Blueprint Genetics

Hypoglycemia, Hyperinsulinism and Ketone Metabolism Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0	Male	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
DNA		

SUMMARY OF RESULTS

TEST RESULTS

Patient is homozygous for *ABCC8* c.3124_3126delACCinsCAGCCAGGAACTG, p.(Thr1042Glnfs*75), which is classified as likely pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
ABCC8	11:17428470	NM_000352.3	ABCC8 c.3124_3126delACCinsCAGCCAGGAACTG,, p.(Thr1042Glnfs*75)	frameshift	HOM	**Likely pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs766033867	2/121052	N/A	N/A	N/A	
		PHENOTYPE				
	OMIM	Hyperinsulinemic hypoglycemia, permanent neonatal diabetes, leucine-induced hypoglycemia, transient neonatal diabetes mellitus		**INHERITANCE** AD&AR	**COMMENT** -	

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Hypoglycemia, Hyperinsulinism and Ketone Metabolism Panel	18	204	34970	34808	171	99.5

TARGET REGION AND GENE LIST

Blueprint Genetics Hypoglycemia, Hyperinsulinism and Ketone Metabolism Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with familial hyperinsulinism: ABCC8, ACAT1, FBP1, GCK, GLUD1*, HADH, HMGCL, HMGCS2, HNF1A, HNF4A, INSR, KCNJ11, OXCT1, PCK1, PCK2, PDX1, SLC16A1 and UCP2. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of familial hyperinsulinism mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Hypoglycemia, Hyperinsulinism and Ketone Metabolism Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

STATEMENT

CLINICAL HISTORY

Patient is a 1-month-old baby boy with severe congenital hyperinsulinism. He has persistent hypoglycaemia with limited response to medical therapy. He was born at 29+3 weeks, large for gestational age and has subtle dysmorphic features. He has been hypoglycemic from day one. He has high insulin levels and large glucose need from birth: currently 23 mg/kg/min. He is currently on Diazoxide, glucagon i.v. at maximum recommended rate and Octreotide i.v. No known family history.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hypoglycemia, Hyperinsulinism and Ketone Metabolism Panel (Metabolic Disorders) identified a homozygous deletion/insertion c.3124_3126delACCinsCAGCCAGGAACTG, p.(Thr1042Glnfs*75) causing a frameshift and leading to a premature stop codon. It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

In ExAC, this delins variant has been reported as three individual insertions. There are two South Asian individuals heterozygous for each of these three insertions. Probably the same variant, referred to as c.3124_3126delACCinsCAGCCAGGACCTG in HGMD and c.3127ins10 in the original publication (and c.1327ins10 in Figure 1) has been identified in compound heterozygous state in a patient with permanent neonatal diabetes (PNDM) (PMID: 17668386). This patient had an activating missense mutation in the other allele. Functional studies showed a reduced response to ATP consistent with an activating mutation that results in reduced insulin secretion. However, this mutational mechanism in which a heterozygous activating mutation resulted in PNDM when a second, loss-of-function mutation was also present was a novel finding. Typically loss-of function mutations cause hyperinsulinemic hypoglycemia (FHI), whereas activating mutations cause PNDM. It was proposed that, in the patients with PNDM resulting from compound heterozygous activating/inactivating mutations, the loss-of-function mutation leads to a decrease in functional protein, and the channels are essentially homomeric for the activating mutation.

ABCC8 encodes the ATP-binding cassette transporter subfamily C member 8. It is expressed primarily in the pancreatic beta cell and in certain specific regions of the nervous system. Together with proteins encoded by *KCNJ11, KCNJ8* and *ABCC9,* ABCC8 forms ATP-sensitive potassium channel KATP that senses metabolic changes in the pancreatic beta-cells and regulates insulin secretion. Inactivating pathogenic variants in either ABCC8 or KCNJ11 result in non-functional or dysfunctional KATP channels. Mutations in *ABCC8* cause autosomal dominant and recessive hyperinsulinemic hypoglycemia, familial, 1 (FHI) (MIM #256450) and dominant hypoglycemia of infancy, leucine-sensitive (MIM #240800). In addition, other *ABCC8* mutations cause autosomal dominant diabetes mellitus, noninsulin-dependent (MIM# 125853), permanent neonatal (MIM #606176) and transient neonatal 2 (MIM #610374). In 43% of affected individuals, *ABCC8*-related permanent neonatal diabetes mellitus (PNDM) is inherited in an autosomal recessive manner from unaffected parents with heterozygous pathogenic variants (PMID: 17919176). No deletions or duplications involving *ABCC8* have been reported to cause permanent neonatal diabetes mellitus (PNDM). *ABCC8* defects related to PNDM are activating pathogenic variants and therefore must be missense. In PNDM KATP channels do not close, and thus glucose-stimulated insulin secretion does not happen. Inactivating pathogenic variants in *ABCC8* result in non-functional or dysfunctional KATP channels. In this case channels fail to open, and thus the cell membrane is depolarized even in the absence of an elevated intracellular ATP/ADP ratio. This results in initiation of the insulin secretion cascade, even in the absence of glucose or other metabolic stimulus.

