Polycystic Kidney Disease (PKD)

Disorder also known as: Polycystic renal disease

Panel Gene List (7 genes): GANAB, HNF1B, PKD1, PKD2, PKHD1, PRKCSH, TSC2*

*Test is designed to identify a contiguous gene deletion involving PKD1 and TSC2, not to identify sequencing and exon-level copy number variants of TSC2.

Clinical Features:
Polycystic kidney disease is a multisystem disorder characterized by the development of multiple cysts in the kidney and other extrarenal features.

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by renal cysts that lead to hypertension, renal insufficiency and end-stage renal disease (ESRD). Onset ranges from the antenatal period to late adulthood and the severity of disease varies, even within the same family. Penetration is believed to be very high with most affected adults eventually developing bilateral renal cysts. While cysts may form in other organs, hepatic cysts are the most common extrarenal feature of ADPKD. Individuals with ADPKD may also have vascular abnormalities including intracranial aneurysms, subarachnoid hemorrhage, and/or dilation of the aortic root.

The majority of ADPKD cases are caused by variants in PKD1 and PKD2. Pathogenic variants in PKD1 and PKD2 cause overlapping phenotypes; however, PKD1 variants tend to be associated with more severe disease, as well as an earlier age of onset and progression to end-stage renal failure. Rarely, pathogenic biallelic variants in PKD1 have been reported in individuals presenting with features similar to autosomal recessive PKD (ARPKD). In these cases, affected individuals have inherited a PKD1 pathogenic variant from each of their unaffected parents. Finally, a subset of individuals have presented with polycystic kidney disease as well as phenotypic features of tuberous sclerosis complex due to a contiguous gene deletion involving PKD1 and the adjacent TSC2 gene.

In regards to other genes tested in this panel, a small number of individuals is expected to have ADPKD due to pathogenic variants in the GANAB gene, and those individuals may or may not show liver disease manifestation. Renal cysts following autosomal dominant inheritance have also been reported in patients with HNF1B and PRKCSH variants, although renal cysts are not the main clinical feature. Variants in PRKCSH are found in AD polycystic liver disease (PLD), which may present with occasional renal cysts. Variants in HNF1B cause maturity-onset diabetes of the young type 5 (MODY5), which is characterized by renal cysts
and diabetes (RCAD) syndrome.\textsuperscript{10,11} Bigenic variants in HNF1B and PKD1 have been reported in individuals with severe PKD.\textsuperscript{12}

Autosomal recessive polycystic kidney disease (ARPKD) is characterized by bilateral renal cystic disease and congenital hepatic fibrosis, typically presenting in the developing fetus with oligohydramnios, enlarged echogenic kidneys and liver abnormalities.\textsuperscript{13,14} Other symptoms may include nephromegaly, hypertension and renal dysfunction, often with onset of ESRD within the first decade of life. While features may be seen already in utero, peak age of onset ranges from birth into young adulthood. A subset of patients have a Caroli phenotype, which presents with cystic dilatation of the intrahepatic bile ducts.\textsuperscript{14} ARPKD is a significant cause of renal and liver-related morbidity and mortality in children, with severe cases leading to neonatal lethality.\textsuperscript{13} The majority of cases of ARPKD are caused by bi-allelic pathogenic variants in the PKHD1 gene.\textsuperscript{12,15}

Genetics:
Polycystic kidney disease is a genetically heterogeneous disorder that may be inherited in an autosomal dominant or autosomal recessive pattern.

ADPKD is a common disorder usually presenting in adulthood, while ARPKD is a rare disorder that frequently presents in the prenatal, neonatal, or early childhood period. The majority of ADPKD cases are due to pathogenic variants in PKD1 and PKD2 (~95%) while most ARPKD cases are due to pathogenic variants in PKHD1 (~75%). Multiple types of pathogenic variants have been reported in PKD1 and PKD2 and are disseminated across the genes without apparent mutation clusters. Large copy number changes, including intragenic or whole gene deletions/duplications or rearrangements account for approximately 4% of pathogenic variants in PKD1 and <1% of variants in PKD2.\textsuperscript{5} Rarely, ADPKD may be caused by gene conversion events between the PKD1 gene and one of its pseudogenes, which may not always be detectable by this test.\textsuperscript{16-21} Truncating and missense variants have been reported in GANAB, HNF1B, PRKCSH, and PKHD1. Additionally, copy number variants encompassing multiple exons or the entire HNF1B gene, and more rarely, copy number variants affecting multiple exons of the PKHD1 gene have been reported.\textsuperscript{22-24}

