Nephrotic Syndrome Panel

The Blueprint Genetics Nephrotic Syndrome panel is an effective genetic diagnostic tool for patients manifesting nephrotic syndrome. It can be used for both childhood and adult-onset forms of the disease. The panel covers 9 genes with strong association with the disorder.

The Nephrotic Syndrome Panel provides a high quality read-out of all clinically relevant genes associated with nephrotic syndrome. Our OS-Seq™ technology provides high coverage clinical grade sequencing and enables reliable diagnostics for patients with significantly lower costs and faster turnaround time (basic service TAT 21 days and express service TAT 7-10 days). The Nephrotic 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 nephrotic syndrome mutation and knowledge database, together with our experienced team of geneticists and clinicians, forms the most efficient nephrotic syndrome diagnostics service in the market. Our variant classification schemes and clinical interpretation processes have been developed and validated with thousands of patients with hereditary disease. Blueprint Genetics publically shares all classified variants identified in nephrotic syndrome 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 nephrotic syndrome patients and their families.

Genes Covered by Panel

ACTN4, CD2AP, INF2, LAMB2, NPHS1, NPHS2, PLCE1, TRPC6, WT1

The test covers 9 genes with evidence of association with nephrotic syndrome. All protein coding exons and exon-intron boundaries are covered. Sequencing is also targeted to other regions with reported pathogenic or likely pathogenic mutations.

Description of Test

Blueprint Genetics offers a comprehensive Nephrotic Syndrome Panel that covers the genes associated with nephrotic syndrome. The genes are carefully selected based on the existing scientific evidence, our experience and existing mutation databases.

Coverage

In our latest validation of the Nephrotic Syndrome Panel, median sequencing depth in the target region was 1065x on a nucleotide level and 99.84% 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 our standardized validation process follows each modification. In our latest validation the Nephrotic Syndrome 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.

Diagnostic yield
Blueprint Genetics provides genetic diagnostics for hundreds of hospitals and clinics around the world. The diagnostic yield varies substantially between hospitals and countries. Diagnostic yield (detecting a pathogenic or likely pathogenic mutation) with Nephrotic Syndrome Panel is currently 28.0% in our laboratory. Please review our variant interpretation strategy here.

Bioinformatics

We have developed a unique nephrotic syndrome sequence analyzer and interpretation pipeline. In the core of our bioinformatics lies our in-house created and curated nephrotic syndrome mutation database, which is a synthesis of original publications on nephrotic syndrome and existing mutation databases. 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 hereditary cardiovascular diseases. At Blueprint Genetics 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, prepare the clinical statement. 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 kidney diseases 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

Nephrotic syndrome is caused by leaky glomerular filtration barrier resulting in extensive proteinuria, hypoalbuminemia and
edema. Majority of the patients (80-90%) with nephrotic syndrome are responsive to steroid treatment and achieve remission with a good long-term prognosis. The remaining 10-20% are considered to have steroid-resistant nephrotic syndrome (SRNS). Some of these patients may respond to other immunosuppressive therapies. The prognosis of steroid-resistant nephrotic syndrome is poor, as 30-40% develop end-stage renal disease requiring dialysis and transplantation (Mekahli et al. 2009). Decades of extensive research have revealed important insights into the molecular genetic structure and function of the glomerular filtration barrier. Identification of nephrin gene (NPHS1) mutation, causing congenital nephrosis of Finnish type, opened up a new era in the understanding of the pathophysiology and genetics of proteinuric diseases. Later on, several genes associated with this barrier harbor SRNS related mutations.

Identified genes, their mutations, and genotype-phenotype correlations are now being translated into everyday clinical practice through genetic testing. Ineffective treatment with steroids and other immunosuppressives can be avoided by utilizing genetic testing in patients with nephrotic syndrome (Büscher et al. 2010). Identification of causative mutations can also be used in the prediction of increased risk of post-transplant proteinuria. Posttransplant recurrence is generally high but almost unknown in patients with a genetic origin of the disease. After establishing genetic diagnosis, transplantation and particularly live related transplantation may be explored as a therapeutic option in earlier phase (Santin et al. 2011). Genetic diagnosis forms the basis of effective genetic counseling, risk evaluation in the family, and allows the possibility for prenatal testing (Gigante et al. 2005). With an increasing number of identified causative genes and genotype-phenotype overlaps, the clinical utility of testing single genes or specific mutations is questionable. The development of next-generation sequencing technologies is allowing rapid, comprehensive and cost-efficient genetic diagnostics for patients with nephrosis without cascade screening (McCarthy et al. 2013). Genetic diagnostics is becoming an important tool in personalizing the diagnostics and treatment of nephrotic syndrome and, thus, improving the management of these patients. Detailed description of epidemiology and genetics of nephritic syndrome is described below.

