Blueprint Genetics

*Sample report as of Feb 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications***

Epileptic Encephalopathy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		3		

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *STXBP1* c.874C>T, p.(Arg292Cys), which is pathogenic.

Del/Dup (CNV) analysis

Negative for explaining the patient's phenotype.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
STXBP1	NM_003165.3	c.874C>T, p.(Arg292Cys)	HET	missense_variant	AD	**Pathogenic**

ID	ASSEMBLY	POS	REF/ALT
	GRCh37/hg19	9:130430438	C/T

gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE
0/0	possibly damaging	deleterious	disease causing	Epileptic encephalopathy, early infantile

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Epileptic Encephalopathy Panel	166	2770	529370	527523	119	99.65

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	10760	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Epileptic Encephalopathy Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABAT, ACTL6B, ADAM22, ADAR, ADPRHL2, ADSL, ALDH7A1, ALG13, AMT, AP2M1, AP3B2, APOPT1, ARHGEF9, ARX#, ASNS*, ATP6V1A, BRAT1, CACNA1A, CACNA1B, CACNA1E, CASK, CDKL5, CHD2, CLCN4, CLTC, CNKSR2, CNPY3, CNTNAP2, COX6B1, CPT2, CYFIP2, D2HGDH, DCX, DENND5A, DNM1*, DNM1L, DOCK7, ECHS1, EEF1A2, ETHE1, FAR1*, FARS2, FGF12, FLNA, FOXG1, FRRS1L, GABBR2, GABRA1, GABRB2, GABRB3, GABRG2#, GAMT, GLDC, GNAO1, GPHN, GRIN1, GRIN2A, GRIN2B, GTPBP3, HCN1, HECW2, HEPACAM, HIBCH, HNRNPU, HTT, KCNA2, KCNB1, KCNMA1, KCNQ2, KCNQ3, KCNQ5, KCNT1, KCNT2, KCTD3, KIF1A, LRPPRC, LYRM7, MBD5, MDH2, MECP2, MED17, MEF2C, MOCS1*, MRPL44, MT-ATP6, MT-ATP8, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-RNR1, MT-RNR2, MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY, MTFMT, MTHFR, NACC1, NDUFAF6, NDUFS2, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NECAP1*, NEUROD2, NRXN1, NUBPL, PARS2, PCDH19, PHACTR1, PIGA*, PIGB, PIGP, PIGQ, PIGS, PLAA, PLCB1, PNKP, PNPO, POLG, PPP3CA, PROSC, PTPN23, PURA, RMND1*, RNASEH2A, RNASEH2B, ROGDI, SAMHD1, SCN1A, SCN1B, SCN2A, SCN3A, SCN8A, SCO1, SDHAF1, SERAC1, SIK1, SLC12A5, SLC13A5, SLC19A3, SLC25A1, SLC25A22, SLC2A1, SLC35A2, SLC6A8*, SLC9A6, SNAP25, SPTAN1, ST3GAL3, ST3GAL5, STXBP1, SYN1, SYNGAP1, SYNJ1, SZT2, TBC1D24, TBCD, TBCE, TBCK, TCF4, TRAK1, TREX1, TRIM8, TSC1, TSC2, TTC19, UBA5*, UBE3A*, UNC80, VARS, WARS2, WDR45, WWOX and ZEB2*.* The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: GABRG2 (NM_198903:6).

*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 3-year-old child with mild intellectual disability, psychomotor developmental delay, muscle weakness and generalized epilepsy. Brain MRI was normal. Negative family history of similar condition. Clinical suspicion of West syndrome.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Epileptic Encephalopathy Panel identified a heterozygous missense variant *STXBP1* c.874C>T, p.(Arg292Cys).

STXBP1 c.874C>T, p.(Arg292Cys)

The *STXBP1* c.874C>T, p.(Arg292Cys) variant has not been observed in the large reference population cohorts of 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 these cohorts. All *in silico* tools utilized predict this variant to be damaging to protein structure and function. This variant has previously been reported in multiple individuals with early infantile epileptic encephalopathy, and several times have occurred as *de novo* (PMID: 26865513, 26993267, 29655203, 29390993, 31054490, 29933521; ClinVar ID: 191238).

STXBP1

The *STXBP1* gene (MIM *602926) encodes a syntaxin-binding protein which appears to play a role in release of neurotransmitters via regulation of syntaxin, a transmembrane attachment protein receptor. Heterozygous pathogenic variants in *STXBP1* cause an encephalopathy phenotype that was initially described in patients with early infantile epileptic encephalopathy (EIEE) (EIEE4; MIM #612164), but is now considered to be have a broader phenotypic spectrum.

