Nephronophthisis: *NPHP1, INVS, NPHP3, and NPHP4* Gene Sequencing Panel

| Test Code: WV | Turnaround time: 6 weeks | CPT Codes: S1479 x1 |

### Condition Description

Nephronophthisis, an autosomal recessive cystic kidney disease, is the most frequent monogenic cause of renal failure in childhood. There are four forms of nephronophthisis caused by mutations in four different genes. Clinically, there is a statistically different age at onset at end-stage renal disease: terminal renal failure develops at median ages of 13 years, 1 year, 19 years, and 11-34 years in NPHP1, NPHP2, NPHP3, and NPHP4 respectively. Hallmarks of familial nephronophthisis are tubular basement membrane disruption, interstitial lymphohistiocytic cell infiltration, and development of cysts at the corticomedullary border of the kidneys. The histology in later stages of NPH always merges into a chronic sclerosing tubulointerstitial nephropathy, which is found in chronic renal failure of all origins.

**Nephronophthisis 1**

Clinical features of familial juvenile nephronophthisis (NPHP1) include anemia, polyuria, polydipsia, isosthenuria, and death in uremia. Hypertension and proteinuria are conspicuous in their absence. Excessive urinary loss of sodium accounts for the rarity of hypertension. Symmetrical destruction of the kidneys involving both tubules and glomeruli (which were hyalinized) is observed. The age at death ranges from about 4 to 15 years. This is the second most common cause of childhood chronic renal failure. 65 to 75% of NPHP1 patients exhibit large homozygous deletions in the 2q13 region that includes the *NPHP1* gene.

**Joubert syndrome** is an autosomal recessive multisystem disease characterized by cerebellar vermis hypoplasia with prominent superior cerebellar peduncles (resulting in the "molar tooth sign," or MTS, on axial MRI), mental retardation, hypotonia, irregular breathing pattern, and eye movement abnormalities. Some individuals with JS have retinal dystrophy and/or progressive renal failure characterized as nephronophthisis. The disorder in such patients is referred to as cerebellooculorenal syndrome, or CORS. Individuals with a mild form of JS have been shown to have a homozygous deletion of the *NPHP1* gene identical, by mapping, to that in subjects with nephronophthisis alone. Senior-Loken syndrome, the association of nephronophthisis with autosomal recessive retinitis pigmentosa, has been observed in patients with homozygous deletion of the *NPHP1* gene.

**Nephronophthisis 2**

In one study, individuals with infantile nephronophthisis (NPHP2) presented within the first months of life with severe renal failure and acidosis, which could be associated with hypertension and/or polyuria and/or severe cholestatic liver disease. A renal biopsy, performed in all patients, showed similar features characterized by a diffuse chronic tubulointerstitial nephritis and particularly by the presence of microcystic dilatation of proximal tubules and Bowman space. Progression of the renal disease was extremely rapid and patients can reach end-stage renal failure before the age of 2 years (11 to 22 months).

In another study, phenotypic presentation ranged from a Potter-like syndrome to hyperechogenic kidneys, renal insufficiency, hypertension, and hyperkalemia. Affected individuals showed rapid deterioration of kidney function, leading to end-stage renal failure within 3 years. The manifestations range from prenatal fetal oliguria and oligohydramnios resulting in postnatal respiratory failure and death to postnatal onset of disease later than 30 months of age. None of the postnatally diagnosed patients had a history of either oligohydramnios or neonatal respiratory symptoms. All affected individuals developed anemia, hyperkalemic metabolic acidosis, and increased serum creatine. None of the affected subjects had polyuria, polydypsia, or associated ocular or hepatic complications.

The specific clinical features of this disease are its early onset and rapid progression to end-stage renal failure. Pathologically, it differs from later-onset nephronophthisis by the absence of medullary cysts and thickened tubular basement membranes and by the presence of cortical microcysts. NPHP2 is caused by mutations in the *INVS* gene (also known as *NPHP2*) (9q31). The protein product of the *INVS* gene, inversion, has been shown to interact with that of the *NPHP1* gene, nephrocystin.

**Nephronophthisis 3**

In one study, most individuals with adolescent nephronophthisis (NPHP3) suffered from anemia when they first came to medical attention. Onset of terminal renal failure occurred significantly later (median age, 19 years) than in juvenile nephronophthisis (median age, 13.1 years). Histologic findings in adolescent nephronophthisis are generally not distinguishable from those of juvenile nephronophthisis. Renal pathology in adolescent NPHP is characterized by alterations of tubular basement membranes, tubular atrophy and dilatation, sclerosing tubulointerstitial nephropathy, and renal cyst development predominantly at the corticomedullary junction.

Mutations in the NPHP3 gene (3q22) cause NPHP3. Mutations have been found in NPHP3 in families with isolated nephronophthisis and in families with nephronophthisis with associated hepatic fibrosis or tapetoretinal degeneration. Studies have shown that the protein product of the NPHP3 gene interacts with the protein products of NPHP1 and INVS.

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Nephronophthisis 4

Mutations in the \textit{NPHP4} gene (1p36) cause nephronophthisis 4 (NPHP4), which has also been referred to as juvenile nephronophthisis. In these families, end-stage renal disease commenced within a wide age range, 11 to 34 years. The NPHP4 protein has been shown to interact with the NPHP1 protein. Mutations in \textit{NPHP4} have been associated with Senior-Loken syndrome-4, the association of nephronophthisis with autosomal recessive retinitis pigmentosa.

Testing is available for each gene individually or as a panel.

For patients with suspected nephronophthisis, sequence analysis is recommended as the first step in mutation identification. For patients in whom mutations are not identified by full gene sequencing, deletion/duplication analysis is appropriate.

\textbf{Genes}

\textbf{INVS}, \textbf{NPHP1}, \textbf{NPHP3}, \textbf{NPHP4}

\textbf{Indications}

This test is indicated for:

- Confirmation of a clinical/biochemical diagnosis of nephronophthisis
- Carrier testing in adults with a family history of nephronophthisis

\textbf{Methodology}

\textbf{Next Generation Sequencing:} In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

\textbf{Detection}

\textbf{Clinical Sensitivity:} Unknown. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's biochemical phenotype.

\textbf{Analytical Sensitivity:} \~99%

\textbf{Specimen Requirements}

Submit only 1 of the following specimen types

* Preferred specimen type: Whole Blood

\textbf{Type: Whole Blood}

Specimen Requirements:

In EDTA (purple top) or ACD (yellow top) tube:
Infants (2 years): 3-5 ml
Older Children & Adults: 5-10 ml

Specimen Collection and Shipping: Refrigerate until time of shipment. Ship sample within 5 days of collection at room temperature with overnight delivery.

\textbf{Type: Saliva}

Specimen Requirements:
Oragene™ Saliva Collection kit (available through EGL) used according to manufacturer instructions.

Specimen Collection and Shipping: Store sample at room temperature. Ship sample within 5 days of collection at room temperature with overnight delivery.

**Special Instructions**

Submit copies of diagnostic biochemical test results with the sample, if appropriate. Contact the laboratory if further information is needed.

Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside of EGL Genetics, please submit a copy of the sequencing report with the test requisition.

**Related Tests**

- Deletion/duplication analysis of the *NPHP1, INVS, NPHP3, and NPHP4* genes by CGH array is available for those individuals in whom sequence analysis is negative.
- Custom diagnostic mutation analysis is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available to couples who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.