

Blueprint Genetics



Retinal Dystrophy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL
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PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		42	Female	
PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE		CUSTOMER SAMPLE ID	
Saliva				

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for two variants in *EYS*: c.2826_2827del, p.(Val944Glyfs*9) is pathogenic and *EYS* c.2652dupT, p.(Lys885*) is likely 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
EYS	6:65612024	NM_001142800.1	c.2826_2827del, p.(Val944Glyfs*9)	frameshift	HET	Pathogenic
	ID rs878853349	EXAC AC/AN 0/0	POLYPHEN N/A	SIFT N/A	MUTTASTER N/A	
	OMIM	PHENOTYPE Retinitis pigmentosa		INHERITANCE AR	COMMENT -	
EYS	6:65612382	NM_001142800.1	c.2652dupT, p.(Lys885*)	frameshift	HET	Likely pathogenic
	ID .	EXAC AC/AN 0/0	POLYPHEN N/A	SIFT N/A	MUTTASTER N/A	
	OMIM	PHENOTYPE Retinitis pigmentosa		INHERITANCE AR	COMMENT -	

Please see [APPENDIX 2: Additional Findings](#)

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Retinal Dystrophy Panel	181	3177	574981	572991	249	99.6

TARGET REGION AND GENE LIST

Blueprint Genetics Retinal Dystrophy Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with retinal dystrophy: ABCA4, ABHD12, ADAM9, ADGRV1, AHI1, AIPL1, ALMS1*, ARL6, ARL13B, ATF6, B9D1, B9D2, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, BEST1, C2ORF71, C5ORF42, C8ORF37, CABP4, CACNA1F, CACNA2D4, CAPN5, CC2D2A, CDH23, CDHR1, CEP41, CEP164, CEP290*, CERKL, CHM, CIB2, CLN3, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL2A1, COL9A1, COL9A2, COL9A3, COL11A1, COL11A2, COL18A1, CRB1, CRX, CSPP1, CYP4V2, DFNB31, DHDDS, DTHD1, EFEMP1, ELOVL4, EYS*,

FAM161A, FBLN5, FLVCR1, FRMD7, FZD4, GNAT1, GNAT2, GNPTG, GPR179, GRK1, GRM6, GUCA1A, GUCY2D, HARS, HK1, HMX1, IDH3B, IFT140, IFT172, IMPDH1, IMPG1, IMPG2, INPP5E, INVS, IQCB1, KCNJ13, KCNV2, KIAA0586, KIF7, KIF11, KLHL7, LCA5, LRAT, LRIT3, LRP2, LRP5*, MAK, MERTK, MKKS, MKS1, MVK, MYO7A, NDP, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NRL, NYX, OAT, OFD1, OPA1, OPA3, OTX2, PANK2, PCDH15, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, PDZD7, PEX1, PEX2, PEX7, PHYH, PRCD, PROM1, PRPF3, PRPF8, PRPF31, PRPH2, RAX2, RBP3, RD3, RDH5, RDH12, RGR, RHO, RLBP1, RP1, RP1L1, RP2, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, SAG, SDCCAG8, SEMA4A, SNRNP200, SPATA7, TCTN1, TCTN2, TCTN3, TMEM67, TMEM107, TMEM126A, TMEM138, TMEM216, TMEM231, TMEM237, TOPORS, TRIM32, TRPM1, TSPAN12, TTC8, TTC21B, TTPA, TULP1, USH1C, USH1G, USH2A, VCAN, VPS13B, WDR19, ZNF423 and ZNF513. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of retinal dystrophy mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELS). In addition, the Retinal Dystrophy Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): <http://blueprintgenetics.com/pseudogene/>. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

STATEMENT

CLINICAL HISTORY

Patient is a 42-year-old female with peripheral pigmentary changes OU and clinical diagnosis of retinitis pigmentosa.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Retinal Dystrophy Panel identified two heterozygous variants in *EYS*: a 2-bp deletion c.2826_2827del, p.(Val944Glyfs*9) (rs878853349) and a 1-bp duplication *EYS* c.2652dupT, p.(Lys885*). Due to the large genomic distance of these variants we could not determine whether they occur in the same or different alleles.

EYS c.2826_2827del results in a frameshift and premature truncation of the protein (952aa out of 3144aa). The variant has not been reported in the Exome Aggregation Consortium (ExAC) database ([ExAC](#)). Off note, this region is only covered in 11,000 individuals in the ExAC, which may indicate a low-quality site. The c.2826_2827del variant has been described in the literature in one patient with autosomal recessive retinitis pigmentosa (arRP) who also carried another mutation in the *EYS* gene (ex 19 del) (PMID: [26806561](#)). Also, the variant has been detected in clinical testing in one patient with retinal dystrophy (ClinVar Variation ID [236446](#)).

EYS c.2652dupT, p.(Lys885*) has not been observed in large reference population cohorts (Exome Aggregation Consortium ([ExAC](#)) or Genome Aggregation Database ([gnomAD](#))). This 1-bp duplication generates a premature truncation of the protein product. To our knowledge, this variant has not been reported in the public mutation databases or described in the literature.

