Blueprint Genetics

Sample report as of Feb 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***

Targeted Variant Testing

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

INDIVIDUAL

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

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

TEST INFO

NUMBER OF VARIANTS
1

SUMMARY OF RESULTS

This individual is hemizygous for *RPGR* c.2426_2427del, p.(Glu809Glyfs*25), which is classified as pathogenic.

Targeted testing was requested for the following variants:

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
RPGR	NM_001034853.1	c.2426_2427del, p.(Glu809Glyfs*25)	HEM	frameshift_variant	X-linked	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	X:38145824	CCT/C		
					PHENOTYPE	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	Cone-rod dystrophy, X-linked, 1, Macular degeneration, X-linked atrophic, Retinitis pigmentosa, Retinitis pigmentosa 3	
	0/0	N/A	N/A	N/A		

STATEMENT

CLINICAL HISTORY

This individual is a 40-year-old male with classical retinitis pigmentosa and a family structure consistent with X-Linked inheritance. Previous testing in another laboratory has detected the *RPGR* c.2426_2427del, p.(Glu809Glyfs*25) variant in this individual. Therefore, targeted variant testing has been requested to confirm this finding.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Targeted Variant Testing identified a hemizygous frameshift variant *RPGR* c.2426_2427del, p.(Glu809Glyfs*25).

RPGR c.2426_2427del, p.(Glu809Glyfs*25)

This variant is absent in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. This variant occurs in the ORF15 exon of *RPGR* and is predicted to generate a frameshift resulting in a premature stop codon at position 25 in the new reading frame. This is likely to lead to a loss of normal protein function, both through premature truncation and alteration of the biochemical properties of the C-terminus of RPGR. This variant is a well-known disease causing variant and has previously been identified in several retinitis pigmentosa (RP) patients from different ethnicities, including in North American, Czech, Swedish, and Japanese patients (referred to as g.ORF15+673_674delAG in some publications) (PMID 23822596, 20064120, 11992260, 10932196, 14566651, 17093403, 27620828). Familial segregation of the *RPGR* c.2426_2427del, p.(Glu809Glyfs*25) variant with an RP phenotype has also been documented (PMID 20064120, 17093403, 14566651).

RPGR

RPGR (OMIM *312610) encodes a protein with a series of six N-terminal repeats comprising RCC1-like domain (RLD), characteristic of the highly conserved guanine nucleotide exchange factors. This protein localizes to the outer segment of rod photoreceptors and is essential for their viability. Pathogenic variants in *RPGR* are mainly associated with X-linked retinitis pigmentosa (XLRP, OMIM #300029). Over 70% of the patients with XLRP are explained by variants in *RPGR*. However, *RPGR* variants have also been described in patients with other retinal dystrophies including cone-rod dystrophy, atrophic macular degeneration and syndromic retinal dystrophy with ciliary dyskinesia and hearing loss. XLRP accounts for 10–20% of families with RP and is the most severe form of RP. In XLRP, affected males are symptomatic from early childhood and most patients are blind by the end of their third decade. Female carriers show a broad spectrum of fundus appearances, ranging from normal to extensive retinal degeneration. Typically, retinal disease in females with XLRP is less severe than that seen in males. In a study by Rozet *et al.*, age at disease onset in affected females was delayed compared to affected males with similar truncating variants (20–40 years vs. 10–20 years; PMID: 11950860).

Mutations in RPGR account for over 70% of the patients with XLRP. The *ORF15* exon of *RPGR* has been identified as a mutational hotspot. *ORF15* encodes 567 amino acids and has a repetitive domain with high glutamic acid and glycine content (PMID: 10932196, 12657579). The shorter RPGR isoform which includes *ORF15* (exon 15) is encoded by exons 1–15 and part of intron 15 (1152 amino acids, transcript ID NM_001034853). The other major isoform of RPGR has 815 amino acids and is encoded by exons 1–19 (NM_000328). Both isoforms share exons 1–15 (residues 1–635). Disease causing variants have been identified in exons 1–15 or in *ORF15*, while no disease-causing variants have been reported in exons 16–19 (PMID: 17195164).

Currently, HGMD lists 239 different disease-causing *RPGR* variants in NM_000328.2 and 265 in NM_001034853.1 (which includes exon *ORF15*) (HGMD Professional 2020.2). The majority of the variants are nonsense and frameshift variants leading to loss of function. The disease-causing missense variants are located within the RCC1-like domain (amino acids 54-367, exons 3-10; PMID: 28863407). There is notable inter- and intrafamilial phenotypic variability in XLRP caused by *RPGR* variants. In particular, patients with variants in exons 1–14 have been shown to demonstrate smaller visual fields than patients with variants in *ORF15* exon (PMID: 14564670). Truncating variants in the C-terminal part of *ORF15* have been associated with XL cone-rod dystrophy (i.e c.2965G>T p.Glu989*, c.3197_3198delAG, c.3300_3301delTA, c.3388_3389delTT) (HGMD; PMID: 23150612, 22264887). It has been concluded that variants located in exons 1–14 and the 5′ end of ORF15

cause RP, and variants at the 3′ end of ORF15 cause cone-rod dystrophy (PMID: 32047640).

Mutation nomenclature is based on GenBank accession NM_001034853.1 (*RPGR*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

A hemizygous *RPGR* c.2426_2427del, p.(Glu809Glyfs*25) variant was detected in this individual. Thus, the result is consistent with the research results. Genetic counselling is recommended.

Variant classification

RPGR c.2426_2427del, p.(Glu809Glyfs*25) is classified as pathogenic, based on currently available evidence supporting its disease-causing role.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

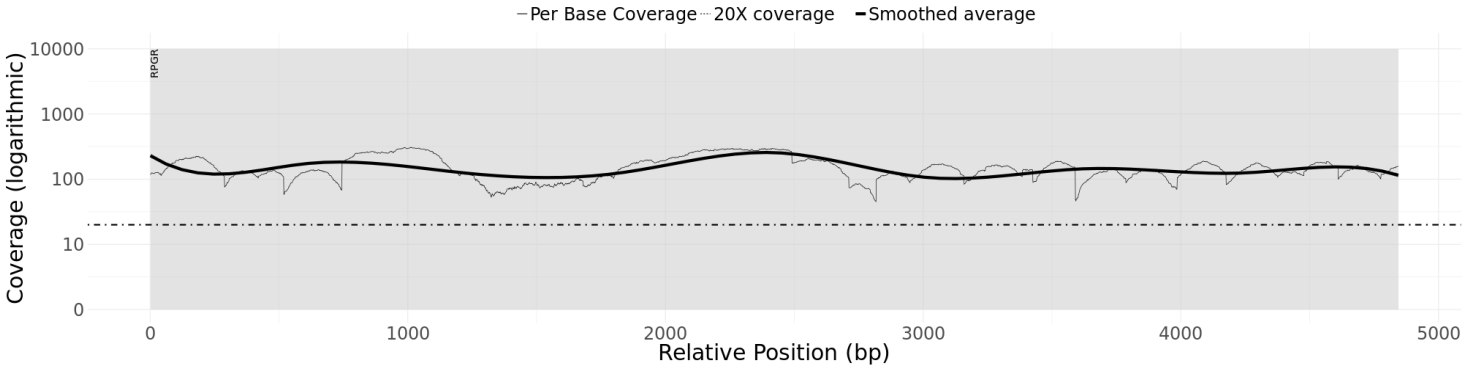
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

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

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.
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: Additional confirmation has been used when the variant quality score (QS) has not met the internal threshold for a true positive/negative call.
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).

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.
NGS = Next Generation Sequencing
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.
WT = wild type (normal allele)