Blueprint Genetics

*Sample report as of Nov 1st, 2020. 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 Kidney Disease Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**

36

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**

SUMMARY OF RESULTS

TEST RESULTS

Both NGS and Sanger sequencing data suggest that the patient is heterozygous for a chimeric *PKD1*/*PKD1P3* gene due to a gene conversion event that has occurred between *PKD1* and one of its pseudogenes *PKD1P3.* This gene conversion affects *PKD1* exon 23. The gene conversion event encompassing three rare amino acid alterations as a whole is classified as likely pathogenic. Please see below for clinical interpretation.

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

PRIMARY VARIANT TABLE: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKD1	NM_001009944.2	c.8279T>C, p.(Met2760Thr)	HET	missense_variant	AD	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	16:2153779	A/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	Possibly damaging	deleterious	disease causing	Polycystic kidney disease	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKD1	NM_001009944.2	c.8282G>C, p.(Arg2761Pro)	HET	missense_variant	AD	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	16:2153776	C/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	Possibly damaging	tolerated	polymorphism	Polycystic kidney disease	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKD1	NM_001009944.2	c.8291T>C, p.(Met2764Thr)	HET	missense_variant	AD	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	16:2153767	A/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	benign	deleterious	disease causing	Polycystic kidney disease	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Polycystic Kidney Disease Panel	12	313	68621	68498	308	99.82

TARGET REGION AND GENE LIST

The Blueprint Genetics Polycystic Kidney Disease Panel (version 3, Oct 19, 2019) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: DNAJB11, DZIP1L, GANAB, HNF1B, JAG1, LRP5*, NOTCH2*, PKD1*, PKD2, PKHD1, 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 36-year-old individual with suspected autosomal dominant polycystic kidney disease. Their renal ultrasound showed enlarged kidneys, some avascular cysts and a few echogenic foci. There were no obvious renal calculi nor hydroureteroneohprosis.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Polycystic Kidney Disease Panel identified a potential heterozygous chimeric *PKD1*/*PKD1P3* gene conversion event. Sanger sequencing, that was optimized to specifically target *PKD1*, detected five distinct nucleotide variants in exon 23. Three of the variants were rare missense variants leading to amino acid alterations: c.8279T>C, p.(Met2760Thr), c.8282G>C, p.(Arg2761Pro) and c.8291T>C, p.(Met2764Thr). Additionally, a relatively common missense variant c.8207C>G, p.(Pro2736Arg) and a synonymous variant c.8235T>G, p.(Ser2745=) were detected. These exon 23 variants were flanked by *PKD1*-specific sequence both upstream and downstream. All five detected variants occur together in one of the *PKD1* pseudogenes, namely *PKD1P3*. Furthermore, bioinformatic analysis of raw sequence data revealed that there was a drop of sequence coverage of *PKD1* exon 23 potentially caused by misalignment of sequence reads to the homologous region in the *PKD1P3* pseudogene. These findings support a gene conversion event where *PKD1* acquired the sequence including the missense variants from the pseudogene, seemingly *PKD1P3*.

Previously, similar event of gene conversion has been reported in patients with autosomal dominant polycystic kidney disease (ADPKD) (PMID: 9285784). In the first family, the index patient harboured five variants p.(Ser2745=), p.(Met2760Thr), p.(Arg2761Pro), p.(Met2764Thr), and p.(Ile2826Thr), and this haplotype segregated with the disease in the family. The haplotype was seen in four affected relatives (including the index patient) whereas it was absent in four unaffected family members. Also, another index patient from an unrelated family harboured a haplotype consisting four overlapping variants p.(Ser2745=), p.(Met2760Thr), p.(Arg2761Pro), (Met2764Thr) and a distinct p.(Leu2763Val) variant. These two index patients were not related and authors suggested that the sets of mutations arose independently by gene conversion events between *PKD1* and its homologs (PMID: 9285784). Furthermore, the variants seen in these two families are listed in the PKD1 Mutation Database as a possible example of exon 23 gene conversion event. A second gene conversion event listed in this database including variants p.(Ser2745=), p.(Met2760Thr), p.(Arg2761Pro), and p.(Met2764Thr) was seen in two independent families (unpublished, PKD1 Mutation Database). Generally, gene conversions cause human disease via different mechanisms altering wild-type protein structure and function that is likely also in relation to this potential gene conversion event.

Rare missense variants in chimeric *PKD1*/*PKD1P3* gene

PKD1 c.8279T>C, p.(Met2760Thr)

This variant has not been observed in the large reference population cohorts of the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). The affected amino acid is well conserved in mammals as well as in evolutionary more distant species, and there is a non-conservative physiochemical difference between the methionine and threonine (Grantham score 81). The variant is predicted damaging by all *in silico* tools used. The variant has been reported previously as part of a possible gene conversion in ADPKD patients (PMID: 9285784).

PKD1 c.8282G>C, p.(Arg2761Pro)

This variant has not been observed in the large reference population cohorts of the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). The affected amino acid is only moderately conserved in mammals as well as in evolutionary more distant species, but there is a large physiochemical difference between the arginine and proline (Grantham score 103). The *in silico* predictions are conflicting for the variant. The variant has been reported previously as part of a possible gene conversion in ADPKD patients (PMID: 9285784).

