The Blueprint Genetics Noonan Syndrome Panel is a comprehensive 12 gene diagnostic tool developed for patients with clinical suspicion of Noonan syndrome or Noonan-like syndrome, also referred as rasopathies. The Noonan Syndrome Panel is a powerful tool for differential diagnostics in patients with features of rasopathies.

The Noonan Syndrome Panel provides a high quality read-out of all genes with well-established association to Noonan syndrome and other rasopathies. Our OS-Seq™ technology provides high coverage clinical grade sequencing and enables reliable diagnostics for rasopathy patients with significantly lower costs and faster turnaround time (basic service TAT 21 days and express service TAT 7-10 days). The Noonan Syndrome Panel has undergone rigorous validation process during its evolution at Blueprint Genetics. Our unique sequencing technology combined with in-house built bioinformatics pipeline with rasopathy mutation and knowledge databases, together with our experienced team of geneticists and clinicians, forms the most efficient rasopathy 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 rasopathy 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 rasopathy patients and their families.

**Genes Covered by Panel**

*BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, SHOC2, SOS1, SPRED1*

The test covers 12 genes with strong evidence of association with rasopathies. All protein coding exons and exon-intron boundaries are covered. Sequencing is also targeted to other regions if reported mutations exist.

**Description of Test**

Blueprint Genetics offers a comprehensive rasopathy NGS panel that covers the genes associated with rasopathies. The genes are carefully selected based on the existing scientific evidence, our experience and existing mutation databases.

**Coverage**

In our latest validation of the Noonan Syndrome Panel, median sequencing depth in the target region was 425x on a nucleotide level and 99.15% 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 Noonan Syndrome Panel had sensitivity of 0.990 and specificity of 1.000 to detect single nucleotide polymorphisms. Our panel had also good performance to detect insertions and deletions. Sensitivity was 1.000 for both indels ranged 1-5 bp and 6-19 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. The Noonan Syndrome Panel is widely used as a differential diagnostics
Blueprint Genetics

panel in patients with any overlap with rasopathies. At Blueprint Genetics, diagnostic yield (detecting a pathogenic or likely pathogenic mutation) with Noonan Syndrome Panel is currently 16.7%. Please review our variant interpretation strategy here.

Bioinformatics

We have developed a unique cardiomyopathy sequence analyzer and interpretation pipeline. In the core of our bioinformatics lies our in-house created and curated cardiomyopathy database, which is a synthesis of hundreds of original publications of rasopathy-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), NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org). In addition, we apply the following in silico variant prediction tools in our analyses: SIFT (http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (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 rasopathies. At Blueprint Genetics the clinical statement is prepared by our geneticists and clinicians, 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 cornerstone 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 rasopathies 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

Noonan syndrome is one of the most common human syndromes with estimated prevalence of 1 in 1000 to 1 in 2500 live births. It is clinically and genetically heterogeneous condition characterized by cardiovascular abnormalities, distinctive facial
features, chest deformity, short stature, and other comorbidities (Romano et al. 2010).

Gene mutations identified in individuals with the NS phenotype are involved in the Ras/MAPK (mitogen-activated protein kinase) signaling pathway and currently explain over 60% of NS cases. Because of the phenotypic variability and the need for multidisciplinary care, it is essential that this condition is identified and managed comprehensively. Because of differences in prognosis, treatment options, and recurrence concerns, accurate diagnosis is critical when assessing NS patients. There are several disorders overlapping with Noonan syndrome phenotype, such as cardiofaciociutaneous (CFC) syndrome, Costello syndrome and LEOPARD syndrome. It has not been a surprise that all these conditions are caused by mutations in genes of the Ras/MAPK pathway. The BpG Noonan Syndrome Panel currently includes NS-associated genes PTPN11, RAF1, SOS1, KRAS, NRAS, BRAF and SHOC2. Current estimates suggest that 50% of NS patents harbor PTPN11 mutations, 3-17% have RAF1 mutations, 10% have SOS1 mutations while rest of the genes are considered as rare causes of the syndrome. Genes associated with CFC are BRAF, KRAS, MAP2K1 and MAP2K2. Costello syndrome is caused by HRAS mutations and LEOPARD syndrome is associated with BRAF, RAF1 and PTPN11. The BpG Noonan Syndrome Panel also includes SPRED1, which is associated with Legius syndrome that manifests with Noonan-like facial features and CBL, which associates to Noonan-like syndrome.

Among the NS-associated genes, many different genotype-phenotype correlations have been established although no phenotypic features are exclusively associated with one genotype. There are, however, significant differences in the risk of various Noonan syndrome manifestations based on the causative gene. For example, PTPN11 mutation carriers are less likely to manifest with hypertrophic cardiomyopathy (HCM), but have more often pulmonary valve stenosis (PS) and atrial septal defect (ASD). PTPN11 mutations are also associated with chest deformity, easy bruising, the characteristic facial appearance, and short stature (Yoshida 2004; Zenker et al. 2004). Patients with SOS1 mutations are more likely to have CFC syndrome–like skin findings, including keratosis pilaris, sparse hair, curly hair and sparse eyebrows. In addition, they are likely to have normal stature and normal cognitive function (Tartaglia et al. 2006). SOS1 mutation carriers have also a higher incidence of PS. Interestingly, up to 95% of RAF1 mutation carriers have HCM (Pandit et al. 2007). A number of other genotype associated clinical features have also been described (Romano et al. 2010).

References


