GENETIC TESTING RESULTS
EpiPanel^{Dx} PLUS

bionano laboratories

Patient Name: SAMPLE	**Provider Name:** SAMPLE	**Sample Type:** Buccal
Patient Date of Birth: SAMPLE	**Provider Phone:** SAMPLE	**Sample Collected:** SAMPLE
Patient Sex: Male	**Provider Fax:** SAMPLE	**Sample Received:** SAMPLE
Patient ICD-10 code(s): F88, F80.1, G40.309	**Provider Institution:** SAMPLE	**Order Date:** SAMPLE
Accession Number: SAMPLE		**Report Date:** SAMPLE

Test Result Summary: Pathogenic

EpiPanel^{Dx} PLUS identified two sequence variants. The variant in the *SHANK3* gene is clinically significant and is consistent with Phelan-McDermid syndrome. The variant in the *RAI1* gene has uncertain clinical significance.

Test performed: Proband Only (mother: N/A; father: N/A)

EpiPanel^{Dx} PLUS Results

Gene (Transcript)	DNA change	Protein Change	Classification	Zygosity	Parental Inheritance	Associated Syndrome (inheritance)
SHANK3 (NM_033517.1)	c.SAMPLE	p.SAMPLE	Pathogenic	Heterozygous	Not Assessed	Phelan-McDermid Syndrome (Autosomal dominant)
RAI1 (NM_030665.4)	c.SAMPLE	p.SAMPLE	Variant of Uncertain Significance	Heterozygous	Not Assessed	Smith-Magenis syndrome (Autosomal dominant)

Clinical Interpretation and Discussion

EpiPanel^{Dx} PLUS identified two sequence variants. The sequence variant reported in the *SHANK3* gene is clinically significant (pathogenic) and is consistent with Phelan-McDermid syndrome. Due to an increased risk of symptoms associated with this condition, additional evaluations may be recommended. The sequence variant reported in the *RAI1* gene has uncertain significance, meaning the clinical consequences are not currently known. Clinical correlation or further evaluations may be helpful. Below is a summary of the current evidence about these findings as well as details about genetic counseling options available to the family.

All sequence variants reported are classified based on the American College of Medical Genetics (ACMG) standards and guidelines (PMID: 25741868).

SHANK3 gene, NM_033517.1, c.SAMPLE, p.SAMPLE, Chr22(GRCh37):g.SAMPLE, pathogenic

A pathogenic sequence variant in one copy (heterozygous) of the *SHANK3* gene was found. This sequence variant is consistent with Phelan-McDermid syndrome and is expected to result in an increased risk of the associated symptoms.

Genetic variants affecting one copy of the *SHANK3* gene cause autosomal dominant Phelan-McDermid syndrome (PMS; PMIDs: 20301377, 23758760, 25784960, 33949759, 34246244, 34559195, 35328058, 35495150). Symptoms of this condition include global developmental delay, moderate-to-profound intellectual disability, severely impaired or absent speech, hypotonia, brain anomalies, sleep disturbances, and neurobehavioral differences such as autism spectrum disorder or impulsivity. Seizures have been reported to affect up to half of individuals, with the most common seizure types including absence and grand mal (PMIDs: 20301377, 34664257, 34325981). Subtle unique facial features may include dolichocephaly, large or prominent ears, epicanthal folds, long eyelashes, supraorbital fullness, full cheeks, and a short or bulbous nose (PMID: 20301377). Developmental regression, affecting speech, motor, and/or self-help skills, has been reported. One study suggested up to 43% of individuals experience a loss of skills, commonly with onset in mid-childhood (PMID: 28346892). There is also an increased risk of psychiatric conditions in adolescents and young adults with PMS, including bipolar disorder, anxiety, mood disorders, catatonia, and possibly schizophrenia (PMIDs: 20385823, 26824576, 31879555, 32050889).

The mechanism of pathogenicity of PMS is loss of function (haploinsufficiency; PMID: 20301377). Most affected individuals have a deletion involving at least the *SHANK3* gene, although approximately 10% of affected individuals have a *SHANK3* sequence variant (PMID: 35495150). Pathogenic sequence variants are typically *de novo* (not inherited; PMID: 20301377). Individuals with PMS due to large deletions involving *SHANK3* are sometimes reported to have more severe clinical presentations, likely due to the inclusion of other genes within the deletion region (PMIDs: 23758760, 33949759, 34325981, 36118903).

