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***

Whole Exome Family

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

0

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

SUMMARY OF RESULTS

PRIMARY FINDINGS

Analysis of whole exome sequence variants in previously established disease genes

The patient has a homoplasmic (94.8%) *MT-ATP6* m.8969G>A, p.(Ser148Asn) variant, which is pathogenic. This variant was not detected in the mother's sample.

Del/Dup (CNV) analysis

Negative

PRIMARY MITOCHONDRIAL FINDINGS: SEQUENCE ALTERATIONS

GENE	POS	AF	LEVEL	GENOTYPE	TRANSCRIPT	NOMENCLATURE	CLASSIFICATION
MT-ATP6	MT:8969	0.948	95%	HOMOPLASMIC	NC_012920.1	m.8969G>A	**Pathogenic**

CONSEQUENCE	PHENOTYPE	INHERITANCE
missense_variant	Ataxia and polyneuropathy, adult-onset, Cardiomyopathy, infantile hypertrophic, Leber hereditary optic neuropathy, Leigh syndrome, Neuropathy, ataxia, and retinitis pigmentosa, Striatonigral degeneration, infantile, mitochondrial	Mitochondrial

SEQUENCING PERFORMANCE METRICS - NUCLEAR GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 20X
Index	152	99.77
Mother	125	99.56
Father	119	99.72

SEQUENCING PERFORMANCE METRICS - MITOCHONDRIAL GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 1000X
Index	3924	100
Mother	6036	100
Father	6330	100

TEST INFORMATION

Blueprint Genetics Whole Exome Family Test (version 3, February 2023) consists of sequence analysis of all protein-coding genes in the genome for the proband and affected/unaffected family members, coupled with Whole Exome Deletion/Duplication (CNV) Analysis. The test targets all protein-coding exons, exon-intron boundaries (± 20 bps), and selected noncoding, deep intronic variants (listed in Appendix). This test should be used to detect single nucleotide variants and small insertions and deletions (INDELs) up to 220 bps, and copy number variations defined as single exon or larger deletions and duplications. In addition, analysis of the mitochondrial genome is included. This test should not be used for the detection of repeat expansion disorders. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

The analysis of the Whole Exome Family Test is primarily focused on established disease genes that have been previously associated with genetic disorders. The genes with known clinical association include those curated by Blueprint Genetics (BpG) and included in BpG diagnostic panels (>4140 genes). These genes are supplemented with genes included in The Clinical Genomics Database (>4320 genes) and the Developmental Disorders Genotype-Phenotype Database (DD2GP) (>2190 genes). The total number of genes that are considered as clinically associated in the Whole Exome Family Plus analysis is >4780 (and the number is constantly updated).

If analysis of exome variants in previously established disease genes is inconclusive, exome variant data is also analyzed for variants that are not located within known clinically associated genes but have properties that make them candidates for potentially disease-causing variants (please see Appendix: Summary of the Test).

STATEMENT

CLINICAL HISTORY

Patient is an infant with spasms, developmental delay, mild ventricular hypertrophy, and failure to thrive.

There is no reported parental consanguinity or family history of similar disease.

Consent has been received to report secondary findings for all family members.

CLINICAL REPORT

Whole-exome sequence analysis of variants in previously established disease genes

Sequence analysis using the Blueprint Genetics (BpG) Whole Exome Family identified a homoplasmic missense variant *MT-ATP6* m.8969G>A, p.(Ser148Asn). This variant was not detected in the mother's sample. The sequencing depth at this genomic position is 5793 reads in the mother.

MT-ATP6 m.8969G>A, p.(Ser148Asn)

The allele frequency in patient population is 0.0017 and in healthy population 0.0007 in the HmtVar database, which contains a dataset of >49,304 complete human mitochondrial genomes from GenBank, of which 44,058 from healthy and 5,246 from diseased individuals (PMID: 30371888, HmtVar). This variant is present in 4 heteroplasmic patients, and 0 homoplasmic in gnomAD v3.1 containing 76,156 whole genomes, of which 56,424 samples passed mitochondrial-specific filters.

