ExomeNext®
Secondary Findings

SAMPLE REPORT

Ambry Genetics®
A TEMPUS COMPANY

PATIENT
Legal Name: **Patient, Sample**
Accession #: 00-306909
DOB: 10/08/2021
Sex Assigned at Birth: Male
MRN: N/A
Indication: Screening

TEST INFORMATION
Portal Order #: 0000000
Family #: 0000000
Specimen #: N/A
Specimen type: Blood EDTA
Collection date: 12/01/2025
Received date: 12/01/2025
Test Started: 12/01/2025
Final Report: 01/01/2026

MEDICAL PROFESSIONAL
Sample Doctor
Sample Facility

ADDITIONAL RECIPIENTS
Sample Genetic Counselor

NEGATIVE: No Reportable Variants Detected

Results and Interpretation

- No reportable variants were detected in the ACMG recommended minimum list of genes v3.3 (Lee, 2025). Only pathogenic and likely pathogenic variants are reported.
- A negative secondary findings result does not rule out the possibility that the individual carries a pathogenic or likely pathogenic variant in a gene on the ACMG list. Not all exons in the genome are sequenced and certain genomic regions may have low coverage.

Notes

- Variant(s) related to this individual's phenotype are reported within the primary report (when applicable), and are not included in secondary findings reports.
- Reclassification reports will be issued for positive secondary findings that are downgraded to a variant of uncertain significance or lower after the time of the report. Results are not systematically reanalyzed for additional secondary findings after the time of the report.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Electronically Signed By Sample Director, on 01/01/2026 at 0:00:00 PM

All content hereafter is supplemental information to the preceding report.

ACMG Recommended Secondary Findings List

(Genes and associated phenotypes as recommended in Lee, 2025)

ABCD1[+], *ACTA2, ACTC1, ACVRL1, APC, APOB, ATP7B*[*] [#], *BAG3, BMPR1A, BRCA1, BRCA2, BTD*[*], *CACNA1S*[#], *CALM1, CALM2, CALM3, CASQ2*[*], *COL3A1*[#], *CYP27A1*[*] [#], *DES, DSC2, DSG2, DSP, ENG, FBN1, FLNC*[#], *GAA*[*], *GLA, HFE*[*] (homozygous c.845G>A p.C282Y only), *HNF1A, KCNH2, KCNQ1, LDLR, LMNA, MAX, MEN1, MLH1, MSH2, MSH6, MUTYH*[*], *MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PALB2, PCSK9, PKP2, PLN, PMS2*[#], *PRKAG2, PTEN, RB1, RBM20, RET, RPE65*[*] [#], *RYR1*[#], *RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM127, TMEM43, TNNC1, TNNI3, TNNT2, TP53, TPM1, TRDN*[*], *TSC1, TSC2, TTN*[^], *TTR, VHL*, and *WT1*[#].

[+]only reported when hemizygous, homozygous, or 2 pathogenic or likely pathogenic variants present

[*]only reported when homozygous or 2 pathogenic or likely pathogenic variants present

[^]only pathogenic or likely pathogenic frameshift and nonsense variants, and variants known to impact the splicing of *TTN* exons with high PSI

[#]sequencing analysis only

Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

- Ambry Genetics Variant Classification Scheme: http://www.ambrygen.com/variant-classification
- Chen S, *et al.* (2024) *Nature*. **625**(7993):92-100. **PMID**: 38057664. Genome Aggregation Database (gnomAD): https://gnomad.broadinstitute.org
- Choi Y, *et al.* (2012) *PLoS One*. **7**(10):e46688. **PMID**: 23056405
- Eppig JT, *et al.* (2012) *Nucleic Acids Res*. **40**(D1):D881-86. **PMID**: 22075990. Mouse Genome Database (MGD): https://www.informatics.jax.org/
- Farwell Hagman KD, *et al.* (2016) *Genet Med*. **19**(2):224-235. **PMID**: 27513193
- Feng BJ. (2017) *Hum Mutat*. **38**(3):243-251. **PMID**: 27995669
- Finger JH, *et al.* (2011) *Nucleic Acids Res*. **39**(Issue suppl_1):D835-D841. **PMID**: 21062809. Mouse Gene Expression Database (GXD): https://www.informatics.jax.org/
- Firth HV, *et al.* (2009) *Am J Hum Genet*. **84**:524-533. **PMID**: 19344873. Database of Genomic Variation and Phenotype in Humans using Ensembl Resources (DECIPHER): https://www.deciphergenomics.org
- Goldfarb T, *et al.* (2025) *Nucleic Acids Res*. **53**(D1):D243-D257. **PMID**: 39526381. RefSeq: http://www.ncbi.nlm.nih.gov/refseq
- Grantham R. (1974) *Science*. 1**85**(4151):862-864. **PMID**: 4843792
- Green RC, *et al.* (2013) *Genet Med*. **15**(7):565-74. **PMID**: 23788249
- Jaganathan K, *et al.* (2019) *Cell*. **176**(3):535-548.e24. **PMID**: 30661751
- Kanehisa M, *et al.* (2014) *Nucleic Acids Res*. **42**(D1):D199-205. **PMID**: 24214961. Kyoto Encyclopedia of Genes and Genomes (KEGG): http://www.genome.jp/kegg
- Karczewski KJ, *et al.* (2020) *Nature* **581**(7809):434-443. **PMID**: 32461654
- Kent WJ, *et al.* (2002) *Genome Res*. **12**(6):996-1006. **PMID**: 12045153. UCSC Genome Browser: https://genome.ucsc.edu
- Landrum MJ, *et al.* (2014) *Nucleic Acids Res*. **42**(D1):D980-5. **PMID**: 24234437. ClinVar: http://www.ncbi.nlm.nih.gov/clinvar
- Lane L, *et al.* (2012) *Nucleic Acids Res*. **40**(D1): D76-D83. **PMID**: 22139911. NeXtProt: http://www.nextprot.org
- Lee K, *et al.* (2025) *Genet Med*. **27**(8):101454. **PMID**: 40568962
- Lek M, *et al.* (2016) *Nature*. **536**(7616):285-91. **PMID**: 27535533
- Lin J, *et al.* (2019) Hum Mutat. **40**(10):1856-1873. **PMID**: 31131953
- Lindeboom RG, *et al.* (2016) *Nat Genet*. **48**(10):1112-8. **PMID**: 27618451
- MacDonald JR, *et al.* (2014) *Nucleic Acids Res*. **42**(D1):D986-92. **PMID**: 24174537. Database of Genomic Variants (DGV): http://dgv.tcag.ca
- Newman S, *et al.* (2015) *Am J Hum Genet*. **96**(2):208-20. **PMID**: 25640679
- Online Mendelian Inheritance in Man (OMIM®). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD). http://www.omim.org
- Pagon RA, *et al.* editors. (1993-) Seattle, WA: University of Washington, Seattle. GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1116
- Petryszak R, *et al.* (2013) *Nucleic Acids Res*. **42**(D1):D926-32. **PMID**: 24304889. Expression Atlas: http://www.ebi.ac.uk/gxa/home
- Rehm HL, *et al.* (2015) *N Engl J Med*. **372**(23):2235-2242. **PMID**: 2601459. Clinical Genome Clinical Validity Classifications: https://clinicalgenome.org/docs/clinical-validity-classifications
- Rhee J, *et al.* (2017) *Sci Rep*. **7**(1):1653. **PMID**: 28490743
- Richards S, *et al.* (2015) *Genet Med*. **17**(5):405-424. **PMID**: 25741868
- Richardson ME, *et al.* (2019) *Genet Med*. **21**(3):683-693. **PMID**: 30054569
- Rivas MA, *et al.* (2015) *Science*. **348**(6235):666-9. **PMID**: 25954003
- Smith ED, *et al.* (2017) *Hum Mutat*. **38**(5):600-608. **PMID**: 28106320
- Solomon BD, *et al.* (2013) *Proc Natl Acad Sci USA*. **110**(24):9851-5. **PMID**: 23696674. Clinical Genomic Database: http://research.nhgri.nih.gov/CGD
- Stenson PD, *et al.* (2014) *Hum Genet*. **133**(1):1-9. **PMID**: 24077912. Human Gene Mutation Database (HGMD®): http://www.hgmd.cf.ac.uk
- Thorvaldsdóttir H, *et al.* (2012) *Brief Bioinform*. **14**(2):178-192. **PMID**: 22517427
- Warde-Farley D, *et al.* (2010) *Nucleic Acids Res*. **38**(Issue suppl_2):W214-20. **PMID**: 20576703. GeneMANIA: http://genemania.org

