Blueprint Genetics

Sample report as of July 7, 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***

Glaucoma Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

PRIMARY SAMPLE TYPE
DNA

SAMPLE COLLECTION DATE

CUSTOMER SAMPLE ID
HN:
PAN: MRN:

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is homozygous for *CYP1B1* c.182G>A, p.(Gly61Glu), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CYP1B1	NM_000104.3	c.182G>A, p.(Gly61Glu)	HOM	missense_variant	AR	Pathogenic
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs28936700	GRCh37/hg19	2:38302350	C/T		
					PHENOTYPE	
					Glaucoma, primary congenital, Glaucoma, primary open angle glaucoma, juvenile-onset, Glaucoma, primary open angle, adult-onset, Peters anomaly	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**		
	67/229534	probably damaging	deleterious	disease causing		

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Glaucoma Panel	19	294	56551	56210	241	99.4

TARGET REGION AND GENE LIST

The Blueprint Genetics Glaucoma Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *CNTNAP2*, *COL4A1*, *CYP1B1*, *FOXC1*, *FOXE3*, *LMX1B*, *LTBP2*, *MAF*[#], *MYOC*, *OPA1*, *OPA3*, *OPTN*, *PAX6*, *PITX2*, *PXDN*, *TBK1*, *TEK*, *TMEM126A* and *WDR36*. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This panel should be used to detect single nucleotide variants and small insertions 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 detect balanced translocations or complex rearrangements, and it may not detect low-level mosaicism.

[#]The gene has suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with a mapping

quality score of MQ>20 reads.

The sensitivity to detect variants may be limited in genes marked with a number sign ([#]).

STATEMENT

CLINICAL HISTORY

Patient is a young child with clinical suspicion of congenital glaucoma. Patient's clinical features include bilateral buphthalmos, megalocornea, high myopia, cataracts, and vitritis (unknown etiology). Patient had goniotomy OU. Family history: patient's sib with congenital glaucoma.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Glaucoma Panel identified a homozygous missense variant *CYP1B1* c.182G>A, p.(Gly61Glu).

CYP1B1 c.182G>A, p.(Gly61Glu)

There are 63 individuals heterozygous and 2 individuals homozygous for this variant in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. The *CYP1B1* c.182G>A, p.(Gly61Glu) variant is predicted to be deleterious by all *in silico* tools utilized. Bejjani *et al.* reported 17 Saudi families with primary congenital glaucoma (PCG) with homozygous *CYP1B1* c.182G>A, p.(Gly61Glu) variant. In three additional families, the *CYP1B1* c.182G>A, p.(Gly61Glu) variant was identified as compound heterozygous with another disease-causing *CYP1B1* variant (PMID: 9463332). The *CYP1B1* c.182G>A, p.(Gly61Glu) variant is the most common disease-causing variant in Saudi Arabian patients with PCG. Subsequently, the variant has been reported in homozygous or compound heterozygous state in patients with juvenile-onset open angle glaucoma (PMID: 24099281, 26164761, 21596299) and also, as heterozygous in patients with primary open-angle glaucoma (PMID: 25091052). Al Jubaidi *et al.* described homozygous *CYP1B1* c.182G>A, p.(Gly61Glu) variant in a patient presenting with congenital anterior staphylomas (PMID: 24591815). The variant has also been submitted to ClinVar by other clinical testing laboratories (variation ID 7730). We have previously detected the *CYP1B1* c.182G>A, p.(Gly61Glu) variant in a homozygous state in two patients with *CYP1B1*-related disease (BpG unpublished observations). Functional studies have shown reduced enzyme activity for *CYP1B1* c.182G>A, p.(Gly61Glu) variant (PMID: 19643970, 19793111).

CYP1B1

The *CYP1B1* gene (Cytochrome P450, subfamily I, polypeptide 1; MIM *601771) encodes an enzyme which belongs to the CYP enzyme superfamily. The CYP enzymes are monooxygenases that catalyze a variety of reactions in drug metabolism and

synthesis of cholesterol, steroids, and other lipids. CYP1B1 expression has been shown to play an important role in the development and function of the trabecular meshwork that controls the outflow of aqueous humor from the eye (PMID: 26005555).

Biallelic pathogenic variants in *CYP1B1* cause autosomal recessive primary congenital glaucoma type 3A (CLG3A; MIM #231300). Primary congenital glaucoma is the most common type of childhood glaucoma, usually diagnosed in the first year of life (GeneReviews NBK1135). It is characterized by elevated intraocular pressure, enlargement of globe (buphthalmos), corneal edema, and optic nerve cupping. The prevalence of primary congenital glaucoma is estimated to range from 1/20,000 (in western countries) to 1/2,500 (in the Middle East), while males are more commonly affected than females and the disease is bilateral in around 70% of individuals (GeneReviews NBK1135). *CYP1B1* pathogenic variants have also been reported in individuals of French ancestry with primary open-angle glaucoma (POAG) (PMID: 15342693); in patients with congenital anterior staphylomas (PMID: 24591815); in individuals with Peters anomaly (OMIM 604229) (PMID: 11403040, 15621878, 15682044) and Axenfeld-Rieger (ARS) malformation (OMIM 180500) (PMID: 20827438); and in patients with Juvenile open-angle glaucoma (JOAG) (OMIM 137750) (PMID: 11774072).