Through reviewing 147 patients with *STXBP1*-associated encephalopathy, Stamberger et al. concluded that all patients had intellectual disability (ID), which is mostly severe to profound (88%). In this cohort, 95% of patients had epilepsy, which tended to have an onset early in life, in the cases in which it was present (median onset age of 6 weeks (range 1 day–12 years)). While one-third of patients presented with Ohtahara syndrome (21%) or West syndrome (9.5%), the majority had a nonsyndromic early-onset epilepsy and encephalopathy (53%) with epileptic spasms or tonic seizures as the main seizure type. There was no correlation between severity of seizures and severity of ID, or between mutation type and seizure characteristics or cognitive outcome. Nine patients had intellectual disability without epilepsy. Neurologic comorbidities including autistic features and movement disorders were frequent. Autism or autistic features were observed in 25 patients. Other behavioral problems were hyperactivity and aggressive behavior. Among the neurologic symptoms, the most frequent findings were (axial) hypotonia, ataxia or ataxic gait, tremor, spasticity, dyskinesia, and dystonia (PMID: 26865513).

As noted by Değerliyurt *et al*. (2019), the presence of ataxia and tremor in children with severe intellectual disability who can walk is an important indicator that *STXBP1* gene analysis may be required, whether epilepsy is present or not. Authors reported the case of a male patient with late-onset epilepsy, ataxia, severe tremor and autistic symptoms such as limited eye contact, rapid breathing, and screaming. While the patient had originally been followed-up for developmental delay and autism, his initial epileptic attacks had started when he was 12 years old. The head circumference in the patient was above +2SD despite the severe intellectual disability. The patient was heterozygous for a small frameshift deletion. Authors highlighted the fact that *STXBP1* pathogenic variants should not only be considered in patients with early-onset epileptic encephalopathy but also in the differential diagnosis in patients with developmental encephalopathy and late-onset epilepsy and even in those with no epilepsy (PMID: 32105008). Indeed, Gburek-Augustat *et al.* (2016), had previously reported on three female patients with an ataxia-tremor-retardation syndrome caused by a *de novo STXBP1* pathogenic variant, thus showing that epilepsy is not a mandatory feature of the *STXBP1*-related disorder (PMID: 27184330).

Pathogenic variants occur typically *de novo*, but parental somatic mosaicism is also possible (PMID: 21062273). More than 200 disease-causing variants in *STXBP1* are reported in HGMD (2020.1). Most of them are loss of function variants (nonsense, frameshift, splicing, deletion of one or more exons or the entire gene) but missense variants have also been reported (30%).

Mutation nomenclature is based on GenBank accession NM_003165.3 (*STXBP1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

STXBP1 c.874C>T, p.(Arg292Cys) is classified as pathogenic, based on currently available evidence supporting its disease-causing role (established association between the gene and the patient's phenotype, the variant is absent in control populations, *in silico* predicted pathogenicity and identification of the variant in multiple individuals with the same phenotype). Disease caused by *STXBP1* variants is inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. *STXBP1*-related disease may be caused by a *de novo* variant. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

