Blueprint Genetics

Sample report as of June 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at ***blueprintgenetics.com/certifications***

Polycystic Liver Disease Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		50	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

TEST RESULTS

The patient is heterozygous for a deletion c.(350+1_351-1)_(598+1_599-1)del, which encompasses exons 6-7 of *PRKCSH*. This alteration is classified as likely pathogenic.

Sequence analysis did not detect any known disease-causing or rare variants which could explain the patient's reported phenotype.

PRIMARY VARIANT TABLE: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
PRKCSH	DELETION	1	HET	PRKCSH:Partial gene	UCSC	**Likely pathogenic**
	OMIM	**PHENOTYPE** Polycystic liver disease			**COMMENT** -	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Polycystic Liver Disease Panel	6	149	33509	33381	239	99.62

TARGET REGION AND GENE LIST

The Blueprint Genetics Polycystic Liver Disease Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *GANAB, LRP5*, PKD1*, PKD2, PRKCSH and SEC63.* This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 50-year-old female with multiple liver cysts seen on CT, stomach pain, and gall stones.

CLINICAL REPORT

Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) Polycystic Liver Disease Panel identified a heterozygous deletion c.(350+1_351-1)_(598+1_599-1)del encompassing exons 6-7 of *PRKCSH*. This deletion is estimated to cover the genomic region chr19:11551993-11553385 and is approximately 1392 base pairs in size. However, the exact breakpoints of the deletion cannot be determined using the present method, and therefore its exact size and genomic position are unknown. Deletions encompassing the *PRKCSH* gene exon 6-7 have been reported in one individual in the Exome Aggregation Consortium (ExAC) control cohorts.

To the best of our knowledge, this variant has not been described in the medical literature or reported in disease-related variation databases such as ClinVar or HGMD. However, loss of *PRKCSH* function is an established disease mechanism, and other truncating variants in the gene have been described in patients with phenotypes consistent with *PRKCSH*-related disease (HGMD).

PRKCSH

PRKCSH (MIM *177060) gene encodes the beta-subunit of glucosidase II, an N-linked glycan-processing enzyme in the endoplasmic reticulum. The encoded protein is an acidic phosphoprotein known to be a substrate for protein kinase C. Pathogenic variants in this gene have been associated with polycystic liver disease 1 (PCLD1; ORPHA:2924). PCLD is an autosomal dominant genetic disorder characterized by the appearance of numerous cysts spread throughout the liver. PCLD has a prevalence of 1- 9/1,000,000, more specifically 1/158,000 in The Netherlands (PMID: 24886261). Women are predominantly affected and have a larger number of cysts than affected males. Cysts are undetectable early in life and usually appear after the age of 40 years. Their number and size increases with age. Symptoms depend on the mass (compression effect) and can include abdominal distension, gastro-esophageal reflux, early satiety, dyspnea, decreased mobility and back pain due to hepatomegaly. Some patients are asymptomatic. Other complications (intracystic hemorrhage or infection, torsion or rupture of cysts) can cause acute abdominal pain. PCLD is genetically heterogeneous with three genes identified: *PRKCSH* (MIM *177060), *SEC63* (MIM *608648) and *LRP5* (MIM *603506). Currently, 27 *PRKCSH*, 22 *SEC63*, and 4 *LRP5* mutations have been identified, with the highest mutation frequency of the *PRKCSH* gene (15%) (PMID 20095989, 16835903 and 26365003). About 80% of patients with a clinical diagnosis of PCLD do not harbour a pathogenic mutation on these loci. Mutations identified in *PRKCSH* include 16% missense, 16% splice site, 44% small insertions and deletions and 24% nonsense variants (PMID: 24886261). ClinVar reports two clinical cases with truncating pathogenic variants in *PRKCSH*.

Mutation nomenclature is based on GenBank accession NM_002743.3 (*PRKCSH*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the identified deletion *PRKCSH* c.(350+1_351-1)_(598+1_599-1)del, affecting exons 6-7, as likely pathogenic based on the established association between the gene and the patient's phenotype, the variant's rarity in control populations, variant type (gross deletion). Disease caused by *PRKCSH* variants is inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. *PRKCSH*-related disease may be caused by a *de novo* variant. Genetic counselling and family member testing are recommended.

CONFIRMATION

Deletion *PRKCSH* c.(350+1_351-1)_(598+1_599-1)del has been confirmed by digital PCR.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

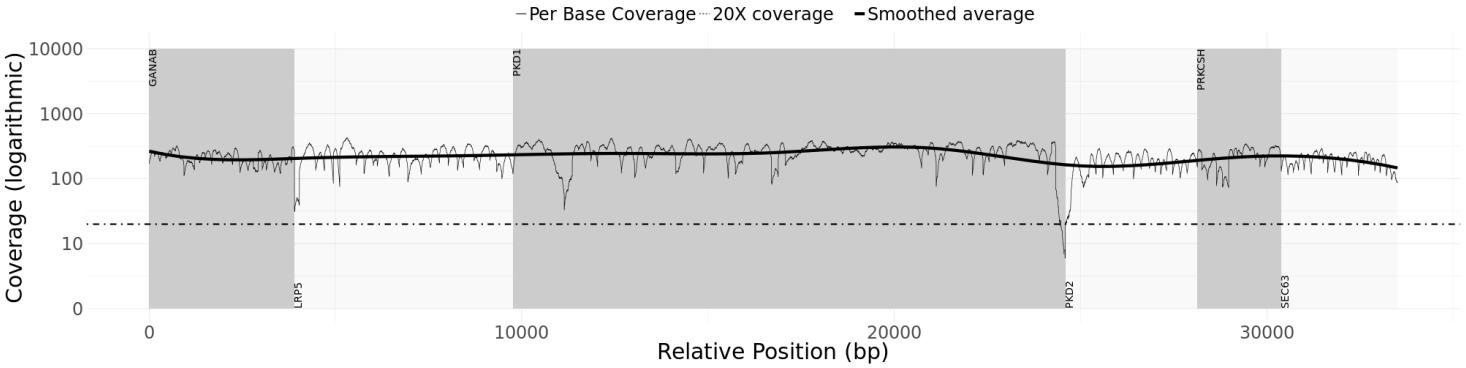
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.
Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.
Variant classification: Our variant classification follows the Blueprint Genetics Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report.
Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.
Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.
Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not

detect very low-level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.

Test restrictions: A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing and digital PCR confirmation).

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_001009944.2(PKD1):c.12445-14T>C
NM_001009944.2(PKD1):c.10167+25_10167+43delGGCTGGGCTGGGGGTCCTG
NM_001009944.2(PKD1):c.9202-16G>A
NM_000297.3(PKD2):c.596-59A>G

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
AR = autosomal recessive
CNV = Copy Number Variation eg, one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)
gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)
HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MT = Mitochondria
MutationTaster = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.