The conservation of serine at position 148 is 100% based on 45 species (of which 40 are mammals) available in the MITOMASTER analysis framework. The variant is predicted to be likely pathogenic by APOGEE (a consensus classifier by MitImpactwhich analyzes and combines input from 13 independent pathogenicity predictors and 6 meta-predictors).

m.8969G>A has been reported as a *de novo* variant in a patient with mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA). Wolff-Parkinson-White was also reported in this case. Studies of fibroblasts derived from the patient revealed a decrease in oligomycin-sensitive respiration, a finding which is consistent with a complex V defect. The cellular respiration studies performed in fibroblasts also revealed a decrease in basal respiration and a decrease in respiratory capacity. The heteroplasmy level in muscle was 88% (PMID 25037980). Another *de novo* patient was a boy who died at age 7.5 months carrying the m.8969G>A mutation in the *MTATP6* gene with 95% heteroplasmy in blood, fibroblasts and skeletal muscle (PMID: 27450679).

Also, it has been reported in a 14-year-old Chinese female who initially developed an isolated IgA nephropathy followed by a complex clinical presentation with brain and muscle problems, showing epileptic episodes and decreased muscle strength, brain atrophy, severe hearing impairment, Wolff-Parkinson-White syndrome, and increased fasting level of glucose. The variant was maternally inherited from the healthy mother. Evaluation of renal biopsy samples revealed a decreased staining of cells induced by COX and NADH dehydrogenase activities, and a strong fragmentation of the mitochondrial network. Increasing the mutation

load in hybrid cell lines was paralleled by the appearance of abnormal mitochondrial morphologies, diminished respiration and enhanced production of reactive oxygen species. The mutation was particularly abundant (89%) in the kidney compared to blood and urine, which is likely the reason why this organ was affected first (PMID 27812026).

Furthermore, this variant was identified in three patients from a cohort of 102 probands with congenital sideroblastic anemia. All showed mild facial dysmorphism, mild neuro-cognitive dysfunction and/or organic aciduria. Patient 1, blood sample showed 81.3% heteroplasmy; patient 2 showed 82.1%, and patient 3 was found to be 99.2% heteroplasmic for the m.8969G>A. Last patient had a more severe phenotype, showing biventricular hypertrophic and dilated cardiomyopathy by echocardiography, facial dysmorphism and failure to thrive soon after birth (PMID 30006447).

Finally, a family with two affected children has been described. Sibling had a mild and stable phenotype with lactic acidosis, poor growth and intellectual disability. Heteroplasmy levels were 75% and 95% in muscle. The variant was inherited from a healthy mother (heteroplasmy level in muscle 9%). The brother with higher level had an early-onset disease, while the other only develop a mild intellectual disability identified at the age of 9. Histologic examination of the muscle biopsy sample and respiratory chain enzyme activities were normal, but ATP production was reduced (PMID 29350304).

The variant has been detected by other laboratories in the context of clinical testing and submitted to ClinVar (variation ID 191364).

MT-ATP6

The *MT-ATP6* gene (MIM *516060) encodes ATP synthase 6 (also known as complex V), a subunit of a large enzyme called ATP synthase. ATP synthase 6 is involved in the final step of oxidative phosphorylation. Variants in *MT-ATP6* have been reported to associate with Leigh syndrome (MILS; maternally inherited form of the Leigh syndrome; MIM #256000) and NARP syndrome (**N**europathy, **A**taxia, and **R**etinitis **P**igmentosa; MIM #551500). *MT-ATP6*-related disease is highly variable clinically, with both pediatric-onset and adult-onset and the disease is generally multi-systemic (PMID: 30763462). In addition to the well-known association with Leigh syndrome and NARP syndrome, recurrent presentations of *MT-ATP6* pathogenic variants include spinocerebellar ataxia, Charcot Marie Tooth, and familial upper motor neuron disease. The ClinGen Mitochondrial Diseases Expert Panel has classified the *MT-ATP6* gene as "Definitive" after Gene-Disease Clinical Validity assessment for Leigh syndrome (MT-ATP; Classification - 06/28/2021).

Isolated cases have also been reported with pathogenic *MT-ATP6* variants causing a range of more diverse clinical presentations, including primary lactic acidosis, cardiomyopathy, 3-methylglutaconic aciduria, isolated optic neuropathy, and retinitis pigmentosa (ClinVar ID 9641; PMID: 30763462). **M**yopathy, **L**actic **A**cidosis and **S**ideroblastic **A**nemia type 1 (MLASA1) has been also associated with a pathogenic variant in this gene.