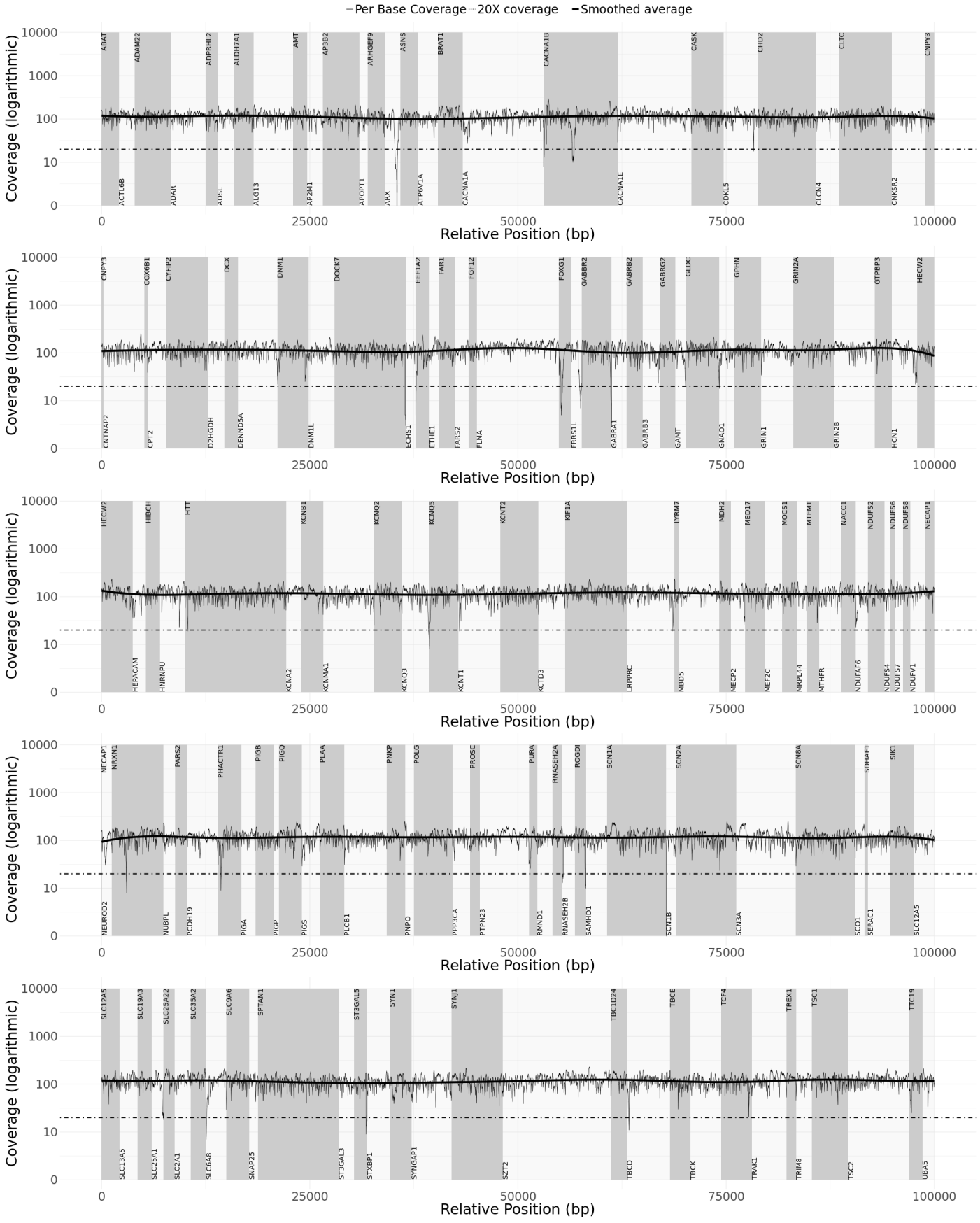
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

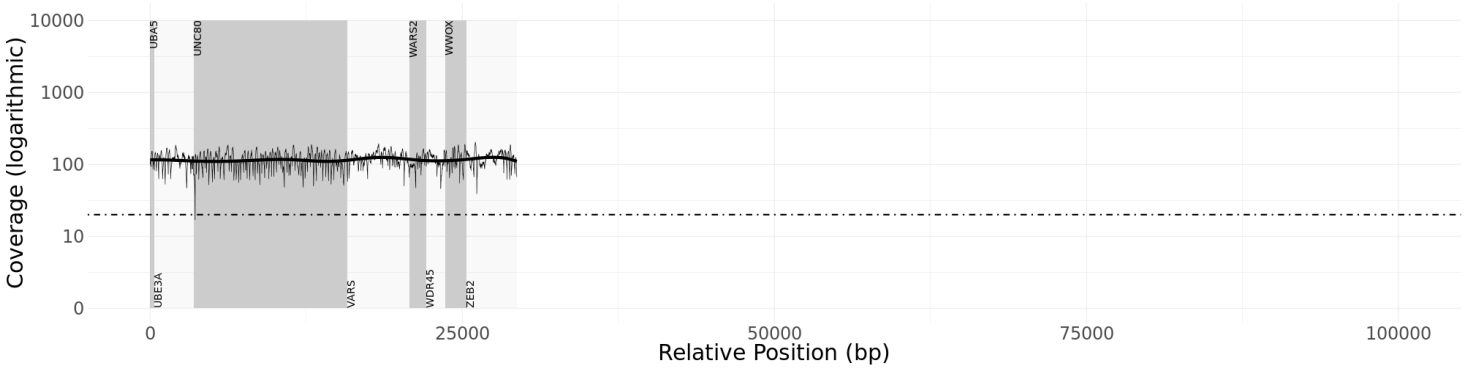
Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.