ExomeNext® Assay Information

General Information: Ambry's ExomeNext® is a cost-effective, comprehensive, integrated whole exome sequencing assay designed to increase the diagnostic yield for genetic disorders that have eluded diagnosis using traditional diagnostic approaches. The exome represents all the protein-coding exons. It is estimated that exons contain about 85% of disease-causing variants. Whole-exome sequencing has been successfully applied to identify both inherited and *de novo* variants in a diverse variety of autosomal dominant, recessive, and X-linked disorders. In addition to the primary analysis, which is performed with the purpose of uncovering the underlying genetic cause for a given clinical presentation, the exome testing may also be utilized to detect secondary findings, which are pathogenic or likely pathogenic variants in select genes that lead to diseases unrelated to the patient's present clinical presentation.

Result Reports: A primary clinical report will only be generated for the proband regardless of number of family members submitted. However, it may be possible to infer information about family members' results based on the proband's report. Pathogenic variant(s) likely to factor into the patient's current clinical presentation are always reported as a relevant finding. Variants previously detected in the proband (not family members) that are provided at the time of testing will receive a report comment if the variant is detected by the assay (limit four variants in three genes). Copy number changes, somatic variants, and variants classified as benign or pseudodeficiency alleles are not eligible for comment. Common reasons a variant may not meet exome reporting criteria include inconsistent zygosity or inheritance, poor molecular support, and/or insufficient clinical overlap with the proband's reported features. Up to 5 genes of interest provided on the TRF were closely reviewed and all clinically significant variants are included on the report. As new scientific information becomes available on a regular basis, this could alter the interpretation of previously reported results. In the event of a change in interpretation, an unsolicited reclassification/amended report may be issued to the ordering clinician. Secondary findings within the ACMG recommended gene list are reported separately unless opted out (Kalia, 2017; Lee, 2025). Expanded childhood onset secondary findings are also available for prenatal exome orders. Gender identity (if provided) is not used in the interpretation of results, and sex assigned at birth is used in the interpretation of results only when necessary.