MLASA1 (OMIM # 600462, OMIM #613561) is a rare mitochondrial disorder characterized by myopathy, lactic acidosis, and sideroblastic anemia. It has been associated with pathogenic variants in the nuclear genes *PUS1* (MIM* 608109) and *YARS2* (MIM* 610957), and with a pathogenic variant in the mitochondrial gene *MT-ATP6*, m.8969G>A. Variable features of this condition include failure to thrive, and developmental delay or intellectual disability. In addition, one patient with MLASA has had pigmentary retinopathy, one patient has been reported with renal involvement and multiple metabolic decompensations and several patients have been described with cardiac involvement, typically hypertrophic cardiomyopathy. The age of onset of symptoms of patients with MLASA varies with some patients diagnosed in infancy with sideroblastic anemia and/or failure to thrive whereas other patients present in childhood or later with exercise intolerance secondary to myopathy. Biochemical investigations in all patients described have demonstrated lactic acidemia, and although respiratory chain studies in fibroblasts may be normal, skeletal muscle biopsies typically display decreased complex I and IV activities with reduced complex III activity in a subset of patients. The lack of pancreatic dysfunction distinguishes MLASA from Pearson syndrome, an mtDNA single deletion disorder that is also associated with sideroblastic anemia (PMID 25037980).

Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA: GenBank Sequence NC_012920.1

Upon request, filtered variant files and raw data files from the whole exome analysis can also be provided.

CONCLUSION

MT-ATP6 m.8969G>A, p.(Ser148Asn) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. The variant was observed as homoplasmic in this tested DNA sample. Disease caused by pathogenic mitochondrial

variants are maternally inherited or occur *de novo*. This variant was not detected in the studied sample of the mother. Maternal identity was confirmed based on the sequencing data. This is consistent with *de novo* occurrence. However, please note that the level of mitochondrial variants may vary highly between tissue types. For pathogenic mitochondrial variants confirmed to be *de novo*, there remains a small risk of recurrence due to the possibility of maternal germline mosaicism. Genetic counseling is 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

APPENDIX 3: SECONDARY FINDINGS

The patient was opted-in for an analysis of secondary findings, which are sequence variants unrelated to the indication for ordering the sequencing but of medical value for patient care. Whole Exome data of the patient was analyzed for secondary findings according to recommendations of American College of Medical Genetics and Genomics (ACMG v3.1; PMID: 35802134).

