Blueprint Genetics

*Sample report as of June 14th, 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.***

Optic Atrophy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

33

PRIMARY SAMPLE TYPE
DNA

SAMPLE COLLECTION DATE **CUSTOMER SAMPLE ID**

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *OPA1* c.983A>G, p.(Lys328Arg), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
OPA1	NM_015560.2	c.983A>G, p.(Lys328Arg)	HET	missense_variant, splice_region_variant	AD,AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	3:193355853	A/G		
					PHENOTYPE	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	Behr synrome, Mitochondrial DNA depletion syndrome 14, Ophthalmoplegia, myopathy, ataxia, and neuropathy, Optic atrophy, Optic atrophy 1, Optic atrophy with or without deafness	
	0/0	probably damaging	deleterious	disease causing		

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Optic Atrophy Panel	39	395	70964	70796	256	99.76

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	3224	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Optic Atrophy Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ACO2*, *AFG3L2**, *ATAD3A**, *AUH*, *C12ORF65*, *C19ORF12*, *CISD2**, *DNAJC19*, *DNM1L*, *FDXR*, *ISCA2*, *MECR*, *MFN2*, *MGME1*, *MT-ATP6*, *MT-ATP8*, *MT-CO1*, *MT-CO2*, *MT-CO3*, *MT-CYB*, *MT-ND1*, *MT-ND2*, *MT-ND3*, *MT-ND4*, *MT-ND4L*, *MT-ND5*, *MT-ND6*, *MT-RNR1*, *MT-RNR2*, *MT-TA*, *MT-TC*, *MT-TD*, *MT-TE*, *MT-TF*, *MT-TG*, *MT-TH*, *MT-TI*, *MT-TK*, *MT-TL1*, *MT-TL2*, *MT-TM*, *MT-TN*, *MT-TP*, *MT-TQ*, *MT-TR*, *MT-TS1*, *MT-TS2*, *MT-TT*, *MT-TV*, *MT-TW*, *MT-TY*, *MTPAP*, *NARS2*, *NDUFAF3*, *NDUFS1*, *NR2F1*, *OPA1*, *OPA3*, *PDSS1#*, *POLG*, *PRPS1**, *RTN4IP1*, *SLC19A2*, *SLC19A3*, *SLC25A46*, *SLC52A2*, *SNX10*, *SPG7*, *SUCLA2*, *TIMM8A**, *TMEM126A*, *TSFM#*, *UCHL1*, *WFS1*, *YME1L1** and *ZNHIT3#*. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: PDSS1 (NM_014317:2), TSFM (NM_001172696:5) and ZNHIT3 (NM_001281432:5).
*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/

#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 an asterisk (*) or number sign (#).

STATEMENT

CLINICAL HISTORY

Patient is a 33-year-old individual referred for genetic testing using the Blueprint Genetics (BpG) Optic Atrophy Panel. Family history: patient's mother affected.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Optic Atrophy Panel identified a heterozygous missense, splice region variant *OPA1* c.983A>G, p.(Lys328Arg).

***OPA1* c.983A>G, p.(Lys328Arg)**

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. The *OPA1* c.983A>G, p.(Lys328Arg) variant affects a highly conserved amino acid in the dynamin type-G domain of the protein, there is small physicochemical difference between Lys and Arg (Grantham score 26, [0-215]), and all in *silico* tools utilized predict the alteration to be deleterious. In addition, the *OPA1* c.983A>G, p.(Lys328Arg) variant substitutes the second-to-last nucleotide of exon 9 of *OPA1*. In *silico* splice prediction tools (SSF, MaxEntScan, NNSPLICE) predict the substitution will slightly weaken the splice donor of intron 9 and may therefore disrupt splicing.

Baris *et al.* first reported the *OPA1* c.983A>G, p.(Lys328Arg) variant as heterozygous in a mother and daughter affected with autosomal dominant optic atrophy (ADOA) (PMID: 14961560). Consistent with the *in silico* predictions, the *OPA1* c.983A>G variant was shown by RT-PCR of patient RNA to result in the *in-frame* skipping of exon 9, which is predicted to lead to the loss of 38 amino acids; however, the RNA from the normal control used in the analysis had significantly weaker expression compared to the RNA from the affected individuals, and no quantification of the skipped transcript was performed; therefore, the functional relevance of this splicing result is unclear (PMID: 14961560). The *OPA1* c.983A>G, p.(Lys328Arg) variant has subsequently been reported in multiple additional patients affected with optic atrophy (PMID: 16418602, 26385429, 22857269), including one family with three individuals affected with both optic atrophy and mild hearing loss (PMID: 23384603). Almind *et al.* described the *OPA1* c.983A>G, p.(Lys328Arg) variant in 10 Danish families, where a common haplotype in nine of the families suggested that they descend from a single founder (PMID: 22857269).

