Blueprint Genetics

Pulmonary Artery Hypertension (PAH) Panel

Test code: CA0601

Is a 23 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical diagnosis of idiopathic or familial pulmonary arterial hypertension.

About Pulmonary Artery Hypertension (PAH)

Pulmonary arterial hypertension (PAH) is characterized by widespread obstruction and obliteration of small pulmonary arteries leading to increased pulmonary vascular resistance, elevated pulmonary arterial pressure and eventually right ventricle failure and death. The clinical diagnosis of PAH can be established by the presence of mean pulmonary artery pressure >25 mmHg at rest or >30 mmHg during exercise when other known causes of pulmonary hypertension are excluded. Initial symptoms of pulmonary arterial hypertension are dyspnea (60%), fatigue (19%), syncope (8%), chest pain (7%), palpitation (5%), and edema (3%) and they correlate with the degree of RV failure. Mean age at diagnosis is 36 years but all age groups can be affected. There is no cure for PAH and current medications form, for some patients, only a bridge to lung transplantation.

Availability

4 weeks

Gene Set Description

Genes in the Pulmonary Artery Hypertension (PAH) Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
ABCC8	Hyperinsulinemic hypoglycemia, Diabetes, permanent neonatal, Hypoglycemia, leucine-induced, Diabetes mellitus, transient neonatal, Pulmonary arterial hypertension (PAH)	AD/AR	170	641
ACVRL1	Hereditary hemorrhagic telangiectasia	AD	140	430
AQP1	Pulmonary arterial hypertension (PAH)	AD	1	5
ATP13A3	Pulmonary arterial hypertension (PAH)	AD	2	11
BMPR1B	Acromesomelic dysplasia, Demirhan, Brachydactyly C/Symphalangism-like pheno, Brachydactyly type A2, Pulmonary arterial hypertension (PAH)	AD/AR	12	23
BMPR2	Pulmonary hypertension, primary, Pulmonary venoocclusive disease	AD	391	572
CAV1	Partial lipodystrophy, congenital cataracts, and neurodegeneration syndrome, Lipodystrophy, congenital generalized, Pulmonary hypertension, primary 3	AD/AR	7	11
EIF2AK4	Pulmonary venoocclusive disease	AR	27	84
ENG	Juvenile polyposis syndrome, Hereditary hemorrhagic telangiectasia	AD	158	491
FOXF1	Alveolar capillary dysplasia with misalignment of pulmonary veins	AD	10	102
GDF2	Hereditary hemorrhagic telangiectasia, type 5, Pulmonary arterial hypertension (PAH)	AD	3	17

KCNA5	Atrial fibrillation	AD	4	25
KCNK3	Pulmonary artery hypertension	AD	7	22
KLF2	Pulmonary arterial hypertension (PAH)	AD		1
NFU1	Multiple mitochondrial dysfunctions syndrome 1	AR	6	15
NOTCH3	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Lateral meningocele syndrome	AD	87	364
RASA1#	Parkes Weber syndrome, Capillary malformation-arteriovenous malformation, Spinal arteriovenous anomalies	AD	55	132
SARS2	Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis	AR	6	5
SMAD4	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, Polyposis, juvenile intestinal, Myhre dysplasia, Hereditary hemorrhagic telangiectasia	AD	179	143
SMAD9	Pulmonary hypertension, primary 2	AD	4	17
SOX17	Pulmonary arterial hypertension (PAH)	AD	2	11
STRA6	Microphthalmia, syndromic, Microphthalmia, isolated, with coloboma	AR	22	33
TBX4	Small patella syndrome	AD	8	58

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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
ABCC8	Chr11:17415959	c.4412-13G>A	NM_000352.3	rs1008906426

ABCC8	Chr11:17427028	c.3399+13G>A	NM_000352.3	rs182340196
ABCC8	Chr11:17449501	c.2041-12C>A	NM_000352.3	
ABCC8	Chr11:17449510	c.2041-21G>A	NM_000352.3	rs746714109
ABCC8	Chr11:17449514	c.2041-25G>A	NM_000352.3	
ABCC8	Chr11:17452526	c.1672-20A>G	NM_000352.3	
ABCC8	Chr11:17465872	c.1333-1013A>G	NM_000352.3	
ABCC8	Chr11:17470268	c.1177-53_1177-51delGTG	NM_000352.3	rs1271038564
ABCC8	Chr11:17498513	c.-190C>G	NM_000352.3	
ACVRL1	Chr12:52314269	c.1378-274C>G	NM_000020.2	
ACVRL1	Chr12:52314327	c.1378-216C>G	NM_000020.2	
ACVRL1	Chr12:52314387	c.1378-156_1378-155invCT	NM_000020.2	
ACVRL1	Chr12:52314412	c.1378-131C>G	NM_000020.2	
ACVRL1	Chr12:52314465	c.1378-78T>G	NM_000020.2	
ACVRL1	Chr12:52314474	c.1378-69C>A	NM_000020.2	
BMPR1B	Chr4:95797053	c.-113+2T>G	NM_001203.2	
BMPR2	Chr2:203241251	c.-947_-946delGCinsAT	NM_001204.6	rs1085307144
BMPR2	Chr2:203241851	c.-347C>T	NM_001204.6	
BMPR2	Chr2:203241919	c.-279C>A	NM_001204.6	
BMPR2	Chr2:203242106	c.-92C>A	NM_001204.6	
BMPR2	Chr2:203395505	c.968-12T>G	NM_001204.6	
CAV1	Chr7:116165023	c.-88delC	NM_001753.4	
ENG	Chr9:130578354	c.1742-22T>C	NM_001114753.2	
ENG	Chr9:130588962	c.361-11T>A	NM_001114753.2	
ENG	Chr9:130616692	c.-58G>A	NM_001114753.2	rs971268057
ENG	Chr9:130616761	c.-127C>T	NM_001114753.2	
ENG	Chr9:130616776	c.-142A>T	NM_001114753.2	
NOTCH3	Chr19:15303132	c.341-26_341-24delAAC	NM_000435.2	

Test Strengths

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 suboptimal coverage in our assay are marked with number sign (#). Gene is considered to have suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. 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 cornerstone 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 healthcare provider at no additional cost, according to our latest follow-up reporting policy.

CPT code(s) *

81405 x3, 81406 x3, 81407, 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

- Abman SH et al. Pediatric Pulmonary Hypertension: Guidelines From the American Heart Association and American Thoracic Society. Circulation. 2015 Nov 24;132(21):2037-99.
- GeneReviews - PAH
- NORD - PAH
- Pulmonary Hypertension Association - UK
- Pulmonary Hypertension Association - USA

- Simonneau G et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2013 Dec 24;62(25 Suppl):D34-41.
- Talwar A et al. Health Disparities in Patients with Pulmonary Arterial Hypertension: A Blueprint for Action. An Official American Thoracic Society Statement. Am J Respir Crit Care Med. 2017 Oct 15;196(8):e32-e47.