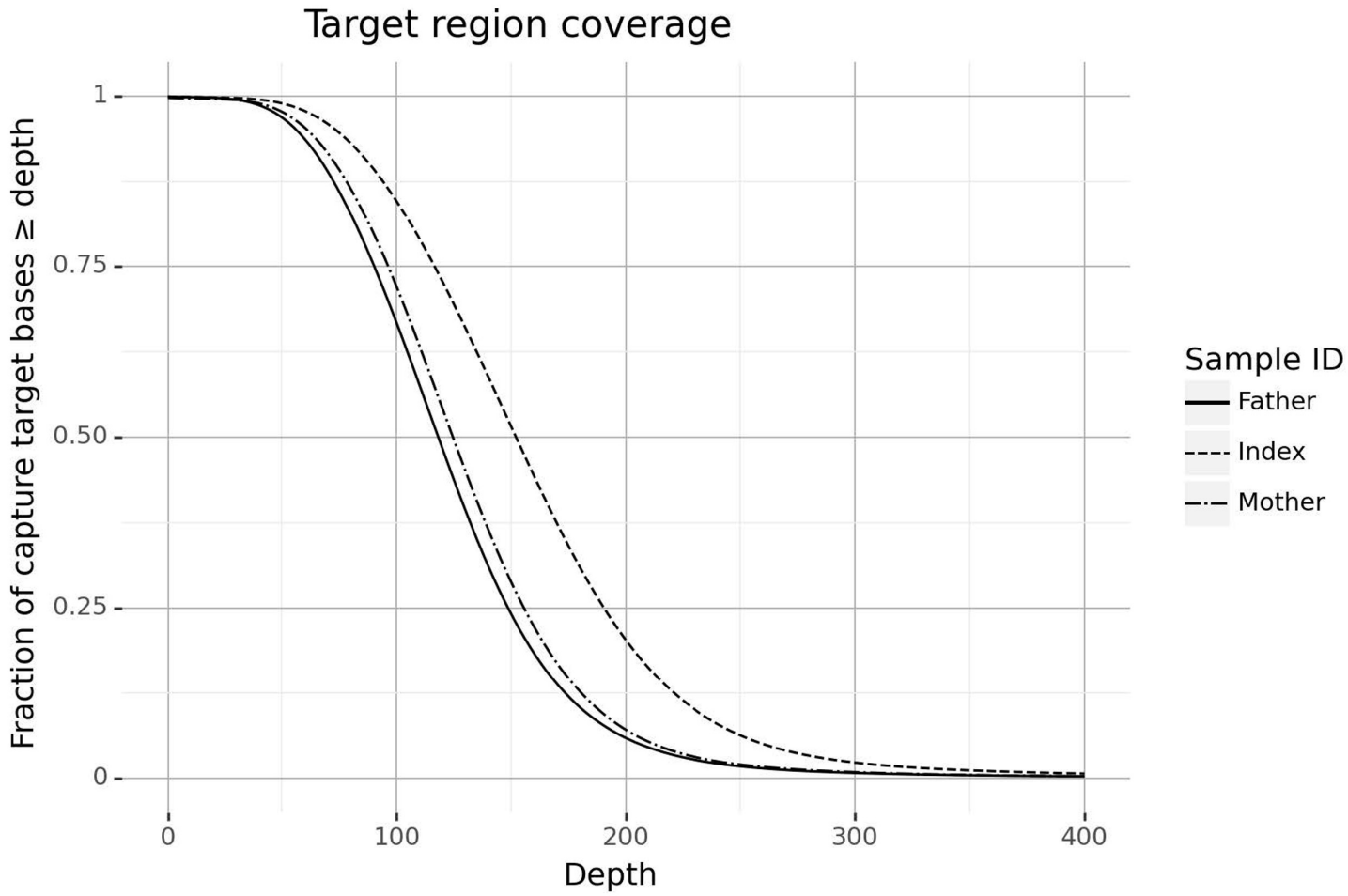
NOTES REGARDING SECONDARY FINDINGS

The analysis was negative for secondary findings.

For any other family members who have opted in for secondary findings analysis, separate statements are available under the order ID in Nucleus.

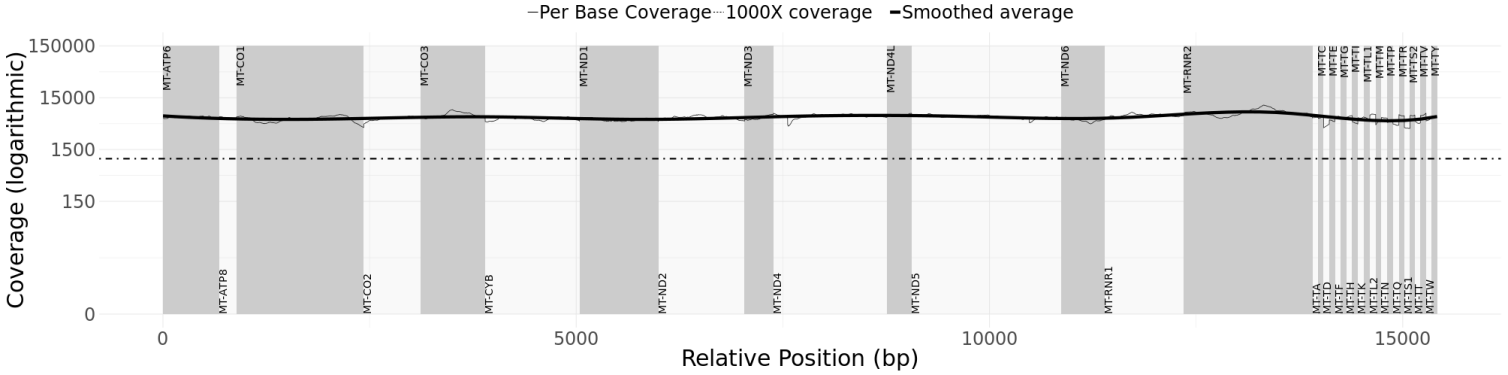
COVERAGE PLOT - NUCLEAR GENES

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.



COVERAGE PLOT- MITOCHONDRIAL GENES

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

WHOLE EXOME

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. Quantity of DNA was assessed using fluorometric method. After assessment of DNA quantity, 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 (2x150 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 has been 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 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 pathogenicity potential of the identified variants was assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as a number of reference population databases and mutation databases such as, but not limited to the 1000 Genomes Project, gnomAD, ClinVar and HGMD Professional. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen and 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 1000 Genomes Project, Database of Genomic Variants, ExAC, gnomAD and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used. 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.
Likely benign and benign variants were not reported. In addition to the analysis of variants in previously established disease genes, variants in genes where disease association has not yet been established were considered as potentially disease-causing mainly using the following scheme:

- For probands who were whole-exome sequenced with parents, all coding region de novo variants were evaluated.

- Novel (absent in gnomAD) heterozygous, truncating variants (nonsense, frameshift, canonical splice site variants) in genes predicted to be intolerant for loss-of-function variation based on gnomAD variant data. Genes were determined as intolerant if probability of loss-of-function intolerance score (pLI) is ≥0.9. The closer the pLI is to one, the more LoF intolerant the gene appears to be. Genes with pLI≥0.9 are defined as an extremely LoF intolerant set of genes.

- Rare (<1% MAF in gnomAD), truncating homozygous or (predicted) compound heterozygous variants, or a combination of rare truncating and rare missense variant that is predicted deleterious by multiple *in silico* tools.

- In addition, only variants in genes whose known expression pattern and function are considered relevant for the phenotype are included (e.g., variants in genes exclusively expressed in a muscular tissue are not considered as a candidate for a central nervous system disease).

For proband and family members who were opted-in for analysis of secondary findings from the WES data, clinically actionable genes were analyzed and reported for secondary findings according to recommendations by ACMG (v3.1; PMID: 35802134). Variants within ACMG genes associated with autosomal dominant phenotypes classified as pathogenic or likely pathogenic are reported. Genes associated with phenotypes inherited in an autosomal recessive manner needs two variants classified as likely pathogenic/pathogenic (or a homozygous variant) to meet the threshold for reporting. Pathogenic and likely pathogenic variants within genes associated with X-linked phenotypes that are apparently hemizygous, heterozygous, compound heterozygous, or homozygous are reported. Secondary findings analysis does not include analysis of CNV data. Secondary findings are not analyzed or reported for deceased individuals or fetal samples.

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. Inquiries regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decisions. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com

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.

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.

Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The validated performance of this whole exome sequencing laboratory assay: sensitivity for SNVs 99.6%, and indels 2-50 bps 97.6%, one-exon deletion 100% and 1-10 exon duplications 82%. Specificity is >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% (5-100% heteroplasmy level), and for simulated gross deletions (500-5000 bp) >99.9%. 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 the 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 mitochondrial DNA testing performed on whole exome sequencing assay).

PERFORMING SITE:

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

Bioinformatic analysis
Interpretation

Please refer to Appendix 7 of the report in Nucleus ordering and reporting portal for full list of non-coding variants included in the Whole Exome analysis.

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
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.
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.
Transcript = GenBank accession for reference sequence used for variant nomenclature

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***

Secondary findings report

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **RELATIONSHIP** **ORDER ID**

Mother

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

SUMMARY OF RESULTS

Negative

SEQUENCING PERFORMANCE METRICS - NUCLEAR GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 20X
Index	152	99.77
Mother	125	99.56
Father	119	99.72

SEQUENCING PERFORMANCE METRICS - MITOCHONDRIAL GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 1000X
Index	3924	100
Mother	6036	100
Father	6330	100

STATEMENT

CLINICAL HISTORY

This individual was opted-in for an analysis of secondary findings.

CLINICAL REPORT

The individual was opted-in for an analysis of secondary findings, which are variants unrelated to the primary purpose of testing but of medical value for patient care. Therefore, the individual's Whole Exome data were analyzed for secondary findings according to the recommendations of the American College of Medical Genetics and Genomics (ACMG v3.1; PMID: 35802134).

The analysis was negative for secondary findings.

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.)

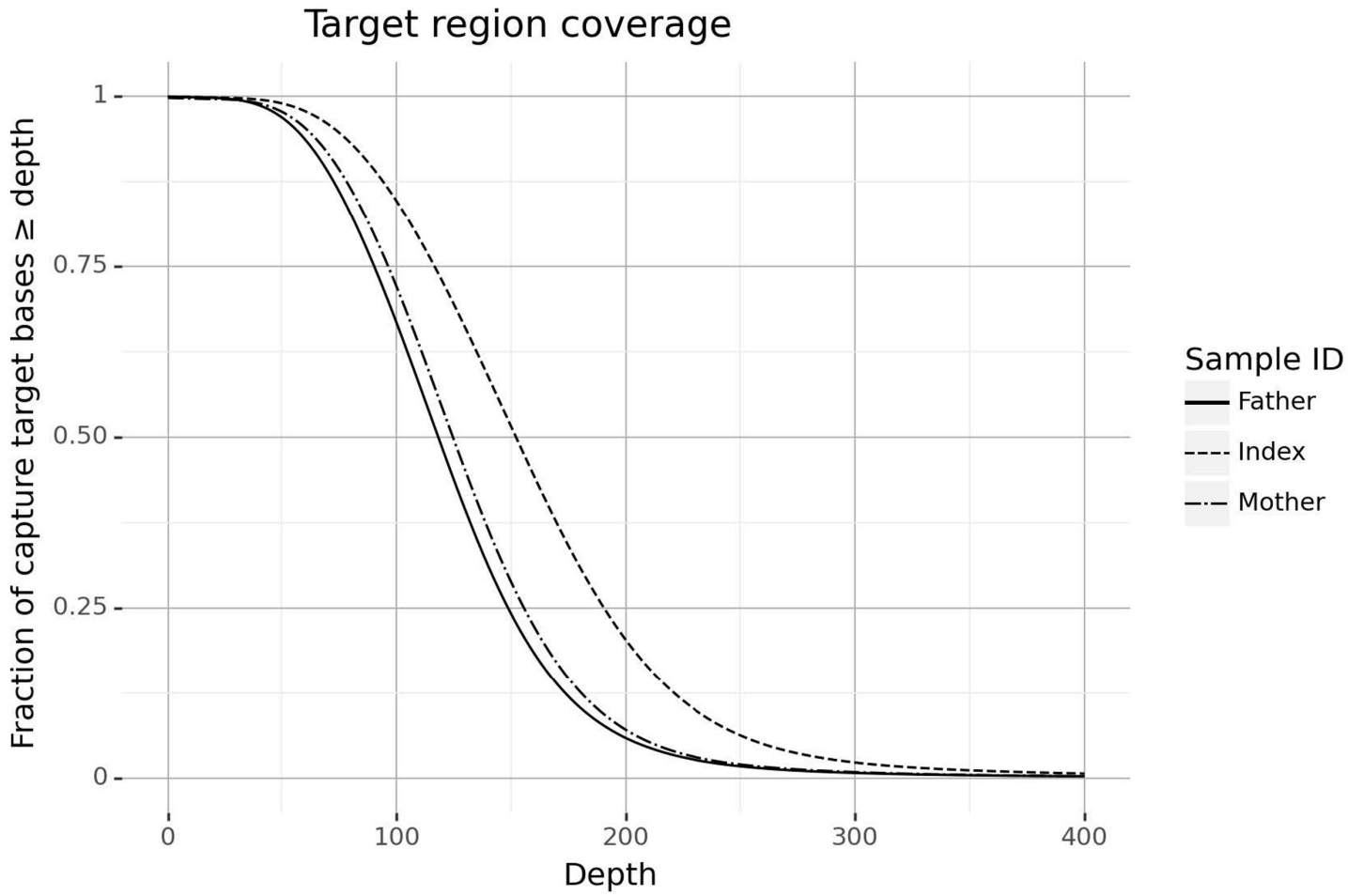
Signature

Name

Title

COVERAGE PLOT - NUCLEAR GENES

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.



COVERAGE PLOT- MITOCHONDRIAL GENES

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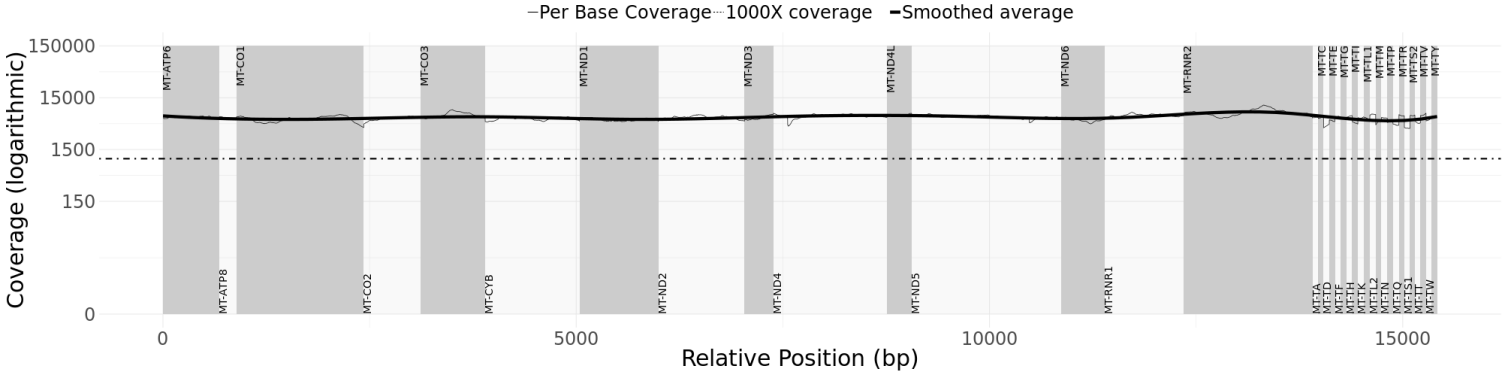

APPENDIX 5: SUMMARY OF THE TEST

SECONDARY FINDINGS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. Quantity of DNA was assessed using fluorometric method. After assessment of DNA quantity, 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 were 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 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.

Interpretation: The pathogenicity potential of the identified variants was assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation, as well as a number of reference population databases and mutation databases such as, but not limited to, the 1000 Genomes Project, gnomAD, ClinVar and HGMD Professional. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen and MutationTaster, were used to assist with variant classification. Reporting is carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. For proband and family members who were opted-in for analysis of secondary findings from the WES data, clinically actionable genes were analyzed and reported for secondary findings according to recommendations by ACMG (v3.1; PMID: 35802134). Variants within ACMG genes associated with autosomal dominant phenotypes classified as pathogenic or likely pathogenic are reported. Genes associated with phenotypes inherited in an autosomal recessive manner needs two variants classified as likely pathogenic/pathogenic (or a homozygous variant) to meet the threshold for reporting. Pathogenic and likely pathogenic variants within genes associated with X-linked phenotypes that are apparently hemizygous, heterozygous, compound heterozygous, or homozygous are reported. Secondary findings analysis does not include analysis of CNV data. Secondary findings are not analyzed or reported for deceased individuals or fetal samples.

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Confirmation: In general, variants reported as secondary findings do not go through Sanger confirmation, which is inline with the ACMG policy.

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 whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, and indels 1-50 bps 99.07%, and specificity >99.9% for most variant types). 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 the misinterpretation of the results.

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 the 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, 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.

PERFORMING SITE:

QUEST DIAGNOSTICS NICHOLS INSTITUTE, 33608 ORTEGA HIGHWAY, SAN JUAN CAPISTRANO, CA 92690 Laboratory Director: IRINA MARAMICA, MD, PhD, MBA, CLIA: 05D0643352

- Next-generation sequencing

BLUEPRINT GENETICS,INC, 2505 3RD AVE, SUITE 204, SEATTLE, WA 98121, USA Laboratory Director: TINA M. HAMBUCH, PHD, FACMG, CLIA: 50D2140410

- DNA extraction and QC
- Confirmation of sequence alterations
- Confirmation of copy number variants

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

- Bioinformatic analysis
- Interpretation

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
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.
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.
Transcript = GenBank accession for reference sequence used for variant nomenclature