

may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: <https://blueprintgenetics.com/pseudogene/>

#The gene has suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#).

STATEMENT

CLINICAL HISTORY

Patient is a 13-months-old girl with global developmental delay (stage of child development - 7 months). She is diagnosed with focal epilepsy since the age of 3 months old. After treatment with levetiracetam was initiated affirmatively the seizures stopped. The patient also tried treatment with vigabatrine before, but with no symptom improvement. The last EEG evaluation showed hypsarrhythmia (april 2019), but there is no clinical evidence of seizures. Treatment with Synacthen was recommended, but it is uncertain if the parents administered the treatment.

Family history is positive for brother of maternal grandmother with 2 epileptic seizures.

CLINICAL REPORT

Blueprint Genetics (BpG) Epileptic Encephalopathy Panel Plus Analysis (Sequence and Del/Dup (CNV)) did not detect any known disease-causing mutation or novel variant that was considered deleterious.

Plus analysis detected variants that were considered as additional findings not explaining the patient's phenotype. Please see APPENDIX 2 for these results.

STEP	DATE
Order date	Jun 10, 2019
Sample received	Jun 12, 2019
Reported	Jun 24, 2019

On Jun 24, 2019 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



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APPENDIX 2: ADDITIONAL FINDINGS

This table includes variants that either are not thought to be the likely cause for patient’s phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient’s phenotype), are secondary findings potentially relevant to patient’s medical care (risk variants, heterozygous pathogenic or likely pathogenic variants for autosomal dominant disorders not related to patient’s current phenotype) or carrier status for pathogenic or likely pathogenic variants for autosomal recessive or X-linked disorder not suspected in the patient.

VARIANT TABLE: ADDITIONAL GENETIC ALTERATIONS

GENE DOCK7	POS 1:62979433	TRANSCRIPT NM_001271999.1	NOMENCLATURE c.4073G>A, p.(Arg1358Gln)	CONSEQUENCE missense_variant	GENOTYPE HET	CLASSIFICATION Variant of uncertain significance
	ID rs150781290	gnomAD AC/AN 20/276928	POLYPHEN benign	SIFT tolerated	MUTTASTER disease causing	
	OMIM	PHENOTYPE Epileptic encephalopathy		INHERITANCE AR	COMMENT -	
GENE SLC13A5	POS 17:6590916	TRANSCRIPT NM_177550.4	NOMENCLATURE c.1507A>T, p.(Met503Leu)	CONSEQUENCE missense_variant	GENOTYPE HET	CLASSIFICATION Variant of uncertain significance
	ID	gnomAD AC/AN 0/0	POLYPHEN probably damaging	SIFT deleterious	MUTTASTER disease causing	
	OMIM	PHENOTYPE Epileptic encephalopathy, early infantile		INHERITANCE AR	COMMENT -	
GENE RNASEH2A	POS 19:12923962	TRANSCRIPT NM_006397.2	NOMENCLATURE c.703C>T, p.(Arg235Trp)	CONSEQUENCE missense_variant	GENOTYPE HET	CLASSIFICATION Variant of uncertain significance
	ID	gnomAD AC/AN 4/245918	POLYPHEN probably damaging	SIFT deleterious	MUTTASTER disease causing	
	OMIM	PHENOTYPE Aicardi-Goutières syndrome		INHERITANCE AR	COMMENT -	

NOTES REGARDING ADDITIONAL FINDINGS

The patient is a heterozygous carrier of the *DOCK7* c.4073G>A,p.(Arg1358Gln), *SLC13A5* c.1507A>T,p.(Met503Leu) and *RNASEH2A* c.703C>T,p.(Arg235Trp) variants. No other known disease-causing or rare, potentially pathogenic variants were detected in the same genes. Disease caused by variants in these genes are inherited in an autosomal recessive manner and, thus, these three variants are not expected to explain patient’s phenotype.

Notably, the *RNASEH2A* c.703C>T,p.(Arg235Trp) variant has been previously identified in one patient with Aicardi-Goutières syndrome, it was identified in combination with *RNASEH2A* c.557G>A, p.(Arg186Gln) (PMID: [24300241](#)).

The figure displays six panels of genomic coverage data across a 100,000 bp region. Each panel shows per-base coverage as grey bars and the smoothed average coverage as a black line. The y-axis represents coverage on a logarithmic scale from 0 to 10,000, with a dashed horizontal line at 20. The x-axis represents the relative position in base pairs (bp) from 0 to 100,000. Gene models are indicated by grey bars above and below the plots, with gene names labeled. The panels show varying levels of coverage, with some regions showing higher coverage than others, and some genes having higher coverage than others.

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 the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call and 2) visual check-up of the variant at IGV is in-line with the variant call. 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). 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. 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_000026.2(ADSL):c.-49T>C
 NM_001182.4(ALDH7A1):c.696-502G>C
 NM_000481.3(AMT):c.-55C>T
 NM_001127221.1(CACNA1A):c.5404-13G>A
 NM_003159.2(CDKL5):c.-162-2A>G
 NM_152783.3(D2HGDH):c.293-23A>G
 NM_021032.4(FGF12):c.*4722T>C
 NM_000806.5(GABRA1):c.-248+1G>T
 NM_000814.5(GABRB3):c.-2204G>A
 NM_000814.5(GABRB3):c.-2290T>C
 NM_138924.2(GAMT):c.391+15G>T
 NM_005943.5(MOCS1):c.*365_*366delAG
 NM_005957.4(MTHFR):c.1753-18G>A
 NM_005957.4(MTHFR):c.-13-28_-13-27delCT
 NM_152416.3(NDUF6F6):c.298-768T>C
 NM_024407.4(NDUFS7):c.17-1167C>G
 NM_025152.2(NUBPL):c.815-27T>C
 NM_007254.3(PNKP):c.1387-33_1386+49delCCTCCTCCCCTGACCCC
 NM_024570.3(RNASEH2B):c.65-13G>A
 NM_006920.4(SCN1A):c.4820-14T>G
 NM_006920.4(SCN1A):c.4306-14T>G
 NM_006920.4(SCN1A):c.964+14T>G
 NM_006920.4(SCN1A):c.384-21T>A
 NM_025243.3(SLC19A3):c.980-14A>G
 NM_006516.2(SLC2A1):c.680-11G>A
 NM_005993.4(TBCD):c.1564-12C>G
 NM_000548.3(TSC2):c.-30+1G>C
 NM_000548.3(TSC2):c.976-15G>A
 NM_000548.3(TSC2):c.2838-122G>A
 NM_000548.3(TSC2):c.5069-18A>G
 NM_014795.3(ZEB2):c.-69-1G>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.
