Blueprint Genetics

Metabolic Liver Failure Panel Plus

THIS IS AN UPDATED REPORT. PLEASE SEE THE FOLLOW-UP REPORT TEXT BELOW. THIS REPORT SUPERSEDES THE REPORT ISSUED ON MAY 08, 2018

FOLLOW-UP REPORT

Follow-up May 18, 2018

Parental testing revealed that the *ATP7B* variants c.2336G>A, p.(Trp779*) and c.3646G>A, p.(Val1216Met) occur in *trans* and the index ID: 27095 is compound heterozygous for them.

Johanna Tommiska, PhD, Geneticist

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
8 Female

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
Blood

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for two variants in *ATP7B:* c.2336G>A, p.(Trp779*) and c.3646G>A, p.(Val1216Met), both of which are classified as 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 ATP7B	**POS** 13:52532466	**TRANSCRIPT** NM_000053.3	**NOMENCLATURE** c.2336G>A, p.(Trp779*)	**CONSEQUENCE** stop_gained	**GENOTYPE** HET	**CLASSIFICATION** **Pathogenic**
	ID rs137853283	**gnomAD AC/AN** 10/277056	**POLYPHEN** N/A	**SIFT** N/A	**MUTTASTER** disease causing	
	OMIM	**PHENOTYPE** Wilson disease		**INHERITANCE** AR	**COMMENT** -	
GENE ATP7B	**POS** 13:52513240	**TRANSCRIPT** NM_000053.3	**NOMENCLATURE** c.3646G>A, p.(Val1216Met)	**CONSEQUENCE** missense_variant	**GENOTYPE** HET	**CLASSIFICATION** **Pathogenic**
	ID	**gnomAD AC/AN** 23/277238	**POLYPHEN** probably damaging	**SIFT** deleterious	**MUTTASTER** disease causing	
	OMIM	**PHENOTYPE** Wilson disease		**INHERITANCE** AR	**COMMENT** -	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Metabolic Liver Failure Panel	16	463	45050	45050	393	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Metabolic Liver Failure Panel (version 1, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ALDOB, ATP7B, FAH, GALE, GALK1, GALT, LIPA, MPI, NPC1, NPC2, PHKA2, PHKB, PHKG2, PYGL, SERPINA1 and SMPD1. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel 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 panel 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.

STATEMENT

CLINICAL HISTORY

Patient is a 8-year-old girl with clinical suspicion of Wilson disease. She has elevated transaminase levels, liver fibrosis, fatty liver and repeatedly low levels of caeruloplasmin.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Metabolic Liver Failure Panel identified two heterozygous variants in *ATP7B*: a nonsense variant c.2336G>A, p.(Trp779*) and a missense variant c.3646G>A, p.(Val1216Met) in *ATP7B*. Due to the large genomic distance between these variants, we were unable to determine whether they occur in the same or in different alleles.

There are 10 individuals heterozygous for *ATP7B* c.2336G>A, p.(Trp779*) in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from this reference population database. The variant causes a premature stop codon and is thus predicted to cause loss of normal protein function either through protein truncation (778 out of 1465 aa) or nonsense-mediated mRNA decay. *ATP7B* c.2336G>A, p.(Trp779*) has been previously reported in Wilson disease patients of North European descent in homozygous or compound heterozygous state in at least 4 families (PMID: 8938442, 22774841). The variant has also been identified in clinical testing and classified as pathogenic (ClinVarID: 156284).

There are 23 individuals heterozygous for *ATP7B* c.3646G>A, p.(Val1216Met) in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). *In silico* tools PolyPhen, SIFT, and MutationTaster predict the variant as deleterious. *ATP7B* c.3646G>A, p.(Val1216Met) has been identified in compound heterozygous state with a disease-causing variant in several patients with Wilson disease and reported in Wilson disease database (PMID: 9671269, 9452121, 10447265, 11043508, 14966923, 15952988, 17587212, 17876883, 18034201). It lies in ATP pocket domain at an amino acid position highly conserved in Cu-dependent ATPases. In structural modelling it was characterised as sensitive to mutation (PMID: 22692182). The variant has also been identified in clinical testing and classified as likely pathogenic (ClinVarID: 188859).

ATP7B

The *ATP7B* gene (MIM *606882) encodes a polypeptide that acts as a plasma membrane copper-transport protein (PMID: 10940336). The product of *ATP7B* is copper-transporting ATPase 2, an intracellular transmembrane copper transporter that is key in incorporating copper into ceruloplasmin and in moving copper out of the hepatocyte into bile. The gene is expressed mainly in liver and kidney. Tissue damage occurs after excessive copper accumulation resulting from lack of copper transport from the liver. Even when no transporter function is present, accumulation of copper occurs over several years. Biallelic *ATP7B* pathogenic variants cause Wilson disease (MIM #277900), which is an autosomal recessive disorder of copper metabolism. It can present with hepatic, haematologic, neurologic, or psychiatric disturbances, or a combination of these. It can present at any age from three years to older than 50 years; symptoms vary among and within families (GeneReviews). The prevalence of Wilson disease is estimated at one in 30,000 in most populations.

