Blueprint Genetics

Sample report as of July 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at ***blueprintgenetics.com/certifications***

Whole Exome Family Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		29		

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 is homozygous for *GSS* c.129+1663A>G, which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS IN ESTABLISHED DISEASE GENES

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GSS	NM_000178.4	c.129+1663A>G	HOM	intron_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	20:33537864	T/C		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	N/A	N/A	N/A	Glutathione synthetase deficiency	

SEQUENCING PERFORMANCE METRICS

SAMPLE	MEDIAN COVERAGE	PERCENT >= 20X
	186	99.49

TEST INFORMATION

Blueprint Genetics Whole Exome Family Plus Test 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 non-coding, 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. This

test should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

Analysis of Whole Exome Family 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 (>2400 genes). These genes are supplemented with genes included in The Clinical Genomics Database (>3350 genes) and the Developmental Disorders Genotype-Phenotype Database (DD2GP) (>1640 genes). Total number of genes that are considered as clinically associated in the Whole Exome Family Plus analysis is >3750 (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). If over time other patients with similar phenotype and variants in the same gene are identified, the variant may be reclassified as a likely cause of the disorder.

STATEMENT

CLINICAL HISTORY

The patient is a 29-year-old individual with intellectual disability, anemia and 5-oxoprolinuria. Also retinitis pigmentosa diagnosed at the age of 17 years. Previous testing with the Blueprint Genetics Retinal Dystrophy panel was negative (order ID: 47647).

The patient's mother is unaffected. The parents are first cousins.

CLINICAL REPORT

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

Given that there is no reported family history of the same disease, the exome data of the patient and mother were analysed for rare heterozygous variants absent in the mother (potential de novo variants), and variants following recessive inheritance pattern. To account for incomplete penetrance of pathogenic variants, also rare heterozygous variants inherited from the mother were analysed.

Sequence analysis using the Blueprint Genetics (BpG) Whole Exome Family Plus identified a homozygous intronic variant *GSS* c.129+1663A>G. The patient's mother is heterozygous for this variant.

GSS c.129+1663A>G

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 variant leads to the substitution of a nucleotide within intron 2 of *GSS*. *GSS* c.129+1663A>G has been reported in the literature as homozygous in multiple individuals with glutathione synthetase deficiency (PMID: 14635114, 26669244, Alqarajeh et al. 2020). Njalsson et al. (2003) identified the variant as homozygous in three unrelated individuals presenting with hemolytic anemia, metabolic acidosis and 5-oxoprolinuria in the neonatal period, and who had severely reduced glutathione synthetase enzyme activity in cultured fibroblasts (PMID: 14635114). RT-PCR analysis showed that the variant led to the inclusion of a pseudoexon from the intronic sequence between exons 2 and 3. Subsequently, Gündüz et al. (2016) reported the variant as homozygous in a young child with metabolic acidosis, seizures, microcephaly, autistic features and abnormalities on brain MRI (PMID: 26669244). Following a treatment regime, the patient was reported to have moderate intellectual disability and seizures only rarely at the age of nine years. Most recently, Alqarajeh et al. (2020) reported the variant as homozygous in a patient who presented with respiratory distress, metabolic acidosis and hemolytic

anemia in the first days of life, and seizures at the age of five. After beginning treatment, the patient was reported to have normal development and was seizure free at the age of six (Alqarajeh et al. 2020).

GSS

The *GSS* gene (MIM *601022) is located on chromosome 20q11.22 and encodes glutathione synthetase. This enzyme functions as a homodimer to catalyze the second step of glutathione biosynthesis, which is the ATP-dependent conversion of gamma-L-glutamyl-L-cysteine to glutathione. However, glutathione is important for a variety of biological functions, including protection of cells from oxidative damage by free radicals, detoxification of xenobiotics, and membrane transport (NCBI-Gene).

Homozygous or compound heterozygous pathogenic variants in *GSS* cause glutathione synthetase deficiency (MIM #266130, 231900, reviewed by Njalsson 2005), a rare, inborn autosomal recessive metabolic disorder characterized by three clinical forms. The mild form presents with hemolytic anemia and variable 5-oxoprolinuria; the moderate form demonstrates hemolytic anemia, constant 5-oxoprolinuria, and metabolic acidosis; and the severe form involves hemolytic anemia, constant 5-oxoprolinuria, metabolic acidosis, and neurologic defects (PMID: 11445798, 9215686). Retinal dystrophies have also been reported in adults and adolescents with severe glutathione synthetase deficiency (PMID: 17206463, 19111905, 1986110). In general, clinical manifestations of glutathione synthetase deficiency usually first appear during the neonatal period. After the neonatal period, although the condition may stabilize, it may be exacerbated during infection due to severe acidosis or electrolyte imbalance.

For patients with clinical findings suggesting glutathione synthetase deficiency, diagnosis relies on the detection of massive excretion of L-5-oxoproline in the urine, decreased activity of GSS, and molecular genetic testing. Treatment involves the correction of metabolic acidosis using parenteral compounds followed by oral maintenance therapy. It has been reported that early supplementation with vitamins C and E may improve the long-term clinical outcome (PMID: 11445798, 26984560).

There are currently 40 variants in *GSS* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2021.2), including missense, truncating and splice variants.

