Pulmonary Arterial Hypertension (PAH) Panel

The Blueprint Genetics Pulmonary Arterial Hypertension (PAH) Panel is the most efficient genetic diagnostic tool on the market for pulmonary arterial hypertension (PAH). This panel covers genes associated with heritable and idiopathic PAH. As there is genetic overlap between PAH and hereditary hemorrhagic telangiectasia (HHT) this panel can be also used as a diagnostic tool also for HHT patients.

The PAH Panel provides a high quality read-out of all genes with well-established association to pulmonary arterial hypertension. Our OS-Seq™ technology provides high coverage clinical grade sequencing and enables reliable diagnostics for PAH patients with significantly lower costs and faster turnaround time (basic service TAT 21 days and Express service TAT 7-10 days). The PAH Panel has undergone rigorous validation process during its evolution at Blueprint Genetics. Our unique sequencing technology combined with in-house built bioinformatics pipeline with pulmonary arterial hypertension mutation and knowledge databases, together with our experienced team of geneticists and clinicians, forms the most efficient PAH diagnostics service in the market. Our variant classification schemes and clinical interpretation processes have been developed and validated with thousands of patients with hereditary cardiovascular disease. Blueprint Genetics publically shares all classified variants identified in pulmonary arterial hypertension patients to improve future diagnostics (ClinVar; http://www.ncbi.nlm.nih.gov/clinvar/). Our mission is to improve the quality of diagnostics and management of PAH patients and their families.

Genes Covered by Panel

ACVRL1, BMPR1B, BMPR2, CAV1, ENG, KCNK3, SMAD9

The test covers 7 genes with evidence of association with pulmonary arterial hypertension. All protein coding exons and exon-intron boundaries are covered except exon 1 in KCNK3. Sequencing is also targeted to other regions if reported mutations exist.

Description of Test

Blueprint Genetics offers a comprehensive NGS panel for pulmonary arterial hypertension that covers the genes associated with PAH disease. The genes are carefully selected based on the existing scientific evidence, our experience and existing mutation databases.

Coverage

In our latest validation of the PAH Panel, median sequencing depth in the target region was 752x on a nucleotide level and 100.00% of the nucleotides had at least 15x coverage.

Analytical validity

Analytical validation is a continuous process at Blueprint Genetics. Our mission is to improve the quality of the sequencing process and each modification is followed by our standardized validation process. In our latest validation the PAH Panel had sensitivity of 1.000 and specificity of 1.000 to detect single nucleotide polymorphisms. Our panel had also good performance to detect insertions and deletions. Sensitivity was 0.963 for indels ranged 1-5 bp. This panel has not been validated to identify larger deletions, insertions or complex rearrangements.

Yield
Blueprint Genetics provides genetic diagnostics for hundreds of hospitals and clinics around the world. The diagnostic yield varies substantially between hospitals and countries. At Blueprint Genetics, the diagnostic yield (detecting a pathogenic or likely pathogenic mutation) in PAH is 28.5% in our laboratory. Please review our variant interpretation strategy here.

**Bioinformatics**

We have developed a unique PAH sequence analyzer and interpretation pipeline. In the core of our bioinformatics lies our in-house created and curated pulmonary arterial hypertension database, which is a synthesis of all publications of PAH-associated and validated mutations. The analysis pipeline includes rigorous quality control steps to ensure validity and consistency of results, and incorporates gene variability data from thousands of publicly available human reference sequences in order to eliminate false positive findings and deliver data of the highest relevance. Reference databases currently used are 1000 Genomes ([http://www.1000genomes.org](http://www.1000genomes.org)), NHLBI GO Exome Sequencing Project (ESP; [http://evs.gs.washington.edu/EVS](http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (ExAC; [http://exac.broadinstitute.org](http://exac.broadinstitute.org)). In addition, we apply the following *in silico* variant prediction tools in our analyses: SIFT ([http://sift.jcvi.org](http://sift.jcvi.org)), Polyphen ([http://genetics.bwh.harvard.edu/pph2/](http://genetics.bwh.harvard.edu/pph2/)), and Mutation Taster ([http://www.mutationtaster.org](http://www.mutationtaster.org)).