More than 800 pathogenic variants have been identified (Wilson Disease Mutation Database, HGMD Professional 2018.1), including nonsense, missense, frameshift, and splice site variants as well as large deletions. The most common pathogenic variant in populations of European origin is an amino acid substitution in a highly conserved motif close to the ATP-binding region (p.His1069Gln) (PMID: 8298641). This pathogenic variant occurs at a frequency of 26%-70% in various populations and is associated with neurologic or hepatic disease. Several studies have found a mean age of onset of 20 to 22 years in individuals homozygous for the common p.His1069Gln pathogenic variant, although earlier onset also occurs (PMID: 15519648, 7666402). The most common pathogenic variant in the Asian population is an amino acid substitution in exon 8, p.Arg778Leu. Pathogenic variants in the promoter region are rare (PMID: 14616767) except in Sardinia, where a 15-bp deletion in the 1-kb promoter region (c.-441_-427del15) predominates (PMID: 10502776). Pathogenic variants that abolish ATP7B function tend to result in a more severe phenotype than some missense variants (PMID: 9055581, 15024742, 14966923).

Mutation nomenclature is based on GenBank accession NM_000053.3 (*ATP7B*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

ATP7B c.2336G>A, p.(Trp779*) and *ATP7B* c.3646G>A, p.(Val1216Met) are classified as pathogenic, considering the current literature and their well-established role as disease-causing variants. Wilson disease caused by *ATP7B* variants is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants occur in *cis* (the same copy of the gene) or in *trans* (different copies of the gene). Compound heterozygosity of the identified variants (variants in *trans*) would explain the patient's disease. If the parents of the affected individual carry one pathogenic variant, then each sibling of the affected individual has a 25% chance of being compound heterozygous and affected, a 50% chance of being an asymptomatic carrier of one of the variants, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended. BpG offers targeted variant testing for the family if requested.

YHTEENVETO

Luokittelemme *ATP7B* c.2336G>A, p.(Trp779*) ja *ATP7B* c.3646G>A, p.(Val1216Met) geenivirheet tautia aiheuttavaksi (pathogenic), koska ne ovat tunnettuja ja hyvin kuvattuja kirjallisuudessa. *ATP7B*-geenin virheet aiheuttavat peittyvästi periytyvän taudin. Koska löydetyt variantit sijaitsevat kaukana toisistaan, ei NGS-datan perusteella pystytä määrittämään sijaitsevatko ne samassa vai eri alleelissa ja tätä varten suosittelemme vanhempien kohdennettua geenitutkimusta. Todetut geenivirheet selittävät potilaan taudin esiintyessään eri vanhemmilta perityissä alleeleissa (compound heterozygosity). Jos kummatkin vanhemmat ovat yhden geenivirheen heterotsygoottisia kantajia, jokaisella sisaruksella on 25 % todennäköisyys periä geenivirhe kummaltakin vanhemmalta ja olla täten sairas, 50 % todennäköisyys olla oireeton geenivirheen kantaja ja 25 % todennäköisyys välttyä geenivirheiltä. Suosittelemme perinnöllisyysneuvontaa ja lähisukulaisten kohdennettua geenitestausta. BpG tarjoaa sukulaisten geenitestauspalvelua.

CONFIRMATION

ATP7B c.2336G>A, p.(Trp779*) and *ATP7B* c.3646G>A, p.(Val1216Met) have been confirmed with Sanger sequencing.