This frameshift variant has been shown by functional studies to result in a truncated protein, lacking several crucial domains involved in mGluR and actin binding (Homer, AbPI, cortactin), and in the synaptic targeting and postsynaptic assembly of the SHANK3 multimer (PMID: 17173049). This variant has been reported in multiple unrelated individuals with PMS in the medical literature, including at least four cases of *de novo* events (PMIDs: 17173049, 29719671, 30763456, 32050889, 33256793). It has also been reported as pathogenic by multiple submitters in ClinVar, a database that curates genetic information from individuals with clinical features (Variation ID: XXXX; PMID: 26582918). This variant was not found in any of the reference alleles in gnomAD, a database of approximately 135,000 individuals without severe genetic conditions (PMID: 32461654).

RAI1 gene, NM_030665.4, c.SAMPLE, p.SAMPLE, Chr17(GRCh37):g.SAMPLE, variant of uncertain significance

A sequence variant of uncertain clinical significance in one copy (heterozygous) of the *RAI1* gene was found. It is currently not known if this sequence variant affects the function of the *RAI1* gene. Therefore, the clinical consequences of this sequence variant are not currently known.

Genetic variants affecting one copy of the *RAI1* gene cause autosomal dominant Smith-Magenis syndrome (SMS; PMIDs: 20301487, 26336863, 26384114, 21199049, 33368193, 35205380). Symptoms of this condition include developmental delay, variable intellectual disability (often mild-to-moderate), sleep disturbance, childhood-onset abdominal obesity, and behavioral concerns. Infants with SMS have feeding difficulties, failure to thrive, hypotonia, hyporeflexia, prolonged napping or need to be awakened for feeds, and generalized lethargy. Behavioral features are typically recognized by 18 months and include stereotypies, sensory problems, and maladaptive or self-injurious behaviors. Additional symptoms may include hearing loss, short stature, scoliosis, constipation, ocular abnormalities, hypercholesterolemia/hypertriglyceridemia, and congenital anomalies affecting the heart, eyes, or genitourinary system. Dysmorphic facial features become more prominent with age, including a broad square-shaped face, brachycephaly, prominent forehead, synophrys, mildly up-slanting palpebral fissures, deep-set eyes, broad nasal bridge, midface retrusion, short/full-tipped nose with reduced nasal height, micrognathia in infancy changing to relative prognathia with age, and a distinct appearance of the mouth, with fleshy everted vermilion of the upper lip.

The majority of individuals with SMS have a *de novo* 17p11.2 deletion involving *RAI1* and other genes; however, *RAI1* sequence variants have been reported in approximately 10% of affected individuals (PMIDs: 20301487, 29138588, 24715852). These sequence variants are often frameshift or nonsense, but missense variants have been reported in some. In most cases, sequence variants are *de novo*, but inheritance of a pathogenic *RAI1* frameshift variant from an affected parent has been reported (PMID: 27683195). Some genotype-phenotype correlations have been described, including individuals with 17p11.2 deletions being more likely to have cardiac and renal anomalies, motor delay, short stature, and hearing loss, while individuals with *RAI1* sequence variants are more likely to develop obesity/overweight, feeding disorders, behavioral concerns, muscle cramping, and dry skin (PMID: 35205380). The mechanism of pathogenicity of SMS is loss of function (haploinsufficiency; PMIDs: 20301487, 29138588).

This missense variant was found in 3 of 272,734 of the reference alleles in gnomAD (PMID: 32461654). The highest allele frequency that this variant has been observed at in any sub-population with available data is less than 0.01%. There are no homozygous control individuals for this variant. This variant has been reported once as likely benign in ClinVar (Variation ID: XXXX; PMID: 26582918). To our knowledge, this variant has not been reported in affected individuals.

Summary

The variant in the *SHANK3* gene is considered pathogenic and is expected to result in an increased risk of symptoms associated with Phelan-McDermid syndrome. **There are published expert opinions on medical management for this condition (PMIDs: 20301377, 25784960, 35328058).**

The *RAI1* variant has uncertain clinical significance and may not provide a genetic diagnosis; however, follow up testing or evaluations may be recommended. Evaluation with a medical geneticist or discussion with a genetic counselor may be considered to determine if additional testing or assessments would be useful.

