PROGENE LAB
For Medical And Genetic Tests
معمل بروجين للتحاليل الطبية

Whole Exome Sequencing Report

Personal Data

Patient Name: *********	اسم المريض: *********
Sample type: Blood sample	**DOB:** *********
Patient phone No.: *******	**Sex:** ********
Referring Doctor: *********	**Country:** ********
Report type: Whole Exome Sequencing	**Order received:** *******
Report date: *********	
Reason of referral: Suspicion of genetic etiology based on clinical features	

Clinical Information: A 7-year-old male child complaining of chronic liver disease, cholestasis and excess copper in urine.

Summary of the result

Summary of The Results

Positive result

Two clinically relevant variants were detected, for clinical correlation and differential diagnosis

Two heterozygous variants in *ABCB4* gene causing Cholestasis, progressive familial intrahepatic 3, with autosomal recessive mode of inheritance

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8 شارع حسين حجازي متفرع من شارع القصر العيني – القاهرة
تليفون: 01101799014 – 01149552098

Full Explanation of the result

Interpretation of the test results:

Primary findings

Gene	Variant Coordinates	Zygosity/Protein effect	Heredity	MAF	Classification
ABCB4	NC_000007.10:g.87076453A>G NM_000443.4:c.902T>C NP_000434.1:p.(Met301Thr)	Heterozygous/ missense	AR	0.00	**Likely Pathogenic**
ABCB4	NC_000007.10:g.87047847A>G NM_000443.4:c.2478+6T>C	Heterozygous/ splice variant	AR	0.00	**Variant of Uncertain Significance (VUS) with pathogenic evidence**

The *ABCB4* gene; ATP-binding cassette, subfamily b, member 4 (OMIM* 171060) is a member of superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. This gene encodes a full transporter and member of the p-glycoprotein family of membrane proteins with phosphatidylcholine as its substrate. The function of this protein has not yet been fully determined; however, it may involve transport of phospholipids from liver hepatocytes into bile. Biallelic variants in *ABCB4* are reported to cause Cholestasis, progressive familial intrahepatic 3 (PFIC3; OMIM# 602347).

Patients with PFIC3 usually exhibit hepatosplenomegaly, discolored stools, jaundice, and pruritus during childhood. Most patients progress to cirrhosis, portal hypertension (PHT), and liver failure during the first two decades of life[1]. Gonzales et al., 2023 described 38 patients with disease causing variants in *ABCB4* gene. Median age at symptom onset was 0.9 years (range 0.1–23.3y) and 5 patients presented after 10 years. At presentation, hepatomegaly (82%), pruritus (79%), and splenomegaly (68%) were the most frequent symptoms recorded. Jaundice was present in 11 patients (29%); a history of transient neonatal jaundice was recorded in four more patients[2]. They reported that ursodeoxycholic therapy was tried in 31 patients (82% of their reported cohort), including seven who presented with cirrhosis at the time of referral (median age 4.2 years, range 0.3–27.3y). Sixteen patients (52%) showed a positive response to ursodeoxycholic acid therapy; partial response was seen in five patients (16%) and no response in 10 (32%)[2].

Significant elevation of copper in urine and in liver biopsy has been additionally reported in several case studies, in which the diagnosis of Wilson disease was initially suspected[3-5].

Two heterozygous variants were detected in the ABCB4 gene in our proband: The first missense variant detected in exon-9; NM_000443.4:c.902T>C, NP_000434.1:p.(Met301Thr) is evaluated as **Likely Pathogenic** according to the American College of Medical Genetics (ACMG) criteria for the classification

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8 شارع حسين حجازي متفرع من شارع القصر العيني – القاهرة
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of pathogenic variants[6]. The variant has been described previously as disease causing in several studies, such as **Lipiński et al., 2021** and **Poupon et al., 2013**[7,8]. Furthermore, the variant is predicted as deleterious by the absolute majority of predictor tools, location is highly conserved among different species and is completely absent in population databases (MAF 0.00 in gnomAD).

The second is a novel splice-site variant; NM_000443.4:c.2478+6T>C and is evaluated as **Variant of Uncertain Significance (VUS) with pathogenic evidence** according to the ACMG criteria for the classification of pathogenic variants[6], that is strongly predicted to cause exon skipping and loss of function. Furthermore, the variant location is highly conserved among different species and is completely absent in population databases (MAF 0.00 in gnomAD).

Information for Table Interpretation:

Human Genome Variation Society (HGVS) recommendations were used to describe sequence variants (http://www.hgvs.org).

Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria according to ACMG guidelines[6], variants were assigned to one of five interpretation categories (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign) and using computational pathogenicity calculators. All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance (VUS).

Recommendation:

- We strongly recommend family segregation and the confirmation of the detected heterozygous variants in *ABCB4* gene with Sanger sequencing.
- Genetic counseling has to be offered to the family.

Core disease genes list:

Whole exome of the patient was sequenced. Analysis was restricted togene panels according to the clinical data. In this case, > 99.9% of the targeted regions were covered by a minimum of 85 high-quality sequencing reads per base. The evaluation of variants is dependent on available clinical information at the time of analysis. This prediction can be complemented with additional in-silico predictions in individual cases. Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

Methods:

Sequencing: The coding and flanking intronic regions were enriched using in solution hybridization technology and were sequenced using the Illumina HiSeq/NovaSeqsystem.NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high quality reads using an

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internally developed method based on sequencing coverage depth. Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage.

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 Freebayes. Variant data was annotated with public variant databases (VcfAnno, VEP). 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.

Analytic validation:

This laboratory-developed test has been independently validated. The sensitivity of this test is expected to be in the same range as the validated next generation sequencing assay used to generate the data (sensitivity for SNVs 99.9%, indels 11-50 bps 99.1%, one-exon deletions 100% and 1-9 exon duplications 75%, specificity >99.9% for most variant types). It does not detect very low-level mosaicism as a variant.

Test restrictions and limitations:

Next generation sequencing (NGS) approaches are now routinely adopted to accurately detect single nucleotide variants (SNVs) and have emerged as a technology with the capability to detect accurately both SNVs and CNVs in a single assay, but CNV analysis via NGS is not yet routinely adopted in diagnosis. CNV calls from NGS data depend on high depth and uniformity of coverage across target sites, and currently available bioinformatics tools are still not sensitive enough to reliably pick up all CNVs.

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.

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8 شارع حسين حجازي متفرع من شارع القصر العيني – القاهرة
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Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered. Specific genetic events like copy number variants, translocations and repeat expansions may not be reliably detected with Exome Sequencing.

In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected. Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g., Multiplex Ligation Dependent Probe Amplification (MLPA). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Disclaimer:

DNA studies don't constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it shouldn't be a sole diagnostic criterion. This test is used for clinical purposes. It should not be regarded as investigational or for research. Any preparation and processing of a sample from patient material provided by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic testing itself is based on the highest and most current scientific and analytical standards.

References:

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With kind regards

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