PKD1 c.8291T>C, p.(Met2764Thr)

This variant has not been observed in the large reference population cohorts of the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). The affected amino acid is well to moderately conserved in mammals as well as in evolutionary more distant species, and there is a non-conservative physiochemical difference between the methionine and threonine (Grantham score 81). The variant is predicted damaging by most of the *in silico* tools used. The variant has been reported previously as part of a possible gene conversion in ADPKD patients (PMID: 9285784). This variant has also been reported in a Taiwanese patient with PKD (PMID: 23985799).

PKD1

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% sequence homology with six known pseudogenes in chromosome 16.

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 (GeneReviews NBK1246). 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 lifetime (ADPKD1 – GeneReviews). Biallelic disease causing variants in *PKD1* have been indicated in early onset ADPKD (PMID: 29038287).

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%. The pLI value (probability for loss-of-function intolerance) for the gene is 1.0 (minimum 0.0 - maximum 1.0) in gnomAD population database, indicating intolerance for loss-of-function type variation in *PKD1*, such as nonsense or frameshift variants. Loss of normal function of *PKD1* is a well-established molecular mechanism leading to polycystic kidney disease 1 with multiple, different types of loss of function variants listed in HGMD® Professional 2019.4 in relation to polycystic kidney disease.

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

These rare missense variants *PKD1* c.8279T>C, p.(Met2760Thr), *PKD1* c.8282G>C, p.(Arg2761Pro) and *PKD1* c.8291T>C, p.(Met2764Thr) identified in the patient likely arose from a gene conversion event between *PKD1* and its pseudogene *PKD1P3*. However, it is not fully known based on the literature if these variants detected in other individuals with PKD are also due to a gene conversion event or have arisen independently in the *PKD1* gene due to another mechanism. Even though determining the

exact consequences of the gene conversion event on *PKD1* function was not possible, we predict that the chimeric *PKD1/PKD1P3* structure could alter the normal function of the *PKD1* gene and classify the conversion event as likely pathogenic. However, additional information is still required to confirm the pathogenicity of the gene conversion event. Disease caused by *PKD1* variants is inherited in an autosomal dominant manner, and thus each offspring of an affected individual is at a 50% risk of inheriting the variant and of being affected. A proband with autosomal dominant disease may have the disorder as a result of a *de novo* event. Genetic counseling and family member testing are recommended.

CONFIRMATION

PKD1 c.8279T>C, p.(Met2760Thr) has been confirmed with bi-directional Sanger sequencing.

PKD1 c.8282G>C, p.(Arg2761Pro) has been confirmed with bi-directional Sanger sequencing.

PKD1 c.8291T>C, p.(Met2764Thr) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On November 1, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

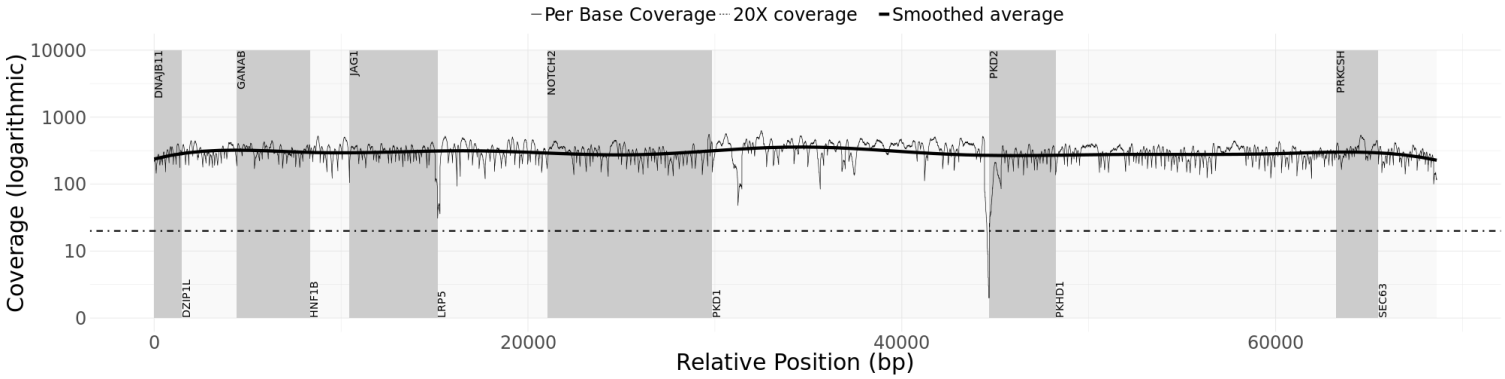
Johanna Huusko, PhD

Geneticist

Juha Koskenvuo, MD, PhD

Lab Director, Chief Medical Officer

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.



*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***

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 was sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Base called raw sequencing data was transformed into FASTQ format. To ensure high quality of the analysis, quality control reference sample was prepared together with patient sample.
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). Variant data was annotated with public variant databases (VcfAnno, VEP). The panel content was sliced from high-quality 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 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 gnomAD, ClinVar, HGMD Professional and Alamut Visual. 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 Database of Genomic Variants, ExAC, gnomAD and 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 the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call and 2) visual check-up of the variant at IGV is in-line with the variant call. 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 exons (heterozygous), less than 3 exons (homo/hemizygous) 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 next generation sequencing assay used to generate the panel

data (sensitivity for SNVs 99.9%, indels 11-50 bps 99.1%, one-exon deletions 100% and 1-9 exon duplications 75%, 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.
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.
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) and accredited by the College of American Pathologists (CAP #9257331). 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_000214.2(JAG1):c.1349-12T>G
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
NM_138694.3(PKHD1):c.8798-459C>A
NM_138694.3(PKHD1):c.7350+653A>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.