Blueprint Genetics

Epileptic Encephalopathy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		1	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

TEST RESULTS

The patient is heterozygous for *SCN1A* c.302G>A, p.(Arg101Gln), which is pathogenic.

The disease cause by pathogenic variants in the *SCN1A* gene is inherited in autosomal dominant manner.

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	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
SCN1A	2:166915161	NM_001165963.1	c.302G>A, p.(Arg101Gln)	missense	HET	**Pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs121917918	0/0	benign	deleterious	disease causing	
	OMIM	**PHENOTYPE** Epileptic encephalopathy, early infantile, Generalized epilepsy with febrile seizures plus, Migraine, familial hemiplegic		**INHERITANCE** AD	**COMMENT** -	

Please see APPENDIX 2: Additional Findings

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Epileptic Encephalopathy Panel	83	1389	251618	251274	282	99.8

TARGET REGION AND GENE LIST

Blueprint Genetics Epileptic Encephalopathy Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with epileptic encephalopathy: ADAR, ADSL, ALDH7A1, ALG13, AMT, ARHGEF9, ARX, CACNA1A, CASK, CDKL5, CHD2, CNTNAP2, CPT2, DCX, DNM1*, DOCK7, EEF1A2, FLNA, FOXG1, GABRA1, GABRB3, GABRG2, GAMT, GLDC, GNAO1, GPHN, GRIN2A, GRIN2B, HCN1, HEPACAM, HNRNPU, KCNA2, KCNB1, KCNQ2, KCNQ3, KCNT1, KIF1A, MBD5, MECP2, MEF2C, MOCS1, MTHFR, NECAP1*, NRXN1, PCDH19, PIGA*, PLCB1, PNKP, PNPO, PURA, RNASEH2A, RNASEH2B, SAMHD1, SCN1A, SCN1B, SCN2A, SCN8A, SIK1, SLC2A1, SLC6A8*, SLC9A6, SLC12A5, SLC13A5, SLC19A3, SLC25A22, SLC35A2, SNAP25, SPTAN1, ST3GAL3, ST3GAL5, STXBP1, SYN1, SYNGAP1, SZT2, TBC1D24, TCF4, TREX1, TSC1, TSC2, UBE3A*, WDR45, WWOX and ZEB2*. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of epileptic encephalopathy mutations known to date and it should

be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Epileptic Encephalopathy Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

STATEMENT

CLINICAL HISTORY

This patient is a soon 2-year-old girl with convulsions with and without fever. There are no affected family members.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Epileptic Encephalopathy Panel identified a heterozygous missense variant c.302G>A, p.(Arg101Gln) in the *SCN1A* gene. This variant is absent in the Exome Aggregation Consortium (ExAC) data set, comprised in total of over 60,000 unrelated individuals. The *in silico* predictions for pathogenicity are conflicting for this variant, as it is predicted benign by PolyPhen, but deleterious by SIFT and MutationTaster.

The *SCN1A* c.302G>A, p.(Arg101Gln) variant has been detected previously in at least six patients with Dravet syndrome, intractable epilepsy, or generalized epilepsy with febrile seizures plus as a result of *de novo* events, or parental mosaicism (PMID: 14738421, 23808377, 24328833, 25885068, 23195492, 23158734).

SCN1A encodes voltage-gated sodium channel alpha subunit 1. Each sodium channel alpha subunit has four homologous domains that contains six transmembrane regions. Each sodium channel is composed of a large pore-forming, glycosylated alpha subunit and two smaller beta subunits. They regulate sodium exchange between intracellular and extracellular spaces and are essential for the generation and propagation of action potentials in muscle cells and neurons. Three of the four RefSeq transcript variants are supported by experimental evidence. Autosomal dominant mutation in *SCN1A* associate with so called *SCN1A*-related seizure disorders ranging from simple febrile seizures to severe epilepsy. Mulley et al found that most *SCN1A* pathogenic variants cluster in the C-terminus and in the pore loops connecting S5 and S6 especially in the first three domains of the protein (PMID 15880351). There are altogether 530 *SCN1A* variants classified without conflicts as pathogenic or likely pathogenic in Clinvar (April 2017), of which 48% are missense variants, 22% deletions or insertions leading to frameshift, 13% nonsense, 7% in-frame deletions or insertions and 7% variants affecting consensus splice site. Despite of few exceptions all the disease causing *SCN1A* variants are absent from ExAC reference population. There are only two individuals with truncating *SCN1A* variant affecting canonical transcript in the ExAC reference database (carrier frequency 1 per 30,000).

SCN1A-related seizure disorders (SRSDs) include Dravet Syndrome (synonym to severe myoclonic epilepsy of infancy, (SMEI) and polymorphic myoclonic epilepsy in infancy (PMEI); OMIM *607208)), generalized epilepsy with febrile seizures plus type 2 (GEFS+; OMIM *604403) and familial hemiplegic migraine type 3 (OMIM *609634). Phenotypes with intractable seizures including Dravet syndrome are usually associated with progressive dementia. Less commonly observed phenotypes include myoclonic-astatic epilepsy, Lennox-Gastaut syndrome, infantile spasms and vaccine-related encephalopathy and seizures. About 85% of Dravet syndrome cases are due to a defective *SCN1A* gene (Orpha33069). Prevalence of SRSDs is unknown. The phenotype of *SCN1A*-related seizure disorders can vary even within the same family as the proportion of probands with an *SCN1A*-related seizure disorder and an affected parent decreases as the severity of the phenotype in the proband increases. Thus, Dravet syndrome commonly arise from a *de novo* mutation. Penetrance varies by phenotype: 70% for the GEFS+ phenotype (PMID 14738422) and 90% for the familial simple febrile seizure phenotype (PMID 16326807). Several antiepileptic drugs which are effective for most forms of epilepsy can make seizures worse in *SCN1A* related disease that respond often optimally to antiepileptic drugs that bind to the GABA receptor. (*SCN1A*-related seizure disorders - GeneReviews).

Mutation nomenclature is based on GenBank accession NM_001165963.1 (*SCN1A*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature, the well-established role of *SCN1A c.302G>A,* p.(Arg101Gln) as a disease-causing mutation, we classify it as pathogenic. Genetic counseling and family member testing is recommended. The disease caused by *SCN1A* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting the mutation. A proband with autosomal dominant *SCN1A*-related seizure disorder may have the disorder as a result of a *de novo* mutation. BpG offers mutation testing for the family if requested.

CONFIRMATION

SCN1A c.302G>A, p.(Arg101Gln) was confirmed with bidirectional Sanger sequencing.

STEP	DATE
Order date	Mar 16, 2017
Sample received	Apr 04, 2017
Reported	Apr 25, 2017

On May 03, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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Lab Director, Chief Medical Officer

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Clinical Consultant

APPENDIX 2: ADDITIONAL FINDINGS

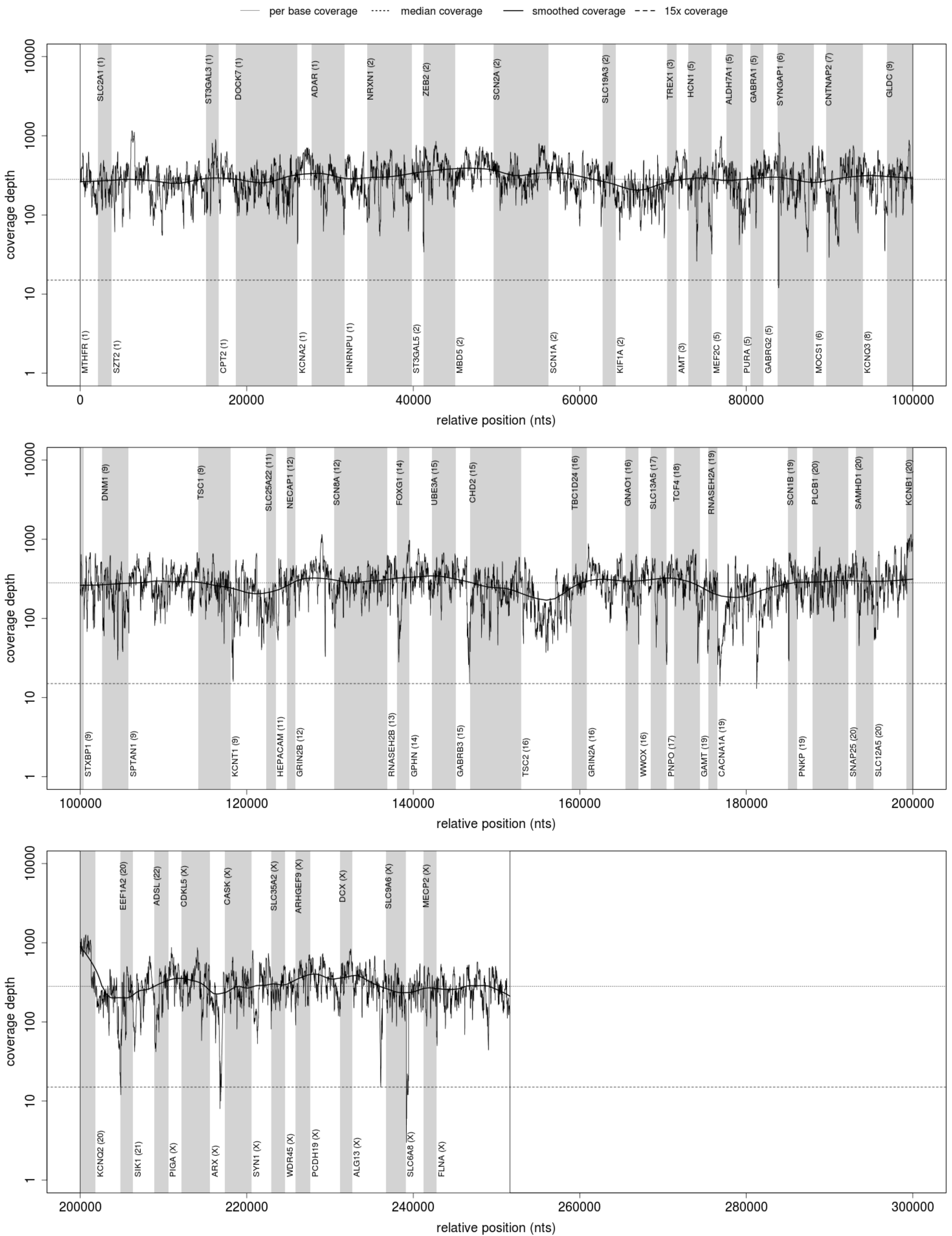
This table includes variants that either are not thought to be the likely cause for patient's phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient's phenotype), 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	**POS**	**TRANSCRIPT**	**NOMENCLATURE**	**CONSEQUENCE**	**GENOTYPE**	**CLASSIFICATION**
STXBP1	9:130435550	NM_003165.3	c.1110+10T>A	intron	HET	**Variant of uncertain significance**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	.	0/0	N/A	N/A	N/A	
	OMIM	**PHENOTYPE** Epileptic encephalopathy, early infantile		**INHERITANCE** AD	**COMMENT** Intronic variant not predicted to affect splicing.	
GENE	**POS**	**TRANSCRIPT**	**NOMENCLATURE**	**CONSEQUENCE**	**GENOTYPE**	**CLASSIFICATION**
ARHGEF9	X:62858101	NM_015185.2	c.1370-12C>T	intron	HET	**Variant of uncertain significance**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	.	0/0	N/A	N/A	N/A	
	OMIM	**PHENOTYPE** Epileptic encephalopathy, early infantile		**INHERITANCE** X-linked	**COMMENT** Intronic variant not predicted to affect splicing.	