Through our online ordering and statement system, Nucleus, the customer can access specific details of the analysis of the patient. This includes coverage and quality specifications and other information on the analysis. This represents our mission to build fully transparent diagnostics where customer has easy access to the details of the analysis.

**Clinical Interpretation**

In addition to our cutting-edge proprietary sequencing technology and bioinformatics pipeline, BpG also provides the customers with the best-informed clinical statement on the market. Clinical interpretation requires fundamental clinical and genetic understanding on pulmonary arterial hypertension. At Blueprint Genetics the clinical statement is prepared by our geneticists and cardiologists, who together, evaluate the results from the sequence analysis pipeline in the context of phenotype information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals, even without training in genetics.

Variants reported in the statement are always classified using the Blueprint Genetics Classification Scheme modified from the ACMG guidelines (Richards et al. 2015), which has been developed by evaluating existing literature, databases and with thousands of clinical cases analyzed in our laboratory. Variant classification forms the corner stone of clinical interpretation and following patient management decisions. Our statement also provides allele frequencies in existing databases and *in silico* predictions. We also provide PubMed IDs to the articles or submission numbers to public databases that have been used in the interpretation of the detected variants. In our conclusion, we summarize all the existing information and provide our rationale for the classification of the variant.

A final component of the analysis is the Sanger confirmation of variants classified as likely pathogenic or pathogenic. This does not only bring confidence to the results obtained by our NGS solution but establishes the mutation specific test for family members. Sanger sequencing is also used occasionally with other variants reported in the statement. In the case of variant of unknown significance (VUS) we do not recommend risk stratification based on the genetic finding. Furthermore, in the case VUS we do not recommend use of genetic information in patient management or genetic counseling. For some cases Blueprint Genetics offers a special service to investigate the role of identified VUS.

We constantly follow the development on the field of pulmonary arterial hypertension and adapt new relevant information and findings to our diagnostics. Relevant novel discoveries can be rapidly translated and adopted into our diagnostics without delay. These processes ensure that our diagnostic panels and clinical statements remain the most up-to-date and relevant on the market.

**Sample Requirements**

- 3ml of EDTA blood
- Purified DNA 10μg
- Saliva (Oragene DNA OG-500 or OGD-500 kit, DNA Genotek)

Details for sample preparations and sending are found [here](#).
About the Disorder

Pulmonary arterial hypertension 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 are involved. There is no cure for PAH and current medications form, for many patients, only a bridge to lung transplantation.

Genetics of PAH has been strongly associated with mutations in genes associated with transforming growth factor beta (TGF-ß) signaling pathway. Heritable PAH associates with BMPR2 gene mutations. Recent studies have clarified that BMPR2 mutations are present in 70% of the familial cases and in up to 40% of cases with idiopathic PAH (Simonneau et al. 2009). Five-year survival is lower and hazard risk higher (12.5x) in BMPR2 mutation carriers than non-carriers (Chida et al. 2012). Furthermore, patients with missense mutations in BMPR2 are younger at time of diagnosis, present with more severe disease and increased mortality compared to patients carrying truncating BMPR2 mutations (Austin et al. 2009). Although BMPR2 mutations cause a dominantly inherited disease the penetrance of these mutations is only 20-58% suggesting the presence of other causative factors. Other causative genes reported in PAH are ACVRL1 (also known as ALK1), BMPR1B, CAV1, ENG, KCNK3 and SMAD9 (Chaouat et al. 2004, Harrison et al. 2005, Shintani et al. 2009, Austin et al. 2012, Chida et al. 2012). Ma et al. published recently an article in New England Journal of Medicine, where they identified several mutations in KCNK3 in both familiar and idiopathic PAH patients, therefore our PAH Panel has been complemented with the KCNK3 gene. Mutations in ACVRL1 and ENG are predominantly associated with HHT, which manifests with spontaneous and recurrent nosebleeds, mucocutaneous telangiectasies and solid organ arteriovenous malformations. PAH patients carrying ACVRL1 mutations manifest their disease younger and show decreased survival compared to BMPR2 mutation carriers and non-carriers (Girerd et al. 2010). Hereditary hemorrhagic telangiectasia HHT is inherited in an autosomal dominant fashion with near complete penetrance (97%) by the age of 60 years (Plauchu et al. 1989).

References