Biological parent samples were not submitted for testing with this individual's sample; hence, it is not known if the *SHANK3* and *RAI1* variants were new (*de novo*) or inherited. If biological parents are available and interested in submitting samples, site-specific parental testing is available. Please contact a Bionano Laboratories genetic counselor for further details.

References

For more information about the genes or conditions in this report, please see the Online Mendelian Inheritance in Man (OMIM) database: http://www.omim.org

The references in this report can be found by searching the PubMed IDs (PMID) from the PubMed home page: http://www.ncbi.nlm.nih.gov/pubmed/

For more information on how to use PubMed, see the following tutorial: http://www.nlm.nih.gov/bsd/disted/pubmedtutorial/cover.html

The Genome Aggregation Database (gnomAD; PMID: 27535533) provides data on how frequently specific genetic variants are seen in presumably unaffected individuals. The sequencing variants in this report can be found by searching the genomic position on the gnomAD browser: http://gnomad.broadinstitute.org/

Genetic Counseling and Family Resources

The EpiPanel[Dx] PLUS looks for sequence variants (spelling changes) as well as deletions and duplications (missing or extra pieces) in the DNA code in over 200 genes related to epilepsy. These genetic changes may be responsible for causing a person to have seizures, epilepsy, and related clinical features. Since everyone has genetic variants, the genetic community is continuously learning about which variants cause symptoms (called pathogenic variants) and which variants do not (called benign variants).

Bionano Laboratories genetic counselors are available by phone to speak with providers or the family about this test result. You can schedule a time to speak with a Bionano Laboratories genetic counselor by calling 801-931-6191.

A genetic counselor can help review what these results mean for an individual and family members, background on genetics, and discuss additional resources or next steps that may be helpful. Additionally, the genetic counselor may review medical, developmental, and family history of the person tested. This will help the genetic counselor better answer questions about how a specific result may impact the family. To best prepare for a genetic counseling session, it may be helpful for a family to create a list of questions. Additionally, it may help to review some information on basic genetic concepts such as what are genes and genetic changes. The following resource is a good place to start for information about a variety of genetic topics:

MedlinePlus: This website can be used to find information about genetic topics, genes, specific genetic conditions, and broad topics like autism spectrum disorder. It also provides links to other websites for more in-depth information about genetic conditions, patient support and advocacy resources, and relevant clinical trials. https://medlineplus.gov/genetics

Epilepsy Foundation: www.epilepsy.com

MyGene2: This is a resource for connecting families with rare conditions to other families, clinicians, and researchers. http://mygene2.org/MyGene2/

Phelan-McDermid Syndrome Foundation: This organization supports families and individuals with Phelan-McDermid syndrome. http://www.pmsf.org

Genes Tested

EpiPanel[Dx] PLUS: 244 genes tested, 99.62% at >20x, 99.7% at 10x.

Genes tested: *ABCD1, ADSL, AKT3, ALDH7A1, AMT, ANKRD11, ARG1, ARHGEF9, ARID1B, ARL13B, ARSA, ARSB, ASAH1, ASPM, ATIC, ATP1A2, ATP2A2, ATP6AP2, ATP6V0A2, ATRX, AUH, B4GALT1, BCKDK, BCS1L, BOLA3, BRAF, BTD, C12orf57, CACNA1A*, CACNA1E, CACNA1H, CACNB4, CASK, CASR, CC2D2A, CDKL5, CENPJ, CEP290, CHD2, CHRNA2, CHRNA4, CHRNB2, CLCN2, CLCN4, CLN3, CLN5, CLN6, CLN8, CNNM2, CNTNAP2, COG8, COL4A1, COQ2, COQ8A, COX10, CPA6, CPT2, CRH, CSTB*, CTSA, CUL4B, DCX, DDC, DEPDC5, DHFR, DLD, DOLK, DPAGT1, DPM1, DPYD, DYNC1H1, DYRK1A, EEF1A2, EHMT1, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, EMX2, EPM2A, FH, FLNA, FOLR1, FOXG1, GABBR2, GABRA1, GABRB3, GABRD, GABRG2, GALC, GAMT, GATM, GCH1, GCSH, GFAP, GLB1, GLDC, GLI3, GLRA1, GLRB, GLUD1*, GNAO1, GNE, GNS, GOSR2, GRIA3, GRIN1, GRIN2A, GRIN2B, HECW2, HEXA, HEXB, HGSNAT, HSD17B10, IDS, IQSEC2, KANSL1, KCNA1, KCNA2, KCNB1, KCNC1, KCNMA1, KCNQ2, KCNQ3, KCNT1, KDM5C, KDM6A, KMT2D, L2HGDH, LAMA2, LGI1, LRPPRC, MAP2K1, MBD5, MECP2*, MEF2C, MFSD8, MGAT2, MLC1, MOCS1, MTOR, NAGLU, NDUFA1, NDUFA2, NDUFS1, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NEDD4L, NEU1, NEXMIF, NF1*, NGLY1, NHLRC1, NIPBL, NPC1, NPC2, NRXN1, NSD1, OFD1, PANK2, PCDH19, PDHA1, PHF6,*

PHGDH, PLA2G6, PLP1, PMM2, PNPO, POLG, PPP3CA, PPT1, PRICKLE1, PRODH, PRRT2, PSAP, PSPH, PURA, QDPR, RAB39B, RAI1, RARS2, RBFOX1, RBFOX3, RELN, RFT1, RNASEH2A, RNASEH2B, RNASEH2C, ROGDI, SAMHD1, SCN10A, SCN1A, SCN1B, SCN2A, SCN3A, SCN4A, SCN5A, SCN8A, SCN9A, SDHA, SERPINI1, SETBP1, SHANK3, SLC17A5, SLC19A3, SLC25A15, SLC2A1, SLC46A1, SLC4A10, SLC6A1, SLC6A5, SLC6A8, SLC9A6, SMARCA2, SMC1A, SMC3, SMS, ST3GAL5, STX1B, STXBP1, SYN1, SYNGAP1, SZT2, TBL1XR1, TBX1*, TCF4*, TPK1, TPP1, TREX1, TSC1, TSC2, TUBA1A, TUBB2B, TWNK, UBE2A, UBE3A, UNC80, VPS13A, WDR45, ZEB2.*

*Indicates gene has specific limitations; see methodology for details.

Bionano Laboratories' Clinical Interpretation Process

Sequence variants, deletions, and duplications identified by EpiPanel[Dx] PLUS are analyzed with a comprehensive interpretation process. Guidelines from the American College of Medical Genetics and Genomics (ACMG) are applied to variants reported (PMID: 25741868). The interpretation process involves collaboration between experts including laboratory directors, variant analysts, and certified genetic counselors. This team completes an up-to-date review of the medical literature, patient databases (PMIDs: 26582918, 12754702) and control datasets (PMIDs: 24174537, 32461654). This process provides comprehensive information about the current evidence for genes and specific variants within them. The relevant evidence from this interpretation process is summarized in each report.

Methodology and Limitations

Methodology: Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). DNA was barcoded, and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were then sequenced using a Next Generation Sequencing technology. Following alignment to the human genome reference sequence (assembly GRCh37), variants were detected in regions of at least 10x coverage. Regions that did not have 10x coverage were not evaluated. Specific specimen coverage is provided in the Genes Tested section of this report. Variants were interpreted manually using locus specific databases, literature searches, and other molecular biological principles. To minimize false positive results, any variants that do not meet internal quality standards are confirmed by Sanger sequencing. Variants classified as pathogenic, likely pathogenic, unknown significance, or risk allele which are located in the coding regions and nearby intronic regions (+/- 20bp) of the genes listed above are reported. Variants outside these intervals may be reported but are typically not guaranteed. When a single pathogenic or likely pathogenic variant is identified in a clinically relevant gene with autosomal recessive inheritance, the laboratory will attempt to ensure 100% coverage of coding sequences either through NGS or Sanger sequencing technologies ("fill-in"). All genes listed were evaluated for large deletions and/or duplications. However, single exon deletions or duplications will not be detected in this assay, nor will copy number alterations in regions of genes with significant pseudogenes. Putative deletions or duplications identified by NGS are confirmed by an orthogonal method (qPCR or MLPA), unless exceeding an internally specified and validated quality score, beyond which deletions and duplications are considered real without further confirmation. New York patients: diagnostic findings are confirmed by Sanger, MLPA, or qPCR; exception SNV variants in genes for which confirmation of NGS results has been performed >=10 times may not be confirmed if identified with high quality by NGS. Bioinformatics: The Fulgent Germline v2019.2 pipeline was used to analyze this specimen.

