Polycystic Liver Disease Panel

Panel Gene List (8 genes): ALG8, GANAB, LRP5, PKD1, PKD2, PKHD1, PRKCSH, SEC63

Clinical Features:
Polycystic liver disease (PLD) is a group of heterogenous disorders which are characterized by progressive development of multiple liver cysts. Although PLD can occur as an isolated disorder restricted to the liver, most cases of liver cysts co-occur with renal cysts in autosomal dominant polycystic kidney disease (ADPKD). Despite larger and greater numbers of liver cysts in isolated PLD patients, the clinical symptoms caused by liver cysts in isolated PLD and ADPKD are similar.

PLD patients are usually asymptomatic with normal hepatic function prior to disease manifestation between the age of 40 and 60 years old. Abdominal pain arising from expansion of liver cysts is the most prominent feature of PLD. The most common complication in symptomatic patients is extensive hepatomegaly, which may lead to malnutrition and can be lethal. Other features and complications caused by an enlarged polycystic liver include cyst haemorrhage, infection and portal hypertension, nausea, vomiting, early satiety, anorexia, shortness of breath and sleep apnea.

Genetics:
Isolated PLD is an autosomal dominant disorder (ADPLD), which is caused by pathogenic variants in the PRKCSH, SEC63, LRP5, ALG8 or GANAB genes. PRKCSH and SEC63 are the major causes of ADPLD, collectively accounting for at least 35% of cases. Heterozygous pathogenic variants in LRP5 contribute to a small number of PLD cases with or without kidney cysts, although LRP5 variants have been commonly associated with both autosomal dominant and recessive disorders of the retina and bone without liver or kidney cysts (see Table below). While ALG8 defects are usually associated with an autosomal recessive congenital disorder of glycosylation (type Ih), heterozygous loss-of-function variants are a less common cause of ADPLD, with or without kidney cyst presentation. ALG8-affected individuals present with variable clinical manifestations ranging from typical ADPLD to severe ADPLD requiring procedural intervention to reduce liver cyst mass. In a limited number of cases, ADPLD has been attributed to variants in the GANAB gene, which also causes ADPKD. Finally, a few patients with clinical presentation of ADPLD showing innumerable small liver cysts who tested negative for PRKCSH and SEC63 variants were found to be heterozygous carriers of PKHD1 pathogenic variants. PKHD1 is the main causative gene for autosomal recessive PKD (ARPKD) and its presence in a PLD genetic testing panel may also be useful for differential diagnosis. ADPKD is characterized by renal cysts that lead to hypertension, renal insufficiency and end-stage renal disease (ESRD). While cysts may form in other organs, liver cysts are
the most common extrarenal feature of ADPKD.\textsuperscript{13} The majority of ADPKD cases are caused by variants in PKD1 and PKD2.\textsuperscript{14}

Truncating and missense variants have been reported in ALG8, GANAB, LRP5, PKHD1, PRKCSH, and SEC63. Additionally, copy number variants affecting multiple exons of the PKHD1 and LRP5 genes have been reported.\textsuperscript{15-19} 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{20} 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{21-26}

**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-CNV). 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. For the ALG8 gene, sequencing but not deletion/duplication analysis is performed. 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{27,28} 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 Ligatton-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 liver disease is a genetically heterogeneous group of disorders. 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. 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>ALG8</td>
<td>α-1,3-Glucosyltransferase</td>
<td>AD/AR</td>
<td>ADPLD 3 with or without kidney cysts / AR CDG, type Ih</td>
<td>~3% of individuals with isolated PLD5,29</td>
</tr>
<tr>
<td>GANAB</td>
<td>Glucosidase II Alpha Subunit</td>
<td>AD</td>
<td>PKD 3, with or without PLD</td>
<td>~2% of individuals with isolated PLD11,29, &lt;1% of individuals with ADPKD 11</td>
</tr>
<tr>
<td>LRP5</td>
<td>Low density lipoprotein receptor-related protein 5</td>
<td>AD/AR</td>
<td>FEVR / LRP5-associated bone disorders / PLD 4 with or without kidney cysts</td>
<td>10-25% of individuals with FEVR30-32, ~2% of individuals with isolated PLD10</td>
</tr>
<tr>
<td>PKD1</td>
<td>Polycystin 1</td>
<td>AD</td>
<td>PKD 1</td>
<td>~80% of individuals with ADPKD33,34</td>
</tr>
<tr>
<td>PKD2</td>
<td>Polycystin 2</td>
<td>AD</td>
<td>PKD 2</td>
<td>~15% of individuals with ADPKD33,34</td>
</tr>
</tbody>
</table>
## Test Information Sheet

<table>
<thead>
<tr>
<th>PKHD1</th>
<th>Fibrocystin</th>
<th>AR</th>
<th>PKD 4, with or without hepatic disease</th>
<th>~75% of individuals with ARPKD(^{35,36})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRKCSH</td>
<td>Glucosidase 2 subunit beta (or Hepatocystin)</td>
<td>AD</td>
<td>PLD 1</td>
<td>15-33% of individuals with isolated PLD(^{3,37,38})</td>
</tr>
<tr>
<td>SEC63</td>
<td>Translocation protein SEC63 homolog</td>
<td>AD</td>
<td>PLD 2</td>
<td>6-15% of individuals with isolated PLD(^{3,10,38})</td>
</tr>
</tbody>
</table>

AD – Autosomal Dominant; AR – Autosomal Recessive; CDG – congenital disorder of glycosylation; PKD – Polycystic Kidney Disease; PLD – Polycystic Liver Disease; FEVR – Familial Exudative vitreoretinopathy

### References: