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***

MODY Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

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

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for deletion *GCK* c.(?_-1)_(45+1_46-1)del, which encompasses exon 1 of *GCK*. This alteration is classified as pathogenic.

PRIMARY FINDINGS: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
GCK	COPY NUMBER LOSS	1	HET	GCK:Partial gene	UCSC	**Pathogenic**
	OMIM	**PHENOTYPE** Diabetes mellitus, permanent neonatal, Hyperinsulinemic hypoglycemia, familial, Maturity-onset diabetes of the young, type 2			**COMMENT** c.(?_-1)_(45+1_46-1)del	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
MODY Panel	17	200	39250	39106	205	99.63

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	7452	100

TARGET REGION AND GENE LIST

The Blueprint Genetics MODY Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABCC8*, *APPL1*, *BLK*, *CEL**, *GATA6*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*, *KLF11*, *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*, *NEUROD1*, *PAX4*, *PDX1*, *RFX6* and *WFS1*.
*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 7-year-old child with glucose intolerance (HbAIC 6.2%, abnormal glucose tolerance test, glucose 10.2 at 2h). Negative anti-GAD antibodies. Strong 3 generation family history of diabetes. High suspicion of MODY. Mother, maternal uncle and maternal grandmother and maternal great-grandfather all diagnosed with diabetes.

CLINICAL REPORT

Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) MODY Panel identified a heterozygous deletion c.(?_-1)_(45+1_46-1)del encompassing exon 1 of *GCK*.

Deletion *GCK* c.(?_-1)_(45+1_46-1)del

This deletion is estimated to cover the genomic region chr7:44228410-44229115 and is approximately 705 base pairs in size. However, the exact breakpoints of the deletion cannot be determined using the present method, and therefore its exact size and genomic position are unknown. Deletions affecting the *GCK* gene have been reported in 2 individuals in the Exome Aggregation Consortium control cohorts, however, this same deletion has not been reported in this database. No gross deletions in *GCK* are reported in the Genome Aggregation Database control cohorts (gnomAD SVs v2.1, ExAC data available in the gnomAD browser).

Heterozygous inactivating variants in *GCK* cause maturity-onset diabetes of the young type 2 (MODY-2), and multiple disease-causing truncating variants as well as larger deletions in *GCK* have been listed in HGMD Professional 2022.2. The deletion of exon 1 of *GCK* has also been previously described in the literature in multiple MODY families (PMID: 19790256, 30912798). Berberich et al. identified a heterozygous whole exon deletion affecting exon 1 of *GCK* in two apparently unrelated patients with clinical features of MODY (PMID: 30912798). The deletion co-segregated with the phenotype in both families across two generations.

GCK

GCK (MIM #*138079*) encodes the enzyme glucokinase, which is a structurally and functionally unique member of a family of enzymes called hexokinases, types I through IV (glucokinase). Phosphorylation of glucose at the sixth carbon position is the first step in glycolysis. Glucokinase is expressed only in mammalian liver and pancreatic islet beta cells. Because of its unique functional characteristics, the enzyme plays an important regulatory role in glucose metabolism. Glucokinase acts as the "glucose sensor" for the pancreas. The rate of glucose metabolism in the liver and pancreas is a function of the activity of the enzyme, thus inactivating variants in glucokinase cause defects in insulin secretion and hepatic glycogen synthesis resulting in mild chronic hyperglycemia, impaired glucose tolerance, or diabetes mellitus while activating variants in *GCK* result in hyperinsulinemia and an ensuing risk of hypoglycemia. Variants in *GCK* have been associated with autosomal dominant maturity-onset diabetes of the young type 2 (MODY-2, MIM #125851), autosomal dominant familial hyperinsulinemic hypoglycemia 3 (MIM #602485), autosomal dominant late-onset non-insulin-dependent diabetes mellitus (MIM#125853), and autosomal recessive permanent neonatal diabetes mellitus 1 (MIM #606176). Heterozygous inactivating variants in *GCK* cause a subtype of maturity-

onset diabetes of the young (MODY-2). Patients with MODY-2 typically present with blood glucose in the range of 5.5 to 8 mmol/l, with a less than a 3 mmol/l increase in blood glucose in 70% of patients in a 2-hour-long oral glucose tolerance test. In contrast, homozygous inactivating variants result in a more severe phenotype presenting at birth as neonatal diabetes mellitus (NDM, MIM #*606176*). *GCK*-related NDM can be caused by missense *GCK* variants located anywhere in the primary sequence with no major hotspots defined (PMID 14517946 and 11508276). *GCK*-hyperinsulinemia variants cluster in the small domain of GK protein, where the allosteric activator site is located (PMID 19373249). There are currently 967 variants in *GCK* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2022.2), the majority of which (64%) are missense variants. The majority of the variants (86%) have been reported in association with MODY.