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNVR). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications
involving coding exons at the exon-level; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by NGS. Reported clinically significant variants are confirmed by an appropriate method. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on coordinates of involved exons, precise breakpoints or probe coordinates when partial exons are involved. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

As sequencing of the PKD1 gene is known to be challenging due to high homology with six known pseudogenes across exons 1-33, the NGS assay is designed to provide superior mapping quality and uniform coverage across the coding region of PKD1, enabling robust detection of variants including indels.\textsuperscript{25,26} Specifically, at a mapping quality of \textgreater 20, a mean coverage of 264 reads with 99.7\% of the targeted nucleotides covered at 20x and 99.1\% covered at 50x or above was obtained. Even at mapping quality of \textgreater 40, mean coverage is 254x. For PKD1, all sequence variants or suspicious regions are confirmed by long-range, nested PCR and capillary sequencing. In addition, Multiplex Ligation-Dependent Probe Amplification (MLPA) is performed to identify or confirm most intragenic deletions or duplications of PKD1, PKD2 and contiguous gene deletions involving the TSC2 gene.

**Technical Test Sensitivity:**

DNA sequencing will identify nucleotide substitutions and small insertions and deletions, while NGS-CNV analysis, array CGH, or MLPA will identify exon-level deletions and duplications. The technical sensitivity of sequencing is estimated to be greater than 99\% sensitive at detecting single nucleotide events and lesser for deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. NGS-CNV analysis and array CGH methods used in this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Some genes, such as PKD1, have inherent sequence properties (including repeats, homology, or pseudogene regions, gene rearrangements, high GC content and rare polymorphisms) that may result in suboptimal data, potentially impairing accuracy of the results.

**Clinical Test Sensitivity:**

Polycystic kidney disease is a genetically heterogeneous disorder. The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient’s clinical phenotype and family history. Although ADPKD and ARPKD are
usually regarded as clinically distinct disorders, overlapping features and presentation, as well as lack of family history may lead to confusion. Panel testing including genes for both AD and ARPKD provides a solution for questionable diagnosis and is essential for genetic counseling. Additional information about the general clinical sensitivity of each gene is included in the table below.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Disease Association</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GANAB</td>
<td>Glucosidase II Alpha Subunit</td>
<td>AD</td>
<td>PKD 3, with or without PLD</td>
<td>&lt;1% of individuals with ADPKD³⁸</td>
</tr>
<tr>
<td>HNF1B</td>
<td>HNF1 Homeobox B</td>
<td>AD</td>
<td>Renal cysts and diabetes syndrome (RCAD/MODY5)</td>
<td>40-70% of individuals with RCAD (MODY5)²³,²⁷</td>
</tr>
<tr>
<td>PKD1</td>
<td>Polycystin 1</td>
<td>AD</td>
<td>PKD 1</td>
<td>~80% of individuals with ADPKD³,²⁸</td>
</tr>
<tr>
<td>PKD2</td>
<td>Polycystin 2</td>
<td>AD</td>
<td>PKD 2</td>
<td>~15% of individuals with ADPKD³,²⁸</td>
</tr>
<tr>
<td>PKHD1</td>
<td>Fibrocystin</td>
<td>AR</td>
<td>PKD 4, with or without hepatic disease</td>
<td>~75% of individuals with ARPKD⁶,²⁹</td>
</tr>
<tr>
<td>PRKCSH</td>
<td>Hepatocystin</td>
<td>AD</td>
<td>PLD 1</td>
<td>15-33% of individuals with polycystic liver disease³⁰,³²</td>
</tr>
<tr>
<td>TSC2</td>
<td>Tuberin (contiguous deletion with PKD1)</td>
<td>AD</td>
<td>Tuberous sclerosis 2</td>
<td>Rare in individuals with ADPKD⁷</td>
</tr>
</tbody>
</table>

AD – Autosomal Dominant; AR – Autosomal Recessive; PKD – Polycystic Kidney Disease; PLD – Polycystic Liver Disease

References: