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

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

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

SUMMARY OF RESULTS

PRIMARY FINDINGS

Analysis of whole exome sequence variants in previously established disease genes

The patient has a homoplasmic (99.7%) *MT-TF* m.616T>C variant, which is pathogenic.

Del/Dup (CNV) analysis

Negative

PRIMARY MITOCHONDRIAL FINDINGS: SEQUENCE ALTERATIONS

GENE	POS	AF	LEVEL	GENOTYPE	TRANSCRIPT	NOMENCLATURE	CLASSIFICATION
MT-TF	MT:616	0.997	100%	HOMOPLASMIC	NC_012920.1	m.616T>C	**Pathogenic**

CONSEQUENCE
non_coding_transcript_exon_variant

PHENOTYPE
Nephropathy; tubulointerstitial,
Encephalopathy: mitochondrial,
Epilepsy; mitochondrial,
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes,
Myoclonic epilepsy with ragged red fibers,
Myopathy; mitochondrial,

INHERITANCE
Mitochondrial

SEQUENCING PERFORMANCE METRICS - NUCLEAR GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 20X
	158	99.59

SEQUENCING PERFORMANCE METRICS - MITOCHONDRIAL GENOME

SAMPLE	MEDIAN COVERAGE	PERCENT > 1000X
	4360	100

TEST INFORMATION

Blueprint Genetics Whole Exome Plus Test consists of sequence analysis of all protein-coding genes in the genome for the proband, 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 Plus 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 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 are 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 a 30-year-old with stage 4 CKD of unknown etiology, asymptomatic hyperuricemia and persistent low C3 levels. No abnormal findings on urinalysis.

There is no reported family history of similar disease.

Consent has been received to report secondary findings for this individual.

CLINICAL REPORT

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

Sequence analysis using the Blueprint Genetics (BpG) Whole Exome Plus identified a homoplasmic mtDNA variant *MT-TF* m.616T>C.

MT-TF m.616T>C

The variant is absent in gnomAD v3.1 (a reference population containing 76,156 whole genomes, of which 56,434 samples passed mitochondrial-specific filters). The variant is present as heteroplasmic in 6 individuals in HelixMTdb (includes >195,000 unrelated adult individuals sequenced at Helix; this population is not enriched for mitochondrial or other disorders).

The *MT-TF* m.616T>C variant disrupts a base pair in the anticodon stem of mt-tRNA for phenylalanine (Phe) at a highly conserved position (Conservation: 100% at Mitomaster) and the variant is scored as confirmed pathogenic in MitoTIP.

This variant has been identified in at least 7 unrelated individuals with kidney disease. It was reported as homoplasmic (in blood samples) in several individuals with mitochondrial tubulointerstitial kidney disease (MITKD) from two pedigrees (PMID 28267784), and in an additional patient with chronic renal insufficiency and epilepsy (PMID 31722346). Functional studies utilizing cybrid experiments confirmed mitochondria dysfunction in patients with homoplasmic m.616T>C variant: patient-derived cybrids showed a reduction in respiration compared with control cybrids, as well as a reduced mitochondrial tRNAPhe level (PMID 28267784). In 2022, Xu et al. identified heteroplasmic and homoplasmic *MT-TF* m.616T>C variant in 3 additional unrelated families co-segregating with isolated chronic kidney disease and hyperuricemia without hematuria, proteinuria, or renal cyst formation (PMID: 35472031). Lymphoblasts cells carrying the variant m.616T>C exhibited swollen mitochondria, underwent active mitophagy, and showed respiratory deficiency, leading to reduced mitochondrial ATP production, diminished membrane potential, and overproduction of mitochondrial ROS (PMID: 35472031). The variant has also been detected as homoplasmic in blood of a patient with seizures and renal tubulopathy as well as at 89% (blood) and 86% (muscle) in the patient's asymptomatic mother (PMID 31965079). This suggests that the variant may have high phenotypic thresholds in different tissues and that apparent homoplasmy may need to be carefully quantified (PMID 31965079). Also, this variant was identified as apparently homoplasmic in all tissue samples (except leukocytes, where traces of the wild-type allele were detectable) of a patient with maternally inherited severe epilepsy as the main symptom of mitochondrial disease (PMID 20142618). The variant was heteroplasmic in buccal swabs from 3 healthy maternal relatives of the patient, thus suggesting that the variant results in mitochondrial failure only at homoplasmy (or very high proportion of the variant) (PMID 20142618).

The variant has been submitted to ClinVar by other clinical testing laboratories (variation ID 9576).

MT-TF

MT-TF encodes mitochondrial tRNA for phenylalanine (Phe) (UUU/C) (tRNA-Phe) and is involved in the tRNA aminoacylation pathway (MIM *590070, NCBI *MT-TF*, GeneCards *MT-TF*). *MT-TF* comprises 71 nucleotides (human mitochondrial map position 577-647). Heteroplasmic mtDNA variants in *MT-TF* have been associated with myoclonic epilepsy associated with

ragged-red fibers syndrome (MERRF; MIM #545000, PMID: 17878308), and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome; MIM #540000, PMID: 9771776). Heteroplasmic *MT-TF* variants have also been reported in severe encephalopathy with epilepsy (PMID: 31009750, MIM *590070), in late-onset myopathy with mild sensorineural hearing impairment (PMID: 16769874, MIM *590070), and in progressive neurodegenerative disorder characterized by frontotemporal dementia, auditory hallucinations, sensorineural deafness, and akinesia-rigidity (PMID: 21060018, MIM *590070). Homoplasmic mtDNA variants in *MT-TF* have been associated with severe epilepsy as the major symptom (PMID: 20142618, MIM *590070) and with infantile-onset of progressive renal failure due to tubulointerstitial nephropathy (PMID: 11231339, MIM *590070).

Homoplasmic mtDNA variants have been reported in patients with tubulointerstitial kidney disease. A variant *MT-TF* m.608A>G, affecting the distal end of the anticodon stem of the tRNA(Phe) molecule, has been reported in 2 siblings with infantile-onset of progressive renal failure due to tubulointerstitial nephropathy. The variant was detected as homoplasmic from paraffin-embedded renal tissue. Both patients were brought to medical attention for failure to thrive and chronic renal insufficiency. Clinical manifestations included also delayed development, brain atrophy, ataxia, anemia, muscle weakness and atrophy, and lesions suggestive of stroke in deep brain regions (PMID:11231339). The variant *MT-TF* m.616T>C was reported in a family with tubulointerstitial kidney disease with no apparent other organ involvement (PMID: 34514217). The variant was detected as homoplasmic in the blood from the 12-year-old proband and his affected mother and maternal uncle. No disease-causing variants were detected in the two most common genes associated with autosomal dominant tubulointerstitial kidney disease, *UMOD* and *MUC1,* supporting the diagnosis of a mitochondrially inherited kidney disease.

There are currently 2 variants in *MT-TF* annotated as confirmed (Cfrm) in the MITOMAP variant database, of which m.583G>A has been associated with MELAS / mitochondrial myopathy and EXIT and m.616T>C with maternally inherited epilepsy / mito tubulointerstitial kidney disease (MITKD).

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-TF m.616T>C is classified as pathogenic based on the established association between the gene and the patient's phenotype, the variant’s rarity in reference populations, in silico predicted pathogenicity, functional studies, and identification of the variant in multiple affected individuals. *MT-TF* m.616T>C was observed as homoplasmic in this tested blood sample. Disease caused by pathogenic mitochondrial variants are maternally inherited or occur de novo. The mother of a proband with a homoplasmic pathogenic variant generally has the mtDNA variant and may or may not have symptoms. A male does not transmit mitochondria to his offspring. 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