FHI is characterized by hypoglycemia that ranges from a severe neonatal-onset, difficult-to-manage disease to a mild childhood-onset disease with few symptoms and difficult-to-diagnose hypoglycemia. KATP-channel inactivating mutations of *ABCC8,* together with mutations in the *KCNJ11*, account for 97% the diazoxide-unresponsive hyperinsulinism (PMID: 26908106). FHI-KATP, caused by pathogenic variants in either *ABCC8* or KCNJ11, is most commonly inherited in an autosomal recessive manner and less commonly in an autosomal dominant manner, although *de novo* pathogenic variants have been reported (PMID: 26908106). The *ABCC8* gene is the most commonly mutated gene in familial hyperinsulinism: proportion of FHI attributed to mutation of *ABCC8* is up to 45% (GeneReviews) (PMID: 9618169, 10204114, 10338089, 12566718, 15579781). In the Ashkenazi Jewish population, two *ABCC8* founder variants are responsible for approximately 97% of FHI (PMID: 21716120). The c.3989-9G>A pathogenic variant has been identified in several different ethnic groups; haplotype analysis suggests that this is a mutation hot spot.

Mutation nomenclature is based on GenBank accession NM_000352.3 (*ABCC8*), with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the identified *ABCC8* c.3124_3126delACCinsCAGCCAGGAACTG, p.(Thr1042Glnfs*75) as likely pathogenic and probable cause for patient's disease considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, identification of the variant in one patient previously (a novel mechanism described for an inactivating loss-of function mutation in compound heterozygous state with an activating missense mutation explaining the different phenotype) and mutation type (frameshift)). However, additional information is still needed to confirm the pathogenicity of the variant, which could allow independent risk stratification based on this mutation. Genetic counseling and family member testing is recommended. Familial hyperinsulinism caused by pathogenic variants in *ABCC8* (FHI-KATP) is most commonly inherited in an autosomal recessive manner and less commonly in an autosomal dominant manner. The patient is homozygous for the variant, which is in line with autosomal recessive inheritance. If both parents are found to be carriers of this mutation, each sibling of an affected individual has a 25% chance of being a homozygous carrier of the mutation and thus being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. BpG offers mutation testing for the family if requested.

CONFIRMATION

ABCC8 c.3124_3126delACCinsCAGCCAGGAACTG, p.(Thr1042Glnfs*75) was confirmed with bidirectional Sanger sequencing.

STEP	DATE
Irder date	Mar 27, 2017
Sample received	Mar 27, 2017
Reported	Apr 17, 2017

On Apr 21, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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Lab Director, Chief Medical Officer