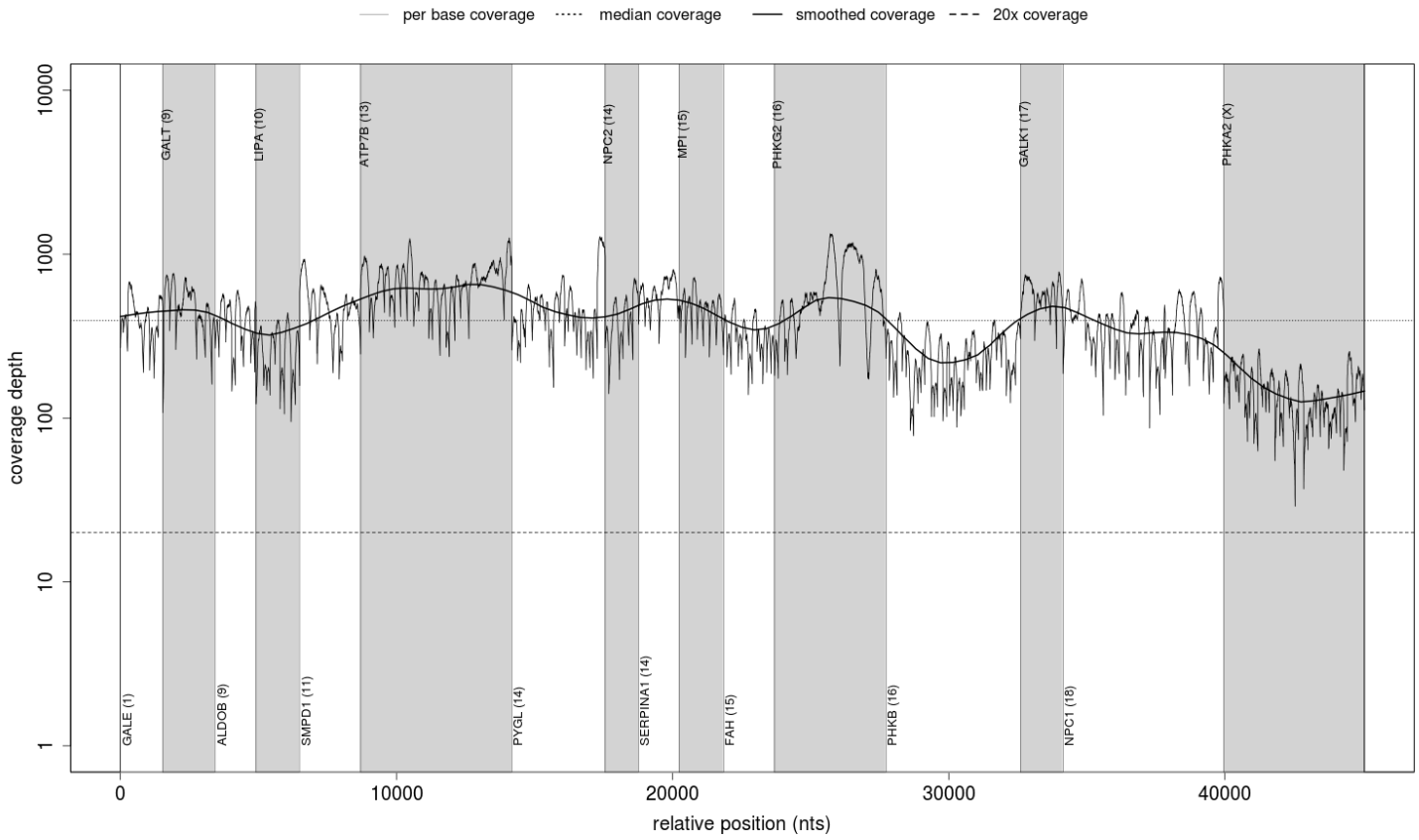
STEP	DATE
Order date	Apr 12, 2018
Sample received	Apr 13, 2018
Reported	May 08, 2018
Last reviewed	May 18, 2018

On May 08, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Tiia Kangas-Kontio, Ph.D., CLG
Senior Geneticist

Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer

Risto Lapatto, MD, Ph.D.
Clinical Consultant


per base coverage
median coverage
smoothed coverage
20x coverage
coverage depth
10000
1000
100
10
1
GALT (9)
LIPA (10)
ATP7B (13)
NPC2 (14)
MPI (15)
PHKG2 (16)
GALK1 (17)
PHKA2 (X)
GALE (1)
ALDOB (9)
SMPD1 (11)
PYGL (14)
SERPINA1 (14)
FAH (15)
PHKB (16)
NPC1 (18)
0
10000
20000
30000
40000
relative position (nts)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean 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). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. 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 as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. 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, which processes aligned sequence reads. 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: 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. Likely benign and benign variants were not reported. 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 the 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.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
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%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, 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 misinterpretation of the results.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000035.3(ALDOB):c.-11+1G>C
NM_000053.3(ATP7B):c.3061-12T>A
NM_000053.3(ATP7B):c.-78A>C
NM_000053.3(ATP7B):c.-123C>A
NM_000053.3(ATP7B):c.-133A>C
NM_000053.3(ATP7B):c.-210A>T
NM_000053.3(ATP7B):c.-442G>A
NM_000154.1(GALK1):c.-22T>C
NM_000155.3(GALT):c.-96T>G
NM_000155.3(GALT):c.83-11T>G
NM_000155.3(GALT):c.687+66T>A
NM_000155.3(GALT):c.820+13A>G
NM_000271.4(NPC1):c.1554-1009G>A
NM_000271.4(NPC1):c.882-28A>G/T
NM_000294.2(PHKG2):c.96-11G>A
NM_000295.4(SERPINA1):c.-5+1G>A

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
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
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.