EYS (MIM [*612424](#)) is an ortholog of the *Drosophila* 'eyes shut' (*eyes*) gene. The predicted protein consists of multiple epidermal growth factor (EGF)-like domains in its N-terminus followed by several laminin G-like domains, interspersed by further EGF-like repeats, at the C-terminus. Based on the known function of the *Drosophila* ortholog, *EYS* is expected to play an important role in retinal morphogenesis and maintenance of its integrity. Homozygous and compound heterozygous mutations in *EYS* have been identified in patients with autosomal recessive retinitis pigmentosa (arRP; PMID: [20537394](#), [21069908](#), [22302105](#), [20333770](#)). Mutations in *EYS* are one of the major causes of arRP, accounting for 5-16% of the cases. Patients with mutations in *EYS* demonstrate classic RP with night blindness as the initial symptom, followed by gradual constriction of the visual field and a decline of visual acuity later in life. The onset of symptoms typically occurs between the second and fourth decade of life. Most of the disease-causing variants in *EYS* are truncating changes (nonsense, frameshift, consensus splice site). The pathogenic missense mutations are mostly located in functional conserved *EYS* domains and are more prevalent in the domains of the second half of the protein (PMID: [21069908](#)). Currently, there are altogether 190 variants associated with RP in HGMD (September 2016). In the ExAC control cohorts, heterozygous truncating *EYS* variants (nonsense, frameshift) are detected in 1 per 523 individuals, however no homozygous truncating *EYS* variants have been reported.

Mutation nomenclature is based on GenBank accession NM_001142800.1 (*EYS*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and well-established role of *EYS* c.2826_2827del, p.(Val944Glyfs*9) as a disease-causing mutation, we classify it as pathogenic. We classify the identified *EYS* c.2652dupT, p.(Lys885*) as likely pathogenic, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, mutation type (frameshift)). Disease caused by *EYS* mutations is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants occur in *cis* (the same copy of the gene) or in *trans* (different copies of the gene). Compound heterozygosity of the identified variants (variants in different alleles) would most likely explain the patient's disease. If both parents are found to be carriers of one of the mutations, each sibling of an affected individual has a 25% chance of being a compound heterozygous for the mutations and thus being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Genetic counseling and family member testing is recommended. BpG offers mutation testing for the family if requested.

CONFIRMATION

EYS c.2826_2827del, p.(Val944Glyfs*9) and *EYS* c.2652dupT, p.(Lys885*) were confirmed by bidirectional Sanger sequencing.

STEP	DATE
Order date	Apr 13, 2017
Sample received	Apr 13, 2017
Reported	May 04, 2017

On May 16, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



Sari Tuupanen, Ph.D.
Geneticist



Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer



Eeva-Marja Sankila, MD, Ph.D.
Clinical Consultant

APPENDIX 2: ADDITIONAL FINDINGS

This table includes variants that either are not thought to be the likely cause for patient's phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient's phenotype), are secondary findings potentially relevant to patient's medical care (risk variants, heterozygous pathogenic or likely pathogenic variants for autosomal dominant disorders not related to patient's current phenotype) or carrier status for pathogenic or likely pathogenic variants for autosomal recessive or X-linked disorder not suspected in the patient.

VARIANT TABLE: ADDITIONAL GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
MVK	12:110029080	NM_000431.2	c.803T>C, p.(Ile268Thr)	missense	HET	Pathogenic
	ID rs104895304	EXAC AC/AN 13/121396	POLYPHEN possibly damaging	SIFT deleterious	MUTTASTER disease causing	
	OMIM	PHENOTYPE Hyper-IgD syndrome, Mevalonic aciduria		INHERITANCE AR	COMMENT -	
GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
COL9A2	1:40768470	NM_001852.3	c.1608_1614del, p.(Leu537Argfs*4)	frameshift	HET	Variant of uncertain significance
	ID .	EXAC AC/AN 0/0	POLYPHEN N/A	SIFT N/A	MUTTASTER N/A	
	OMIM	PHENOTYPE Multiple epiphyseal dysplasia type 2 (EDM2), Stickler syndrome		INHERITANCE AR	COMMENT -	

NOTES REGARDING ADDITIONAL FINDINGS

MVK c.803T>C, p.(Ile268Thr) is a pathogenic variant associated with hyperimmunoglobulin D and periodic fever syndrome (ClinVar ID 11932). The variant has also been reported in compound heterozygous state with p.(Ala334Thr) in one RP proband and her affected brother (PMID: [24084495](#)). Disease caused by *MVK* mutations is inherited in an autosomal recessive manner, thus the identified variant is not sufficient to cause disease without another disease causing variant in the same gene. Genetic counseling is recommended.

Mutations in *COL9A2* have been associated with autosomal dominant multiple epiphyseal dysplasia and in one study, autosomal recessive Stickler syndrome (MIM *120260). The clinical significance of the identified truncating variant *COL9A2* c.1608_1614del, p.(Leu537Argfs*4) is unknown.

APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.

Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.

Confirmation. Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.

Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

Accreditation. 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.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.

Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).

Reporting. Reporting was done using an HGNC-approved gene nomenclature.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

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GLOSSARY OF USED ABBREVIATIONS:

POS = genomic position of the variant in the format of chromosome:position

ID = rsID in dbSNP

Transcript = GenBank accession for reference sequence used for variant nomenclature

Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database

AD = autosomal dominant

AR = autosomal recessive

OMIM = Online Mendelian Inheritance in Man®

ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)

het = heterozygous

hom = homozygous

Del/Dup = Deletion and Duplication

CNV = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.
