Blueprint Genetics

Cystic Kidney Disease Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		26	Male	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
DNA		

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *PKD1* c.4957C>T, p.(Gln1653*), which is classified as pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
PKD1	16:2160211	NM_001009944.2	c.4957C>T, p.(Gln1653*)	stop_gained	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		0/0	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Polycystic kidney disease		AD	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Cystic Kidney Disease Panel	40	1882	205017	204925	408	99.9

TARGET REGION AND GENE LIST

The Blueprint Genetics Cystic Kidney Disease Panel (version 2, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ANKS6, CEP83, CEP164, CEP290*, COL4A1, CRB2, DCDC2, DZIP1L, EYA1, GANAB, GLIS2, HNF1B, IFT172, INVS, IQCB1, JAG1, LRP5*, MAPKBP1, NEK8, NOTCH2*, NPHP1, NPHP3, NPHP4, OFD1, PAX2, PKD1*, PKD2, PKHD1, RPGRIP1L, SDCCAG8, SEC61A1, SIX5, TMEM67, TSC1, TSC2, TTC21B, UMOD, VHL, WDR19 and ZNF423. 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) up to 220 bps 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 26-year-old male diagnosed with polycystic kidney disease at 4 years of age. There is a family history of autosomal dominant polycystic kidney disease (ADPKD) in multiple generations.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Cystic Kidney Disease Panel identified a heterozygous nonsense variant c.4957C>T, p.(Gln1653*) in exon 15 of *PKD1*. It has not been observed in large reference population cohorts of Genome Aggregation Database, (gnomAD, n>120,000 exomes and >15,000 genomes). The variant causes a premature stop codon and is thus predicted to cause loss of normal protein function either through protein truncation (1653 out of 4303 aa) or nonsense-mediated mRNA decay. The variant has been listed as pathogenic in the PKD1 Mutation Database, where it has been reported in four families with autosomal dominant polycystic kidney disease (ADPKD).

PKD1 gene encodes polycystin-1 protein. The encoded glycoprotein contains a large N-terminal extracellular region, multiple transmembrane domains and a cytoplasmic C-tail. It is an integral membrane protein that functions as a regulator of calcium permeable cation channels and intracellular calcium homoeostasis. Polycystin-1 forms a complex with polycystin-2 that regulates multiple signaling pathways to maintain normal renal tubular structure such as cilium length and function. It is involved in fluid-flow mechanosensation by the primary cilium in renal epithelium. Mutations in *PKD1* cause autosomal dominant polycystic kidney disease type 1 (ADPKD1; OMIM *601313). Genomic region encompassing exons 1-33 of the *PKD1* (chr16:2147418-2187265) shows 97.7% sequence homology with six known pseudogenes in chromosome 16. Thus, next generation sequencing technologies with short sequencing reads have limitations to detect disease causing variants from this region, which may result in false positive results. There are altogether 1273 *PKD1* variants classified as definitely pathogenic, highly likely pathogenic or likely pathogenic in PKD1 Mutation Database (Feb 2018); frameshifts 35.3%, missense 24.6%, nonsense 20.7%, splice site 9.7%, in-frame deletions/insertions 8.1% and gross deletions/duplications 1.6%.

Autosomal dominant polycystic kidney disease (ADPKD) is generally a late-onset multisystem disorder characterized by: bilateral renal cysts; cysts in other organs including the liver, seminal vesicles, pancreas, and arachnoid membrane; vascular abnormalities including intracranial aneurysms, aortic dilatation and dissection and abdominal wall hernias. Renal manifestations include hypertension and renal insufficiency. Approximately 50% of individuals with ADPKD have end-stage renal disease (ESRD) by the age of 60 years. The prevalence of intracranial aneurysms is higher in those with a positive family history of aneurysms or subarachnoid hemorrhage (22%) than in those without such a family history (6%). Mitral valve prolapse occurs in up to 25% of affected individuals. Substantial variability in severity of renal disease and extrarenal manifestations occurs even within the same family. Blood pressure monitoring, follow up of renal function and ultrasound as well as MRI angiography for screening of intracranial aneurysms in patients at high risk is recommended. Also, echocardiography in patients with a heart murmur and those with a family history of a first-degree relative with a thoracic aortic dissection is indicated.

Prevalence of ADPKD is between 1:400 and 1:1,000. It is estimated that *PKD1* explains 85% and *PKD2* 15% of ADPKD cases. Approximately 10% of persons receiving renal transplants have ADPKD. Penetrance is very high: practically all older adults with a *PKD1* or *PKD2* pathogenic variant develop multiple bilateral cysts. Penetrance is reduced for ESRD although majority of individuals with truncating *PKD1* variants experience ESRD during their lifetimes. (ADPKD1 – GeneReviews).

Mutation nomenclature is based on GenBank accession NM_001009944.2 (*PKD1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and the established role of *PKD1* c.4957C>T, p.(Gln1653*) as a disease-causing variant, we classify it as pathogenic. Genetic counseling and family member testing are recommended. Disease caused by *PKD1* variants is inherited in an autosomal dominant manner, and thus each child of an affected individual has a 50% chance of inheriting the variant. A proband with autosomal dominant disease may have the disorder as a result of a *de novo* event. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

PKD1 c.4957C>T, p.(Gln1653*) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Apr 30, 2018
Sample received	Apr 30, 2018
Reported	May 30, 2018

On May 30, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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Geneticist

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Lab Director, Chief Medical Officer