Mutation nomenclature is based on GenBank accession NM_000178.4 (*GSS*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

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

CONCLUSION

GSS c.129+1663A>G is classified as pathogenic, based on the established association between the gene and the patient’s phenotype, the variant's absence in reference populations, deleterious effect on splicing, and identification as homozygous in multiple individuals with glutathione synthetase deficiency. Disease caused by *GSS* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is consistent with autosomal recessive inheritance. The patient's mother is heterozygous for this variant. If both of the patient’s parents are each confirmed to be carriers of this variant, any siblings of the patient will have a 25% chance of being homozygous for the variant and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	

STEP	**DATE**
Sample in analysis	
Reported	

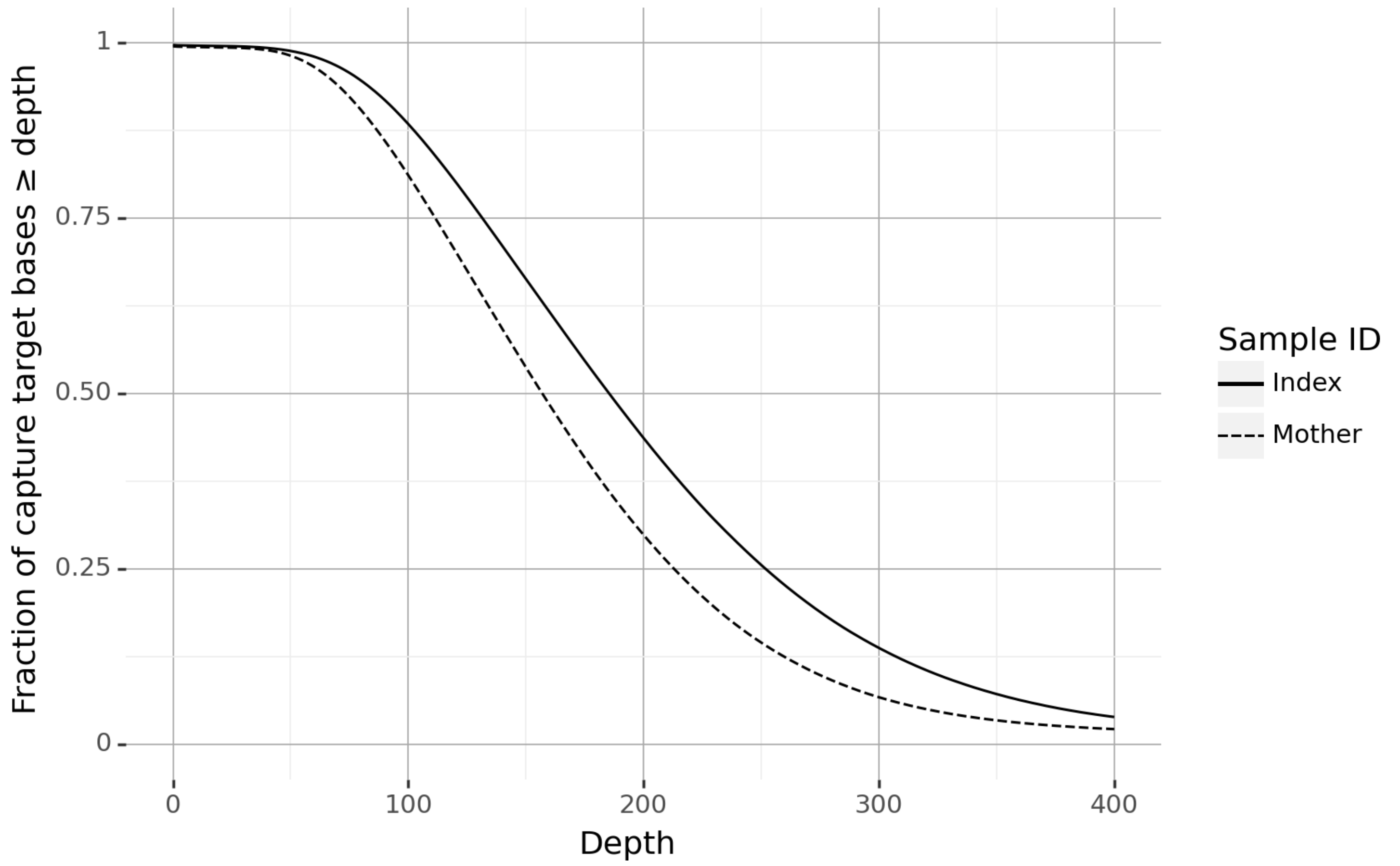
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



APPENDIX 5: SUMMARY OF THE TEST

WHOLE EXOME

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 pathogenicity potential of the identified variants were 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. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, 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, DECIPHER. 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 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 using the following scheme:

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

- 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 ExAC variant data. Genes were determined as intolerant if probability of loss-of-function intolerance score pLI≥0.9 . The closer 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). Candidate variants are not validated by Sanger sequencing, but their quality is inspected by visualization of sequence reads and evaluation of quality metrics, and only likely true variants are reported.

For proband and family members who were opted-in for analysis of secondary findings from the WES data, 59 clinically actionable genes were analyzed and reported for secondary findings according to recommendations by ACMG (PMID 27854360) with minor modifications aiming to increase the clarity of the classifications of the reportable variants (please see our website/clinical interpretation). Secondary findings are not analyzed or reported for deceased individuals or fetal samples. 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

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: 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%, one-exon deletion 92.3% and two exons CNV 100%, 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.

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 digital PCR confirmation).

PERFORMING SITE:

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

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