The *OPA1* c.983A>G, p.(Lys328Arg) variant has also been detected by other laboratories in the context of clinical testing

and submitted to ClinVar (variation ID 95733). In addition, we have previously detected the *OPA1* c.983A>G, p.(Lys328Arg) variant in patients with *OPA1*-related optic atrophy (BpG unpublished observations).

OPA1

The *OPA1* gene (MIM *605290) encodes a protein that localizes to the inner mitochondrial membrane and regulates several important cellular processes including stability of the mitochondrial network, mitochondrial bioenergetic output, and sequestration of proapoptotic cytochrome c oxidase molecules within the mitochondrial cristae spaces. *OPA1* produces multiple mRNA isoforms as a result of the alternative splicing of exon 4 and exons named 4b and 5b (PMID: 11810270). Kamei *et al*. (2005) have shown that rat transcripts from brain, retina, and retinal ganglion cells exhibited similar *OPA1* isoform patterns with a predominance of the one lacking exons 4b and 5b, as found in the corresponding human tissues (PMID: 16249510). Out of 30 exons total (including exons 4b and 5b), Deletter *et al*. (2001) have screened a cohort of 19 unrelated patients with dominant optic atrophy, and demonstrated that majority of the variants were truncating (65%) and located in exons 8 to 28, but a number of them were amino acid changes predominantly found in the GTPase domain (exons 8 to 15) (PMID: 11810270). They suggested that alteration in GTPase activity and loss of the last seven C-terminal amino acids that putatively interact with other proteins were critical in protein function and variants in these domains lead to dominant optic atrophy.

Pathogenic variants in *OPA1* have been associated with autosomal dominant optic atrophy 1 (MIM #165500), optic atrophy plus syndrome (MIM #125250), and autosomal recessive Behr syndrome (MIM # 210000). Autosomal dominant optic atrophy is characterized by an insidious onset of visual impairment in early childhood with moderate to severe loss of visual acuity, temporal optic disc pallor, color vision deficits, and centrocecal scotoma of variable density (MIM #165500). The predominance of *OPA1* null mutations in patients with optic atrophy suggests that the mechanism underlying optic atrophy is haploinsufficiency (PMID: 11440988, 11810270). Syndromic optic atrophy, also known as DOA+ syndrome (MIM #125250), is an autosomal dominant neurologic disorder characterized most commonly by an insidious onset of visual loss and sensorineural hearing loss in childhood with variable presentation of other clinical manifestations including progressive external ophthalmoplegia, muscle cramps, hyperreflexia, and ataxia. A few cases with Behr syndrome (MIM #210000) presenting with compound heterozygous *OPA1* variants have been reported. Behr syndrome is characterized by early-onset optic atrophy accompanied by neurologic features, including ataxia, pyramidal signs, spasticity, and intellectual disability. Heterozygous carriers of a single Behr syndrome-causing variant may present with a milder phenotype (MIM #210000, PMID: 21636302, 25012220). Bi-allelic *OPA1* variants may also lead to childhood-onset, complex and severe multi-system recessive mitochondrial disorders, where optic atrophy might not represent the main feature (PMID: 28494813).

More than 560 distinct disease-causing (DM) *OPA1* variants are listed in the HGMD Professional variant database (transcript NM_015560.3; HGMD Professional 2021.4). The majority are truncating variants (nonsense, frameshift, variants affecting splicing). Approximately one third are missense variants and the rest are frame-retaining alterations or gross deletions or insertions. In addition, 34 disease-causing variants are listed in the alternative isoform (NM_130837.3; opa1tv8).

Mutation nomenclature is based on GenBank accession NM_015560.2 (*OPA1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

OPA1 c.983A>G, p.(Lys328Arg) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *OPA1* c.983A>G, p.(Lys328Arg) is expected to be inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected.

Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

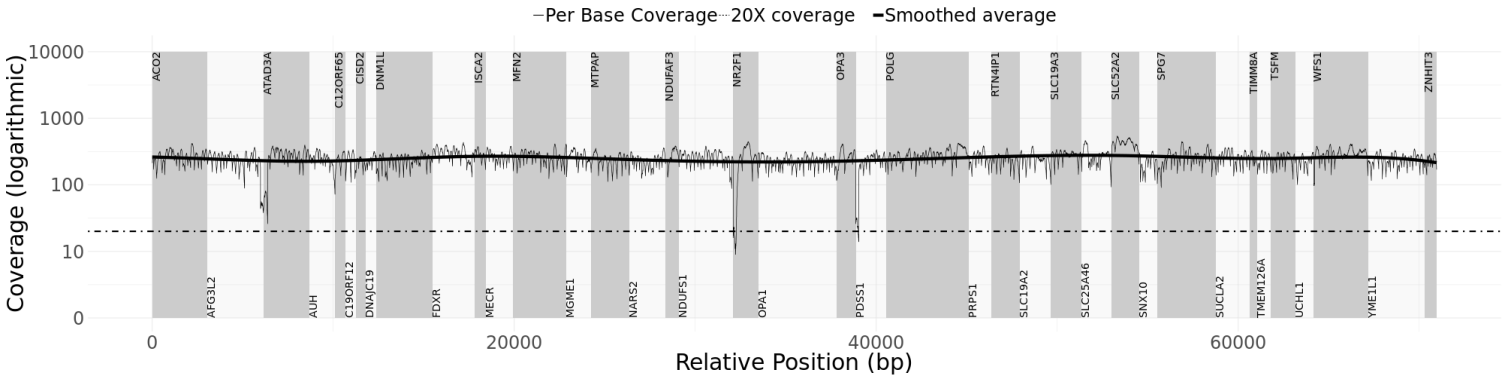
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

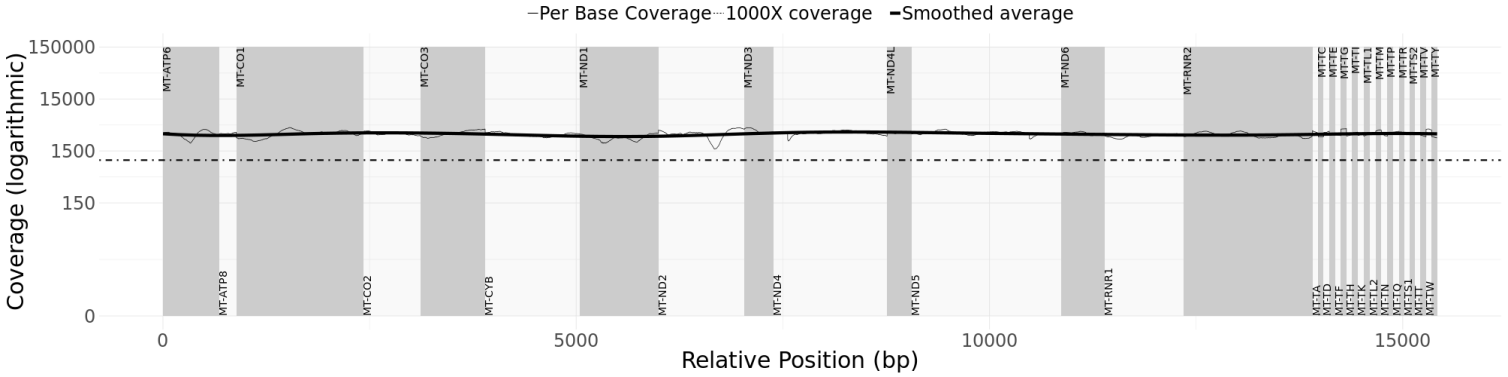
Name

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.



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_025243.3(*SLC19A3*):c.980-14A>G, NM_130837.2(*OPA1*):c.449-34dupA, NM_130837.2(*OPA1*):c.2179-40G>C, NM_006005.3(*WFS1*):c.-43G>T, NM_024531.4(*SLC52A2*):c.-110-1G>A, NM_004085.3(*TIMM8A*):c.133-23A>C

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.