Limitations: These test results and variant interpretation are based on the proper identification of the submitted specimen, accuracy of any stated familial relationships, and use of the correct human reference sequences at the queried loci. In very rare instances, errors may result due to mix-up or co-mingling of specimens. Positive results do not imply that there are no other contributors, genetic or otherwise, to this individual's phenotype, and negative results do not rule out a genetic cause for the indication for testing. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (https://www.genenames.org) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation is based on the available clinical and family history information for this individual, collected published information, and Alamut annotation available at the time of reporting. This assay is designed and validated for detection of germline variants only. It is not designed or validated for the detection of low-level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions (eg. trinucleotide or hexanucleotide repeat expansion). DNA

alterations in regulatory regions or deep intronic regions (greater than 20bp from an exon) may not be detected by this test. Unless otherwise indicated, no additional assays have been performed to evaluate genetic changes in this specimen. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical, computational, or human error, DNA variants may be missed. Although next generation sequencing technologies and our bioinformatics analysis significantly reduce the confounding contribution of pseudogene sequences or other highly homologous sequences, sometimes these may still interfere with the technical ability of the assay to identify pathogenic alterations in both sequencing and deletion/duplication analyses. Deletion/duplication analysis can identify alterations of genomic regions which are two or more contiguous exons in size; single exon deletions or duplications may occasionally be identified, but are not routinely detected by this test. When novel DNA duplications are identified, it is not possible to discern the genomic location or orientation of the duplicated segment, hence the effect of the duplication cannot be predicted. Where deletions are detected, it is not always possible to determine whether the predicted product will remain in-frame or not. Unless otherwise indicated, deletion/duplication analysis has not been performed in regions that have been sequenced by Sanger.

*Gene Specific Limitations:
CACNA1A: The current testing method does not assess trinucleotide repeat expansions in this gene.
CSTB: The current testing method does not assess repeat expansions in this gene.
GLUD1: Pseudogene interference for *GLUD1* (NM.005271.3) is known to limit the sensitivity of partial gene deletion/duplication analysis. Some detected potential del/dup signals by NGS may not be amenable to orthogonal confirmation. Such variants may not be reported unless specific signs of hyperinsulinism-hyperammonemia syndrome are present in the tested individual.
MECP2: Currently available technologies (NGS and qPCR) are not amenable to detection of single exon deletions/duplications of exon 1 of the *MECP2* gene due to the genomic sequence context (high GC/AT ratio).
NF1: The ordered testing method does not guarantee the detection of potentially causative somatic changes in *NF1*.
SHANK3: Two regions of this gene (NM_033517.1) are not currently evaluated by this test due to separate technical limitations: exon 1 (high GC content)and portions of exon 12 (mappability).
TBX1: The current testing method does not assess polyalanine repeat expansions in this gene.
TCF4: The current testing method does not assess trinucleotide repeat expansions in this gene.

Disclaimer: This test was developed and its performance characteristics determined by Fulgent Genetics CAP #8042697 CLIA #05D2043189; 4978 Santa Anita Ave., Suite 205, Temple City, CA 91780. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. It is recommended that patients receive appropriate genetic counseling to explain the implications of the test result, including its residual risks, uncertainties and reproductive or medical options. Fulgent CLIA Laboratory Director: Hanlin Gao, M.D.

Lineagen, Inc.'s (DBA Bionano Laboratories) reporting service is certified under CLIA (CLIA# 46D2042721).
Bionano Laboratories, 2677 E Parleys Way Salt Lake City, Utah 84109, USA. T: 801-931-6200 F: 801-931-6201
www.bionanolaboratories.com

Lineagen CLIA Laboratory Director: SAMPLE

Report Reviewed By: SAMPLE