Family history, age of onset, ethnicity and histological findings give indications of the underlying genetic defect of different SRNSs. The family history suggests an autosomal dominant (AD) pattern of inheritance when there are affected men and women in every generation, whereas autosomal recessive (AR) pattern is usually present when there are affected individuals in only one generation/family. In a recent study with 36 patients aged 0-16 years, 70% of the patients with familial disease and 15% of the sporadic cases carried a definitive or probably pathogenic mutation. In this study the hit rate was 86% in patients presenting NS before the age of two (McCarthy et al. 2013). By analyzing four genes (NPHS1, NPHS2, exons 8 and 9 of WT1 and enzymatic screening of LAMB2), disease-causing mutations were found in 66.3% (53 of 80) of the families with an index patient presenting nephrotic syndrome in the first year of life (Hinkes et al. 2007). Majority of the mutations were in NPHS1 and NPHS2 genes (NPHS122.5%, NPHS2 37.5%, WT1 3.8% and LAMB2>2.5%, respectively). NPHS1 mutations occurred only in congenital nephrotic syndrome (0-3 months), where mutations were found more often than in the infantile onset (4-12 months) disease (85% vs. 44).

To date over 600 mutations have been described in nine genes associated with SRNS. These genes are NPHS1 (nephrin), NPHS2 (podocin), PLCE1 (phospholipase C, epsilon-1), CD2AP (CD2-associated protein), LAMB2 (laminin, beta 2), INF2 (inverted forming FH2 And WH2 domain containing), WT1 (Wilms tumor 1), TRPC6 (transient receptor potential cation channel, subfamily C, member 6), and ACTN4 (actinin, alpha 4).

NPHS1 and NPHS2 are the most common defective genes behind AR steroid-resistant NS. Mutations in these two genes cause congenital and early-onset SRNS (Kestila et al. 1998, Hinkes et al. 2007). Both homozygous and compound heterozygous mutations are described in both genes (Weber et al. 2004, Schultheiss et al. 2004). A third gene strongly associated with recessive SRNS is PLCE1. Homozygous or compound heterozygous mutations in PLCE1 are known to cause nephrotic syndrome, type 3. Majority of patients with PLCE1 mutations manifest severe early-onset nephrotic syndrome, gross proteinuria, steroid resistance, edema and rapid progression to end-stage renal disease (Hinkes et al. 2006). The importance of CD2AP in SRNS is currently unclear, as only a few cases have been described. (Kim et al. 2003, Löwik et al. 2007). Recessive mutations in LAMB2 is known to cause Pierson syndrome characterized by microcoria, congenital nephrotic syndrome and diffused mesangial sclerosis (DMS) in renal biopsy (Hinkes et al. 2007).

Autosomal dominant heterozygous mutations in INF2 have been reported to cause non-syndromic focal segmental glomerulosclerosis (FSGS) (Brown et al. 2010). In this study, patients had microscopic hematuria, hypertension and many present with nephrotic-range proteinuria. Seven of the nine distinct missense mutations in INF2 occurred in close proximity to exon 4, and all nine located within the diaphanous inhibitory domain. Age at diagnosis ranged from 11 to 72 years with progression to end-stage renal disease ranging from 13 to 67 years. Mutations in WT1, Wilms' tumor suppressor gene, are associated with isolated Wilms' tumor, WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies and mental retardation), Frasier syndrome, Denys–Drash syndrome and nephrotic syndrome type 4 (NPHS4) (Jeanpierre et al. 1998, Bücher et al. 2010, Schumacher et al. 1998). Mutations in TRPC6 cause typically late-onset autosomal dominant FSGS (Winn et al. 2005). However, there are case reports where children with SRNS and FSGS have also presented with TRPC6 mutations.
Gene defects in ACTN4 have also been associated with progressive familial adult-onset autosomal dominant FSGS (Kaplan et al. 2000). Patients usually show nephrotic- or non-nephrotic-range proteinuria during adolescence or adulthood and FSGS in renal biopsy. The disease gradually progresses to end-stage renal disease. ACTN4 mutations can also manifest as childhood-onset disease (Weins et al. 2005).

**References**