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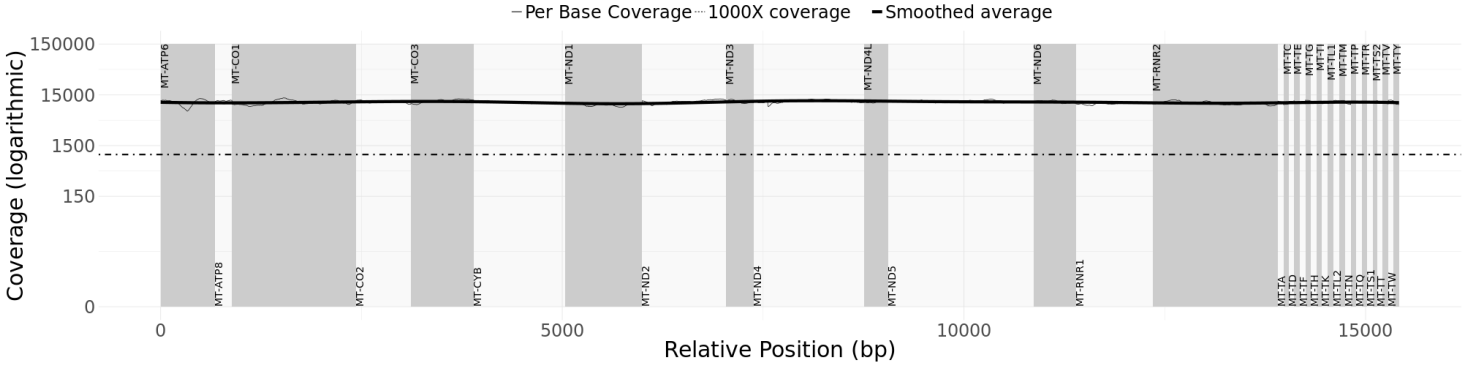

*For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications***

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based 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. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.
Bioinformatics and quality control: Base called raw sequencing data were transformed into FASTQ format using Illumina's software (bcl2fastq). 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) for nDNA. Variant data for were annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. 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 including assessments for contamination and sample mix-up. 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. 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 were adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.
Variant classification: Our variant classification follows the Blueprint Genetics 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.
Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the 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 gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants, specific databases (eg, Mitomap, HmtVar and 1000G) were used.
Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed. **Confirmation of copy number variants:** CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, 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. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.

Test restrictions: 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.

Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. 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 (excluding mtDNA testing and digital PCR confirmation).

NONCODING VARIANTS COVERED BY THE PANEL:

NM_000026.2(ADSL):c.-49T>C
NM_000481.3(AMT):c.-55C>T
NM_001127221.1(CACNA1A):c.*1500_*1504dupCTTTT
NM_001127221.1(CACNA1A):c.5404-13G>A
chr19:g.13617793-13617793
NM_003159.2(CDKL5):c.-162-2A>G
NM_152783.3(D2HGDH):c.293-23A>G
chr19:g.44031407-44031407
NM_021032.4(FGF12):c.*4722T>C
NM_001110556.1(FLNA):c.6023-27_6023-16delTGACTGACAGCC
NM_000806.5(GABRA1):c.-248+1G>T
NM_000814.5(GABRB3):c.-53G>T
NM_000814.5(GABRB3):c.-902A>T
NM_000814.5(GABRB3):c.-2204G>A
NM_000814.5(GABRB3):c.-2290T>C
NM_138924.2(GAMT):c.391+15G>T
NM_002397.4(MEF2C):c.-510_-497delTCTTCCTCCTCCTC
NM_005943.5(MOCS1):c.*365_*366delAG
NM_005943.5(MOCS1):c.*7+6T>C
NM_005943.5(MOCS1):c.251-418delT
NM_005957.4(MTHFR):c.1753-18G>A
NM_005957.4(MTHFR):c.-13-28_-13-27delCT
NM_152416.3(NDUFAF6):c.298-768T>C
NM_152416.3(NDUFAF6):c.420+784C>T
NM_007254.3(PNKP):c.1387-33_1386+49delCCTCCTCCCCTGACCCC
NM_024570.3(RNASEH2B):c.65-13G>A
NM_024570.3(RNASEH2B):c.511-13G>A
NM_024589.2(ROGDI):c.46-30_45+37delGGCGGGGC
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.474-13T>A
NM_032861.3(SERAC1):c.92-165C>T
NM_032861.3(SERAC1):c.92-239G>C
NM_025243.3(SLC19A3):c.980-14A>G
NM_006516.2(SLC2A1):c.680-11G>A
NM_006516.2(SLC2A1):c.-107G>A
NM_001130438.2(SPTAN1):c.6690-17G>A
NM_005993.4(TBCD):c.1564-12C>G
NM_000368.4(TSC1):c.363+668G>A
NM_000548.3(TSC2):c.-30+1G>C
NM_000548.3(TSC2):c.600-145C>T
NM_000548.3(TSC2):c.848+281C>T
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_017775.3(TTC19):c.-42G>T
NM_007075.3(WDR45):c.236-18A>G
NM_014795.3(ZEB2):c.-69-1G>A
NM_014795.3(ZEB2):c.-69-2A>C

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
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
MT = Mitochondria
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.