APPENDIX 3: SECONDARY FINDINGS

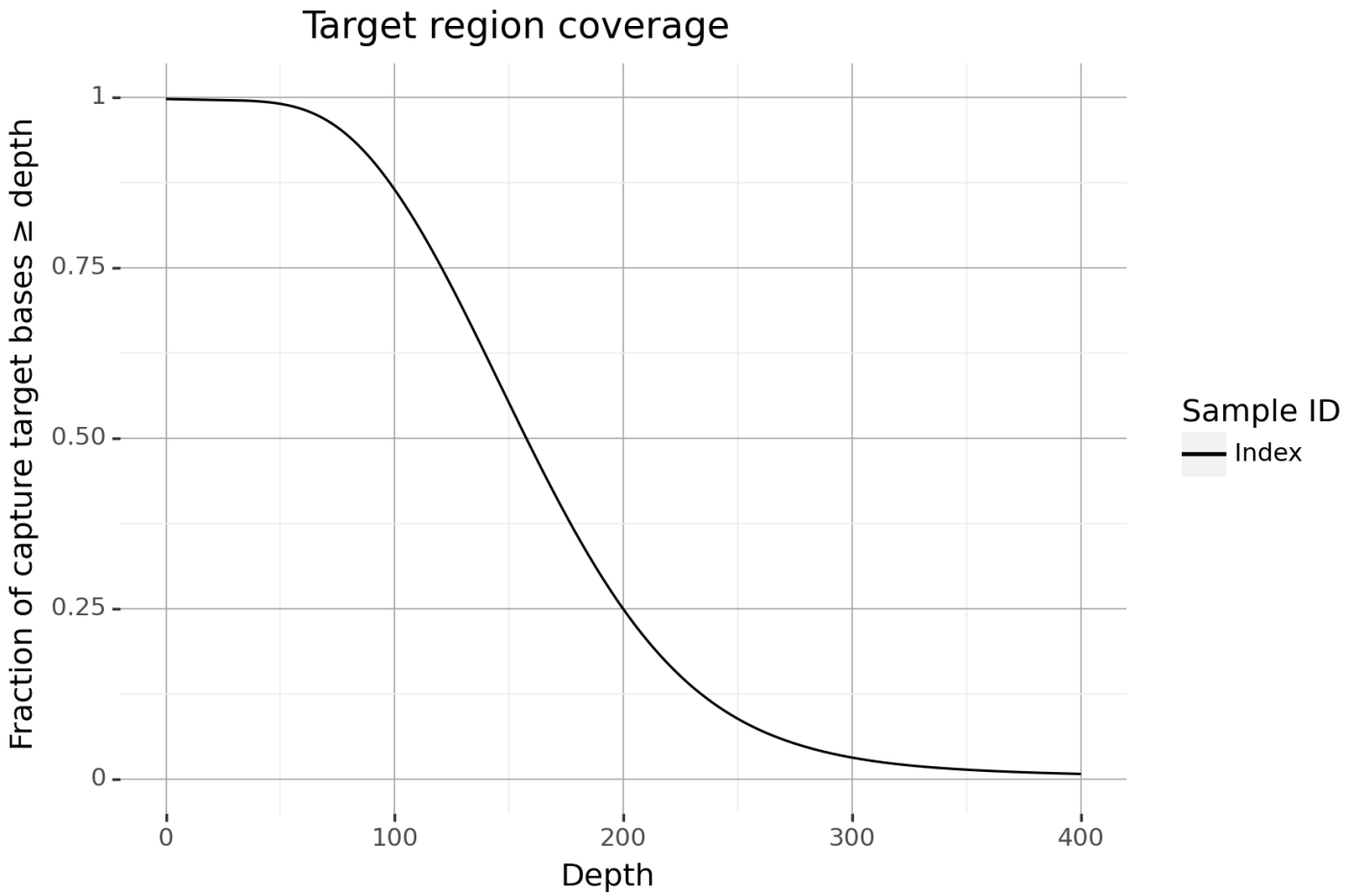
The patient was opted-in for an analysis of secondary findings, which are sequence variants unrelated to the indication for ordering the sequencing but of medical value for patient care. Whole Exome data of the patient was analyzed for secondary findings according to recommendations of American College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

NOTES REGARDING SECONDARY FINDINGS

The analysis was negative for secondary findings.

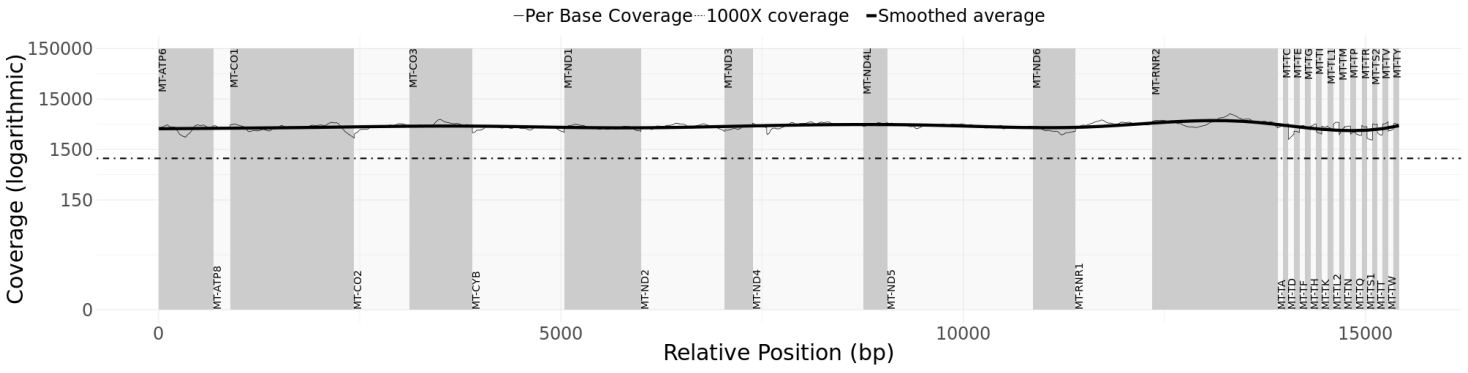
COVERAGE PLOT - NUCLEAR GENES

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

- DNA extraction and QC
- Next-generation sequencing
- Bioinformatic analysis
- Confirmation of sequence alterations
- Confirmation of copy number variants
- 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