Test Limitations: This test was developed and its performance characteristics determined by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration (FDA), which does not require this test to go through premarket review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings and does not represent medical advice. Any questions or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or other skilled medical provider. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The following types of variants are detectable: nucleotide substitutions, small deletions/insertions, small indels, and gross deletions/duplications. The overall coverage of each gene varies and each individual may have slightly different coverage yield. Accurate exon-level gross deletion and duplication detection depends on several factors such as inherent sequence properties of the targeted regions, including shared homology, exon size, depth-of-coverage, capture efficiency, and degree of read depth variation in the reference samples. Therefore, the specificity and sensitivity of gross deletions and duplications may be reduced. Exome sequencing is not intended to analyze the following types of variants: gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, trinucleotide repeat sequences, variants involved in tri-allelic inheritance, certain mitochondrial genome variants, epigenetic effects, oligogenic inheritance, and X-linked recessive variants in females who manifest disease due to skewed X-inactivation. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare undetected variant. Although molecular tests are highly accurate, rare diagnostic errors may occur such as sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, rare genetic variants that may interfere with analysis, or other sources.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's provided specimen. Samples are prepared using the IDT xGen Exome Research Panel V1.0 (IDT). Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with exome baits. Captured DNA is eluted and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina NovaSeq, NextSeq or HiSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools using genome assembly GRCh37/hg19. Data is annotated with the Ambry Variant Analyzer tool (AVA), including: nucleotide and amino acid conservation, population frequency, and predicted functional impact. Data analysis is focused on small insertions and deletions, canonical splice site variants, and non-synonymous variants. Gross deletion/duplication analysis is assessed for proband only for genes within the targeted exome using a custom pipeline based on coverage and/or breakpoint analysis from NGS data and is followed by a confirmatory orthogonal method as needed. The following sites are used to search for previously described variants: the Human Gene Mutation Database (HGMD), gnomAD, and online search engines (e.g., PubMed). Variants are then filtered further based on applicable inheritance models. Co-segregation studies are performed if family members are available. All relevant findings undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via coverage and alternate read ratios above established confidence thresholds with manual review by molecular geneticists using integrated genomics software (IGV). Gross deletions/duplications are confirmed by SNP Microarray (Affymetrix® CytoScan™ HD Array), in-house targeted array, MLPA, or Sanger sequencing. Co-segregation results may be confounded by many factors which cannot be completely ruled out including reduced penetrance, age-of-onset, and/or variable expressivity. Relevant findings are evaluated from among the genes in Ambry's internal, dynamic gene database which classifies genes as characterized or uncharacterized Mendelian disease genes based on clinical validity (Smith, 2017). Characterized genes are those currently known to underlie at least one Mendelian genetic condition. Uncharacterized genes are those with no or insufficient evidence to be associated with a Mendelian genetic condition. Characterized genes are analyzed first, followed by reflex analysis of uncharacterized genes for potential identification of a candidate gene finding. The analysis of candidate gene findings is only performed when an informative trio is received for testing and focuses on *de novo*, autosomal recessive, or X-linked inherited variants. Each variant remaining after inheritance model filtering is manually analyzed to identify the most likely causative variant(s). Interpretation is based on the clinical and family information provided by the referring provider and the current genetic knowledge at the time of reporting. Screening and analysis of known mtDNA pathogenic variants related to the proband's clinical phenotype is included if ordered. Amplification of the entire mitochondrial genome is carried out by long distance PCR and sequencing of mtDNA is performed separately on Illumina MiSeq. If ordered, ribonucleic acid (RNA) is isolated from the patient's whole blood. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR). RNA analysis is performed for reportable germline DNA variants expected to affect splicing, provided such studies are likely to meaningfully inform variant classification. Variants in genes with limited expression in whole blood, limited gene-disease validity, or an inconsistent mechanism of disease do not qualify for RNA analysis. Additionally, secondary findings variants do not qualify for RNA analysis. For eligible variants, primers are designed to amplify the relevant region of the pertinent gene from cDNA. The splicing patterns in variant carriers are then compared to control individuals to identify aberrant splicing. The presence of aberrantly spliced RNA transcripts meeting quality thresholds is incorporated as evidence for the assessment and classification of the DNA variants.

Analysis of Variants: The following lines of evidence are used to assess the pathogenicity of a variant: presence in affected and healthy populations, co-segregation, functional studies, variant type, conservation, in silico predictions, and presence in a functional protein domain.

Analytical range: Approximately 75% of bases are expected to have quality scores of Q30 or higher, which translates to a base-calling error rate of 1:1000 and accuracy of 99.9%. Additionally, 90% and 95% of the exome will be covered at ≥20x and ≥10x respectively under current run conditions, generally sufficient for high quality heterozygous and homozygous variant calling for germline variants. For any given individual ~10% of the targeted exome is not sequenced well enough to make a confident call. Each individual may have slightly different coverage yield distributions within the exome. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. The pipeline detects deletions and duplications >5 exons in size in sequences with sufficient resolution. The minimum depth of coverage for targeted mitochondrial bases is 1,000X.