

Molecular Testing by Next Generation Sequencing for mutations associated with Hereditary Nephrotic Syndrome/ Focal Segmental Glomerulosclerosis (FSGS)/ Alport Syndrome

The UNC Hospitals Molecular Genetics Laboratory performs DNA sequencing of 17 genes to detect mutations that are associated with Hereditary Nephrotic Syndrome, Focal Segmental Glomerulosclerosis, and Alport Syndrome by massively parallel (Next Generation) sequencing on the Illumina MiSeq.

Biology of the Disease:

Nephrotic syndrome (NS) is a common kidney disorder caused by damage of glomeruli and is characterized by significant proteinuria, hematuria and edema. The incidence of NS in the USA has been estimated to be 3 per 100,000 patients, with Focal Segmental Glomerulosclerosis (FSGS) comprising 15-20% of the total. Although most patients with NS respond to steroids with remission, approximately 10% of patients continue to show proteinuria after 4 weeks of steroid therapy and are considered to have steroid-resistant nephrotic syndrome (SRNS). Half of the patients with SRNS even after renal transplantation are reported to develop end-stage renal disease (ESRD). Inherited forms of SRNS have been found due to genetic mutations in multiple different genes associated primarily with the structure and function of kidney podocytes. The age-of-onset of hereditary forms of NS can vary from congenital to adult and can be associated with either autosomal recessive or dominant inheritance due to mutations in specific genes. Hereditary NS presents with extensive genetic heterogeneity and multiple chromosomal loci have been identified including the genes targeted in the panel.

Alport syndrome is a heterogeneous disorder characterized by progressive renal disease to ESRD, hearing loss and ocular lesions. Mutations in the type IV collagen genes that encode for structural components of the basement membrane are the underlying cause of Alport syndrome. It has an estimated prevalence of approximately 1:50,000 live births and is predominantly an X-linked disease, with mutations in *COL4A5* gene accounting for approximately 80% of Alport syndrome. Approximately 20% of patients have mutations in the *COL4A3* and *COL4A4* genes with either autosomal recessive or rarely autosomal dominant inheritance.

Clinical Indications for Molecular Genetic Testing:

Molecular Genetic testing should be considered for confirmation of Hereditary Nephrotic syndrome in patients with a family history of NS or suspected to have a hereditary form of SRNS. The results can also be useful in predicting the clinical outcomes, responsiveness to immunosuppressive drugs, rate of progression to ESRD, and risk of post kidney transplant recurrence. Indications for genetic testing in NS include: 1) family history of SRNS, 2) congenital or infantile onset of SRNS, 3) lack of response to immunosuppressive drugs, 4) histological findings of FSGS on renal biopsy, 5) reduced renal function or renal failure. Testing should be considered for confirmation of a clinical diagnosis of Alport Syndrome, particularly when the family history suggests X-linked or autosomal recessive inheritance. Testing is also useful for identification of at-risk relatives and carriers once a specific disease-causing mutation has been identified in the family.

Laboratory Testing:

The preferred sample is ACD anticoagulated blood (pale yellow top) which may be refrigerated up to 48 hours before analysis. Testing can also be performed on formalin-fixed, paraffin-embedded (FFPE) kidney biopsy samples. Ten unstained sections from tissue on plain, uncoated glass slides (5 microns thick) or scrolls are required.

The test is performed by sequencing the complete coding region of 17 genes that have been associated with hereditary nephrotic syndrome or Alport syndrome using a massively parallel sequencing assay developed in our laboratory. This assay utilizes a custom target amplicon TruSeq amplicon low input dual pool reagent (Illumina) to generate libraries for custom massively parallel sequencing on an Illumina MiSeq instrument. The Table below shows a list of the genes included in the panel.

Results are reported as negative for sequence variant(s) or sequence variant(s) detected. Any variant identified is interpreted as pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign or benign, per ACMG guidelines. Benign population variants are not reported. Results are interpreted as consistent with disease diagnosis or carrier status based on the inheritance pattern for that gene. Obtaining informed consent for testing is the responsibility of the ordering physician. Genetic counseling is recommended; for help with genetic counseling at UNC please call 919-966-4380.

Resources:

1. National Institute of Diabetes and Digestive and Kidney Disease
<https://www.niddk.nih.gov/health-information/kidney-disease>
2. National Kidney Foundation: <https://www.kidney.org/>

References:

1. Online Mendelian Inheritance in Man (OMIM) <http://omim.org/search/>
2. Mori et al., Comprehensive genetic testing approach for major inherited kidney disease, using next-generation sequencing with a custom panel. Clin. Exp. Nephrol. 2016, Feb;21(1):63-75.
3. Benoit et al. Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutation. Pediatr Nephrol 2010, 25:1621-1632.
4. Ha et al. Genetics of hereditary nephrotic syndrome: a clinical review. Korean J Pediatr 2017, 60(3): 55-63.
5. Laurin et al. Podocyte-associated gene mutation screening in a heterogeneous cohort of patients with sporadic focal segmental glomerulosclerosis. Nephrol Dial Transplant. 2014 Nov;29(11):2062-9.

Table. List of 17 Genes included in the Hereditary Nephrotic Syndrome sequencing panel.

<i>ACTN4</i>	<i>APOL1</i>	<i>CD2AP</i>
<i>COL4A3</i>	<i>COL4A4</i>	<i>COL4A5</i>
<i>COL4A6</i>	<i>INF2</i>	<i>LAMB2</i>
<i>MYH9</i>	<i>MYO1E</i>	<i>NPHS1 (NEPHRIN)</i>
<i>NPHS2 (PODOCIN)</i>	<i>PLCE1</i>	<i>SLC17A5</i>
<i>TRPC6</i>	<i>WT1</i>	

Questions?

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