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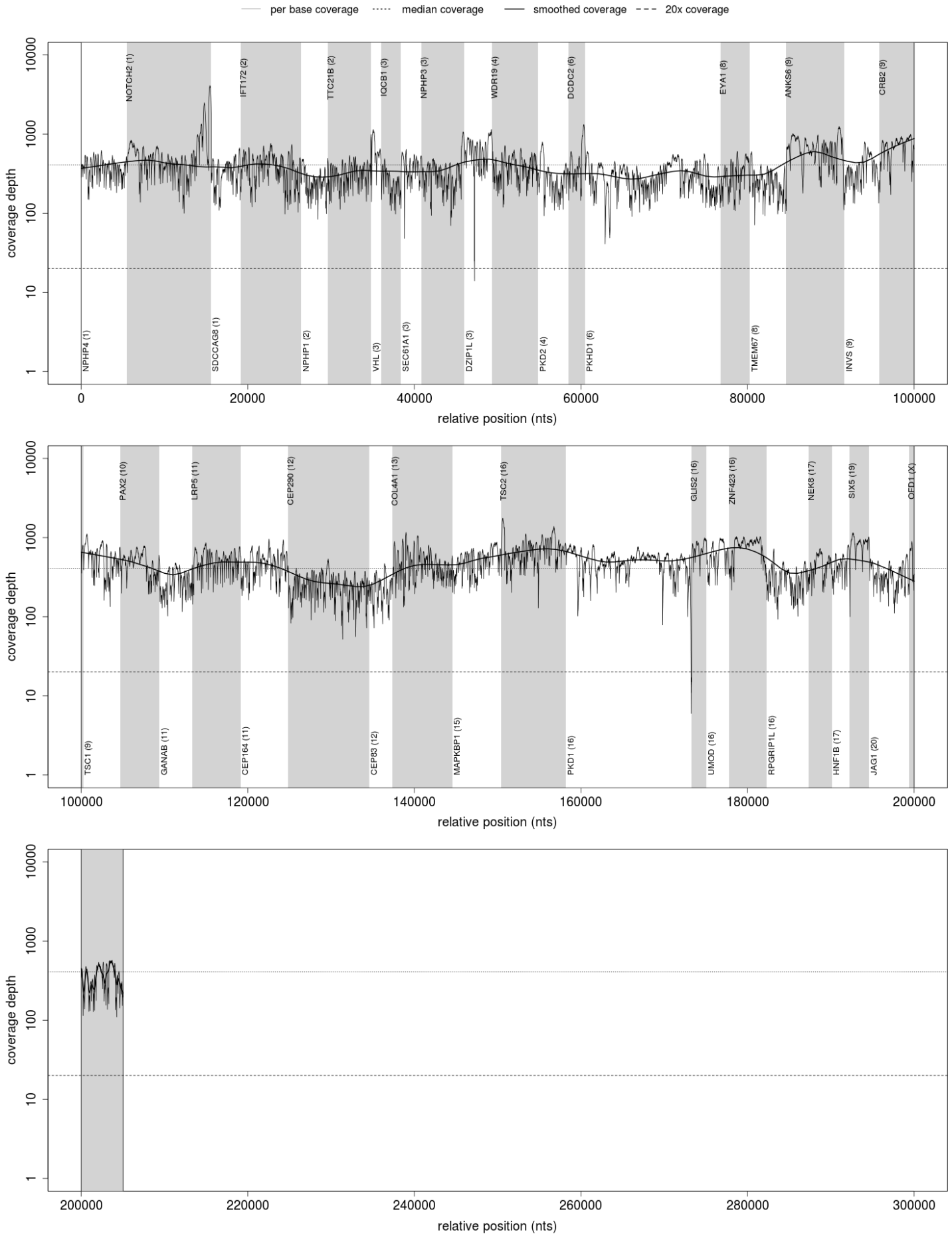
per base coverage
median coverage
smoothed coverage
20x coverage
coverage depth
relative position (nts)
NPHP4 (1)
NOTCH2 (1)
SDCCAG8 (1)
IFT172 (2)
NPHP1 (2)
TTC21B (2)
VHL (3)
IQCB1 (3)
SEC61A1 (3)
NPHP3 (3)
DZIP1L (3)
WDR19 (4)
PKD2 (4)
DCDC2 (6)
PKHD1 (6)
EYA1 (8)
TMEM67 (8)
ANKS6 (9)
INVS (9)
CRB2 (9)
TSC1 (9)
PAX2 (10)
GANAB (11)
LRP5 (11)
CEP164 (11)
CEP290 (12)
CEP83 (12)
COL4A1 (13)
MAPKBP1 (15)
TSC2 (16)
PKD1 (16)
GLIS2 (16)
UMOD (16)
ZNF423 (16)
RPGRIP1L (16)
NEK8 (17)
HNF1B (17)
SIX5 (19)
JAG1 (20)
OFD1 (X)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column 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 and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean 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). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. 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 as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. 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, which processes aligned sequence reads. 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: Our variant classification follows the 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. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon 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 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_025114.3(CEP290):c.6012-12T>A
NM_001845.4(COL4A1):c.*35C>A
NM_001845.4(COL4A1):c.*32G>T
NM_001845.4(COL4A1):c.*31G>T
NM_000503.4(EYA1):c.1051-12T>G
NM_000503.4(EYA1):c.640-15G>A
NM_000214.2(JAG1):c.1349-12T>G
NM_001009944.2(PKD1):c.12445-14T>C
NM_138694.3(PKHD1):c.7350+653A>G
NM_000548.3(TSC2):c.976-15G>A
NM_000548.3(TSC2):c.2838-122G>A
NM_000548.3(TSC2):c.5069-18A>G

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation e.g. 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
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.