Over 200 *CYP1B1* variants are annotated as disease causing (DM) in HGMD Professional 2021.4, of which the majority (around 60%) are missense variants. The common missense variants p.(Gly61Glu) and p.(Arg469Trp) have been shown in functional studies to decrease CYP1B1 activity compared to the wild-type enzyme, likely contributing to the observed disease pathology (PMID: 11740343). An incomplete penetrance has been observed in association with *CYP1B1* variants which suggests that additional genetic and/or environmental factors may modify their effect (PMID: 10655546; 11740343).

Mutation nomenclature is based on GenBank accession NM_000104.3 (*CYP1B1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

CYP1B1 c.182G>A, p.(Gly61Glu) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *CYP1B1* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is consistent with autosomal recessive inheritance. If the patient's parents are each confirmed to be carriers of this variant, any siblings of the patient will have a 25% chance of being homozygous for the variant and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier.

Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

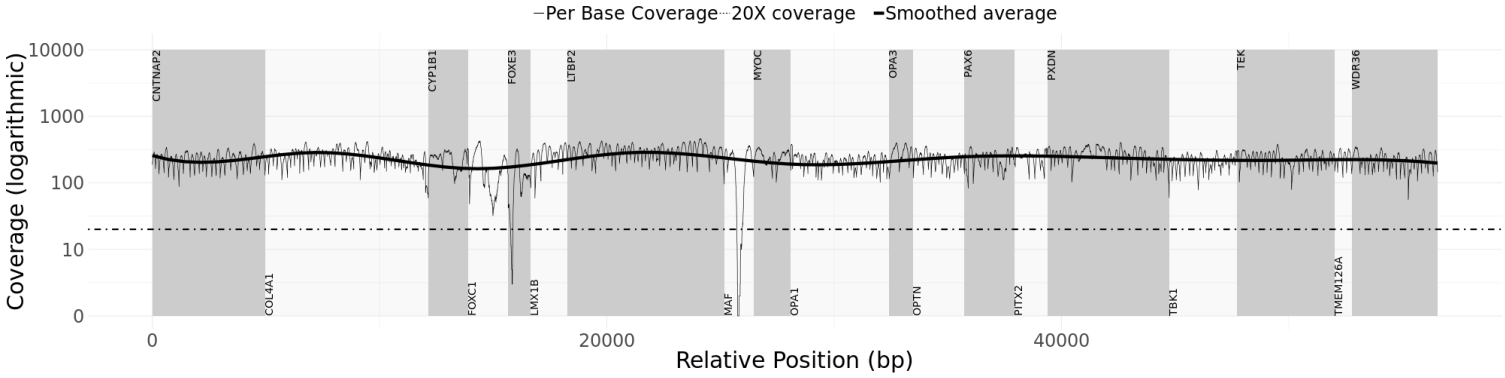
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

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 at Blueprint Genetics. 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.These steps were performed at Blueprint Genetics.
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. Bioinformatics and quality control processes were performed by Blueprint Genetics.
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. The interpretation was performed at Blueprint Genetics.
Variant classification: 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. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com
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.The confirmation of sequence alterations was performed at Blueprint Genetics.

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.The confirmation of copy number variants was performed at Blueprint Genetics.

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).

PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: MD, PhD, CLIA: 99D2092375

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000280.4(*PAX6*):c.*125537G>T, NM_000280.4(*PAX6*):c.1033-42_1033-26delATGTGTTCCTCAGTAACinsG, NM_000280.4(*PAX6*):c.524-41T>G, NM_000280.4(*PAX6*):c.142-14C>G, NM_000280.4(*PAX6*):c.-52+5delG, NM_000280.4(*PAX6*):c.-52+3_-52+6delAAGTinsTG, NM_000280.4(*PAX6*):c.-52+3_-52+4delAA, NM_000280.4(*PAX6*):c.-52+1delG, NM_000280.4(*PAX6*):c.-52+1G>A, NM_000280.4(*PAX6*):c.-115_-112delACTA, NM_000280.4(*PAX6*):c.-118_-117delTT, NM_000280.4(*PAX6*):c.-125dupG, NM_000280.4(*PAX6*):c.-128-1G>T, NM_000280.4(*PAX6*):c.-128-2delA, NM_000280.4(*PAX6*):c.-138_-129+3delCCTCATAAAGGTG, NM_000280.4(*PAX6*):c.-129+2T>A, NM_000280.4(*PAX6*):c.-129+1G>A, NM_001845.4(*COL4A1*):c.*35C>A, NM_001845.4(*COL4A1*):c.*32G>A/T, NM_001845.4(*COL4A1*):c.*31G>T, NM_000104.3(*CYP1B1*):c.-322A>C, NM_000104.3(*CYP1B1*):c.-337G>T, NM_130837.2(*OPA1*):c.449-34dupA, NM_130837.2(*OPA1*):c.2179-40G>C, NM_000325.5(*PITX2*):c.*520_*522delTAT, NM_000325.5(*PITX2*):c.412-11A>G, NM_153426.2(*PITX2*):c.-1214_-1213delAT, NM_001453.2(*FOXC1*):c.-429C>G, NM_001174146.1(*LMX1B*):c.140-37_140-21delGGCGCTGACGGCCGGGC

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 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
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.