PROGENE LAB
For Medical And Genetic Tests
معمل بروجين للتحاليل الطبية

Trio Whole Exome Sequencing Report

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

Father Name: **********	**اسم الأب:** **********
Sample type: Blood sample	**DOB:** **********

Mother Name: **********	**اسم الأم:** **********
Sample type: Blood sample	**DOB:** **********

Clinical Information: A 4.5-year-old female child born to consanguineous parents complaining of global developmental delay, poor vision, horizontal nystagmus, dysmorphic features, microcephaly, hypotonia, hyporeflexia and white matter hypomyelination.

Summary of The Results

Positive result

A variant was detected that may account for the patient's phenotype for clinical correlation and differential diagnosis

A homozygous variant in *CA2* gene causing carbonic anhydrase II (CA II) deficiency syndrome, with autosomal recessive mode of inheritance

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Interpretation of the test results:

Primary findings:

	Gene	Variant Coordinates	Zygosity/Protein effect	Heredity	MAF	Classification
Proband	*CA2*	NC_000008.10:g.86377699G>A NM_000067.3:c.232+1G>A	**Homozygous**/ Splice Donor	AR	0.00	**Pathogenic**
Father	*CA2*	NC_000008.10:g.86377699G>A NM_000067.3:c.232+1G>A	Heterozygous/ Splice Donor	AR	0.00	**Pathogenic**
Mother	*CA2*	NC_000008.10:g.86377699G>A NM_000067.3:c.232+1G>A	Heterozygous/ Splice Donor	AR	0.00	**Pathogenic**

Carbonic anhydrases (CAs) are a family of zinc metalloenzymes. Carbonic Anhydrase II gene (*CA2*; OMIM* 611492) is one of seven human carbonic anhydrase isozymes and is expressed in the cytoplasm of some cells of virtually every human organ[1]. These enzymes participate in a variety of biologic processes, including respiration, calcification, acid-base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid[2]. Biallelic variants of *CA2* gene have been reported to cause many syndromes with neurological features including Osteopetrosis 3 (OPTB3; OMIM# 259730).

Carbonic anhydrase II (CA II) deficiency syndrome is manifested mainly with osteopetrosis, renal tubular acidosis and repeated fractures. Clinical manifestations of this autosomal recessive syndrome comprise mainly of increased bone density, intracerebral calcifications, mental retardation, growth failure, and facial dysmorphism. Visual and hearing impairment are common and are usually attributed to nerve compression by the deformed skull bones[3].

The homozygous splice donor variant in intron 2 splice site of the *CA2* gene in our proband, **NM_000067.3:c.232+1G>A** is evaluated as **Pathogenic**, according to the American College of Medical Genetics (ACMG) criteria for the classification of pathogenic variants[4]. Loss-of-function is a known mechanism of the disease (*CA2* has 15 reported pathogenic LOF variants). The variant has been described previously as disease causing in many studies, such as **Alsharidiet al. 2015** and **Alsemariet al. 2018**[5,6]. Furthermore, the variant is predicted as deleterious by all predictor tools, location is highly conserved among different species and is 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[4], variants were assigned to one of five

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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.

Recommendation:

- Genetic counseling has to be offered to the family.

Core disease genes list:

Whole exome of the patient was sequenced. Analysis was based ongene 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 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

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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.

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.

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

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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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3- Borthwick KJ, Kandemir N, Topaloglu R, Kornak U, Bakkaloglu A, Yordam N, Ozen S, Mocan H, Shah GN, Sly WS, Karet FE. A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. J Med Genet. 2003 Feb;40(2):115-21. doi: 10.1136/jmg.40.2.115.
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6- Alsemari A, Alsuhaibani M, Alhathlool R, Ali BM. Potential oligogenic disease of mental retardation, short stature, spastic paraparesis, and osteopetrosis. Appl Clin Genet. 2018 Nov 8;11:129-134.

With kind regards

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