MODY-2

MODY (maturity-onset diabetes of the young) is a rare, familial, clinically, and genetically heterogeneous form of diabetes characterized by the young age of onset (generally 10-45 years of age) with the maintenance of endogenous insulin production, lack of pancreatic beta-cell autoimmunity, absence of obesity and insulin resistance and extra-pancreatic manifestations in some subtypes (ORPHA: 552). MODY is the most common form of monogenic diabetes. Prevalence is estimated to be about 1/10,000 in adults and 1/23,000 in children. No specific ethnic predilection has been reported. It has been estimated that around 80% of cases are misdiagnosed as type 1 or type 2 diabetes, thus complicating prevalence and incidence estimations. The clinical features of MODY vary depending on the genetic etiology. The most frequent subtypes are HNF1A-MODY (30-50%), GCK-MODY (30-50%), HNF4A-MODY (10%), and HNF1B-MODY (1-5%). The latter is also known as renal cysts and diabetes syndrome. At least 9 other genetic subtypes have been described but are very rare. Patients with *HNF1A* and *HNF4A* mutations have slowly progressing beta-cell dysfunction and respond well to treatment with low-dose sulfonylureas, which are recommended as first-line therapy. Vascular complications of diabetes are observed with a similar frequency to type 1 or type 2 diabetes. Low C-Reactive Protein is seen in HNF1A-MODY and neonatal hypoglycaemia and macrosomia are reported in babies with HNF4A-MODY. GCK-MODY is characterized by asymptomatic non-progressing mild fasting hyperglycemia with low post-prandial glucose excursions from birth, is not associated with vascular complications and does not require treatment. However, GCK-MODY may have clinical relevance during pregnancy. If there is evidence that the fetus of a mother with the condition is growing too rapidly on antenatal ultrasound scans, mothers may be treated with insulin in an attempt to control maternal blood sugar and hence the baby’s growth. However, there is currently a debate about the role of insulin in this situation.

Mutation nomenclature is based on GenBank accession NM_000162.5 (*GCK*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Deletion *GCK* c.(?_-1)_(45+1_46-1)del, affecting exon 1, is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *GCK* variants is 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	

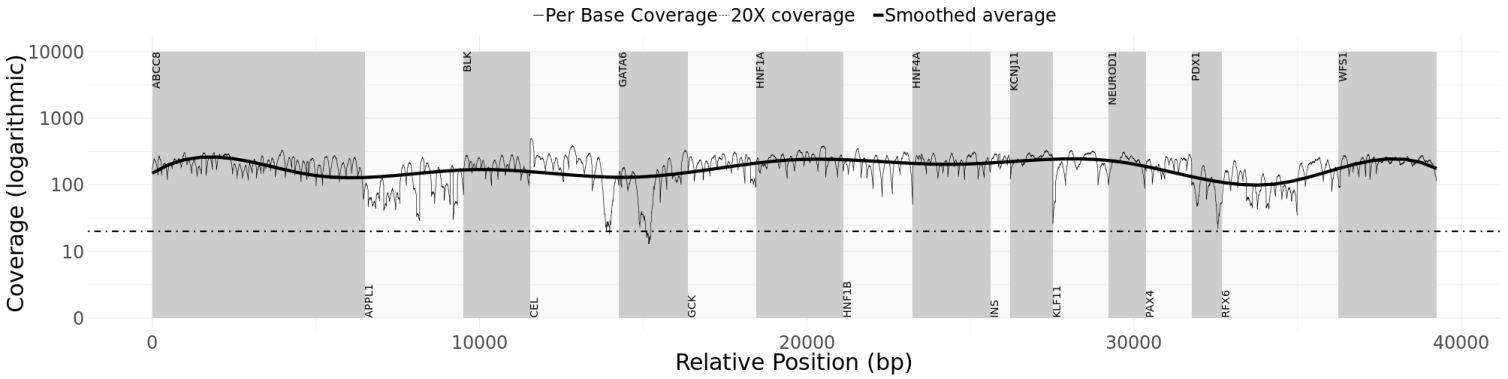
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