NOTES REGARDING ADDITIONAL FINDINGS

As there is not enough data to support or rule out pathogenicity, we classify the identified *STXBP1* c.1110+10T>A and *ARHGEF9* c.1370-12C>T as variants of uncertain significance (VUS). Additional information is needed to assess their clinical significance. Both variants are intronic, and they are absent in the ExAC control population. All five components of the Alamut splicing software (SSF, MaxEnt, NNSPLICE, GeneSplicer, HSF) predict that these variants have no effect on splicing. The patient is heterozygous for these two variants. The disease caused by pathogenic variants in *STXBP1* is inherited in an autosomal dominant manner, while the disease caused by pathogenic variants in *ARHGEF9* is inherited in an X-linked manner. Screening of the variants should not be used for risk evaluation within family members. Management of the patient and family should be based on clinical evaluation and judgment.


per base coverage
median coverage
smoothed coverage
15x coverage
coverage depth
relative position (nts)
SLC2A1 (1)
ST3GAL3 (1)
DOCK7 (1)
ADAR (1)
NRXN1 (2)
ZEB2 (2)
SCN2A (2)
SLC19A3 (2)
TREX1 (3)
HCN1 (5)
ALDH7A1 (5)
GABRA1 (5)
SYNGAP1 (6)
CNTNAP2 (7)
GLDC (9)
MTHFR (1)
SZT2 (1)
CPT2 (1)
KCNA2 (1)
HNRNPU (1)
ST3GAL5 (2)
MBD5 (2)
SCN1A (2)
KIF1A (2)
AMT (3)
MEF2C (5)
PURA (5)
GABRG2 (5)
MOCS1 (6)
KCNQ3 (8)
DNM1 (9)
TSC1 (9)
SLC25A22 (11)
NECAP1 (12)
SCN8A (12)
FOXG1 (14)
UBE3A (15)
CHD2 (15)
TBC1D24 (16)
GNAO1 (16)
SLC13A5 (17)
TCF4 (18)
RNASEH2A (19)
SCN1B (19)
PLCB1 (20)
SAMHD1 (20)
KCNB1 (20)
STXBP1 (9)
SPTAN1 (9)
KCNT1 (9)
HEPACAM (11)
GRIN2B (12)
RNASEH2B (13)
GPHN (14)
GABRB3 (15)
TSC2 (16)
GRIN2A (16)
WWOX (16)
PNPO (17)
GAMT (19)
CACNA1A (19)
PNKP (19)
SNAP25 (20)
SLC12A5 (20)
EEF1A2 (20)
ADSL (22)
CDKL5 (X)
CASK (X)
SLC35A2 (X)
ARHGEF9 (X)
DCX (X)
SLC9A6 (X)
MECP2 (X)
KCNQ2 (20)
SIK1 (21)
PIGA (X)
ARX (X)
SYN1 (X)
WDR45 (X)
PCDH19 (X)
ALG13 (X)
SLC6A8 (X)
FLNA (X)

APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.
Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.
Confirmation. Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.
Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.
Accreditation. 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.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.
Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).
Reporting. Reporting was done using an HGNC-approved gene nomenclature.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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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GLOSSARY OF USED ABBREVIATIONS:

POS = genomic position of the variant in the format of chromosome:position
ID = rsID in dbSNP
Transcript = GenBank accession for reference sequence used for variant nomenclature
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database
AD = autosomal dominant
AR = autosomal recessive
OMIM = Online Mendelian Inheritance in Man®
ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)
het = heterozygous
hom = homozygous
Del/Dup = Deletion and Duplication
CNV = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.