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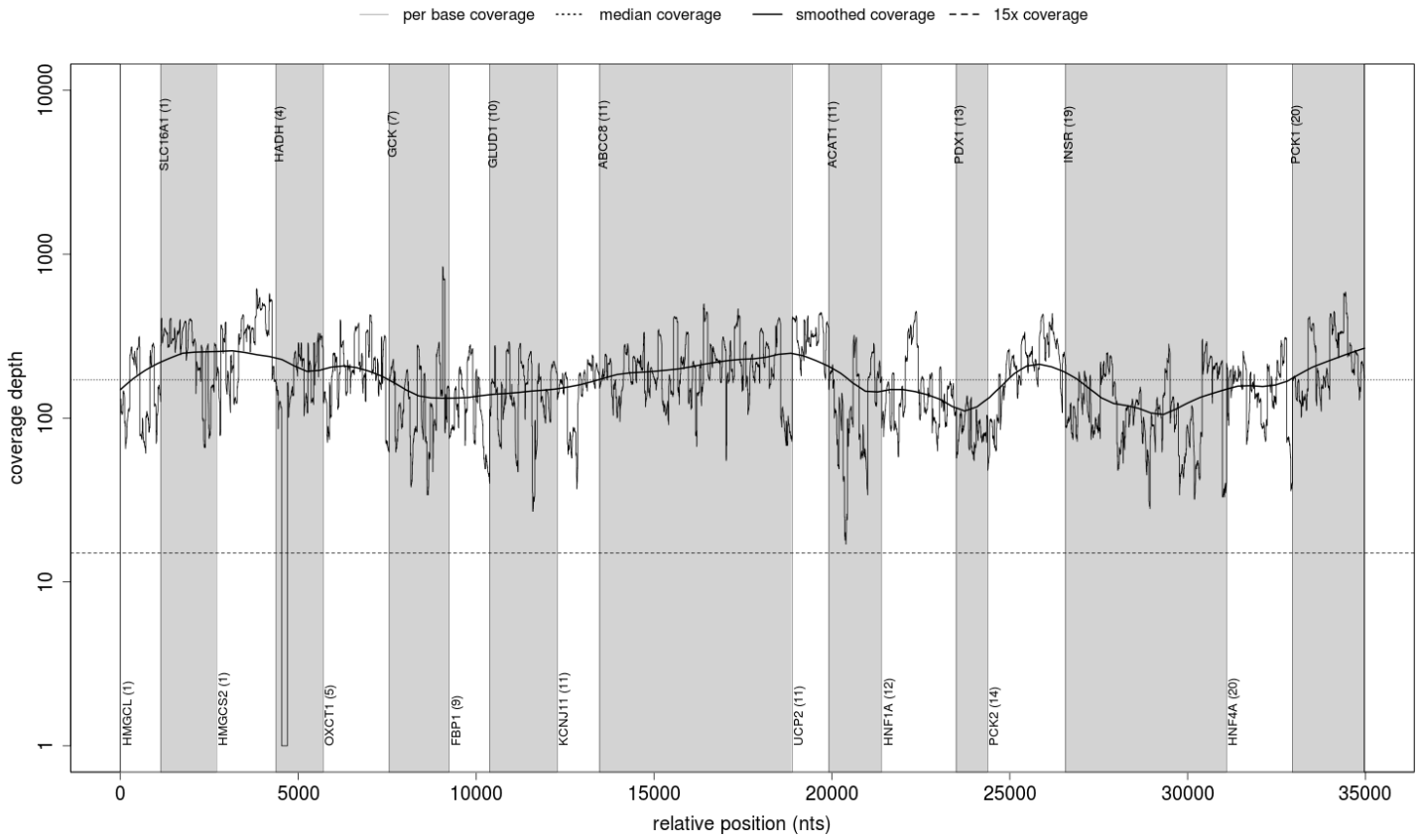
Clinical Consultant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median coverage
smoothed coverage
15x coverage
coverage depth
relative position (nts)
SLC16A1 (1)
HADH (4)
GCK (7)
GLUD1 (10)
ABCC8 (11)
ACAT1 (11)
PDX1 (13)
INSR (19)
PCK1 (20)
HMGCL (1)
HMGCS2 (1)
OXCT1 (5)
FBP1 (9)
KCNJ11 (11)
UCP2 (11)
HNF1A (12)
PCK2 (14)
HNF4A (20)

APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.
Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.
Confirmation. Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.
Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.
Accreditation. 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.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.
Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).
Reporting. Reporting was done using an HGNC-approved gene nomenclature.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.
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GLOSSARY OF USED ABBREVIATIONS:

POS = genomic position of the variant in the format of chromosome:position
ID = rsID in dbSNP
Transcript = GenBank accession for reference sequence used for variant nomenclature
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database
AD = autosomal dominant
AR = autosomal recessive
OMIM = Online Mendelian Inheritance in Man®
ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)
het = heterozygous
hom = homozygous
Del/Dup = Deletion and Duplication
CNV = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.