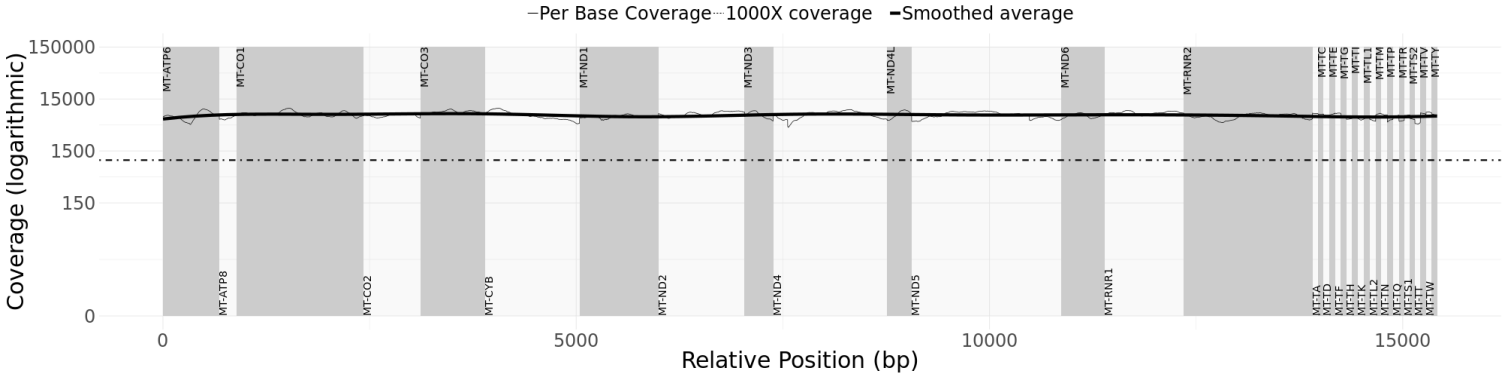
Name

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. 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 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.
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.
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
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.
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: 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).

The sample was analyzed using CE marked Blueprint Genetics CES Platform and/or Blueprint Genetics WES Platform in vitro diagnostic medical device manufactured by Blueprint Genetics Oy. For more information please see Accreditations and Certifications.

PERFORMING SITE:

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

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

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000207.2(*INS*):c.*59A>G, NM_000207.2(*INS*):c.188-15G>A, NM_000207.2(*INS*):c.188-31G>A, NM_000207.2(*INS*):c.187+241G>A, NM_000207.2(*INS*):c.-39A>C, NM_000207.2(*INS*):c.-152C>G, NM_000207.2(*INS*):c.-152C>A, NM_000207.2(*INS*):c.-153C>G, NM_000207.2(*INS*):c.-187_-164del, NM_000525.3(*KCNJ11*):c.-54C>T, NM_000525.3(*KCNJ11*):c.-134G>T, NM_000352.3(*ABCC8*):c.4412-13G>A, NM_000352.3(*ABCC8*):c.3399+13G>A,

NM_000352.3(*ABCC8*):c.2041-12C>A, NM_000352.3(*ABCC8*):c.2041-21G>A, NM_000352.3(*ABCC8*):c.2041-25G>A, NM_000352.3(*ABCC8*):c.1672-20A>G, NM_000352.3(*ABCC8*):c.1333-1013A>G, NM_000352.3(*ABCC8*):c.1177-53_1177-51delGTG, NM_000352.3(*ABCC8*):c.-190C>G, NM_000545.5(*HNF1A*):c.-538G>C, NM_000545.5(*HNF1A*):c.-462G>A, NM_000545.5(*HNF1A*):c.-291T>C, NM_000545.5(*HNF1A*):c.-287G>A, chr12:g.121416285-121416285, NM_000545.5(*HNF1A*):c.-283A>C, NM_000545.5(*HNF1A*):c.-258A>G, NM_000545.5(*HNF1A*):c.-218T>C, NM_000545.5(*HNF1A*):c.-187C>A/T, chr12:g.121416385-121416385, chr12:g.121416385-121416385, chr12:g.121416391-121416391, chr12:g.121416437-121416437, chr12:g.121416446-121416446, NM_000545.5(*HNF1A*):c.-119G>A, NM_000545.5(*HNF1A*):c.-97T>G, chr12:g.121416508-121416508, NM_005257.4(*GATA6*):c.-530A>T, NM_005257.4(*GATA6*):c.-409C>G, NM_002500.4(*NEUROD1*):c.-162G>A, NM_175914.4(*HNF4A*):c.-192C>G, NM_175914.4(*HNF4A*):c.-181G>A, NM_175914.4(*HNF4A*):c.-174T>C, NM_175914.4(*HNF4A*):c.-169C>T, NM_175914.4(*HNF4A*):c.-146T>C, NM_175914.4(*HNF4A*):c.-136A>G, NM_000457.4(*HNF4A*):c.291-21A>G, NM_006005.3(*WFS1*):c.-43G>T, NM_173560.3(*RFX6*):c.224-12A>G, NM_033507.1(*GCK*):c.1022+18G>A, NM_033507.1(*GCK*):c.49-15_49-11delCCCCTinsGGGAGGG, NM_000162.3(*GCK*):c.-457C>T, NM_000162.3(*GCK*):c.-557G>C, NM_001715.2(*BLK*):c.*505G>T

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.