviews - Congenital Central Hypoventilation Syndrome.
- GeneReviews - Hirschsprung Disease
- GeneReviews - Hirschsprung Disease Overview.
- International Foundation for Functional Gastrointestinal Disorders
- NORD - CCHS
- NORD - Hirschsprung Disease
- Neuroblastoma UK