ExomeNext®-*Trio*

Sequencing and Analysis of the Exome

SAMPLE REPORT

Ambry Genetics®

PATIENT
Legal Name: **Patient, Sample**
Accession #: 00-306909
DOB: 01/01/2021
Sex Assigned at Birth: Male
MRN: N/A
Indication: Diagnostic

TEST INFORMATION
Portal Order #: 0000000
Family #: 0000000
Specimen #: N/A
Specimen type: Blood EDTA
Collection date: 01/01/2024
Received date: 01/01/2024
Test Started: 01/01/2024
Final Report:

MEDICAL PROFESSIONAL
Sample Doctor
Sample Facility

ADDITIONAL RECIPIENTS
Sample Genetic Counselor

NEGATIVE: No clinically relevant alterations detected

Reportable Findings		
Single gene alterations	**Contiguous gene deletions/duplications**	**Mitochondrial genome**
None	**None**	**Not Ordered**

Indication for Testing

Intellectual disability, developmental delay

Results and Interpretation

- **These results indicate that the underlying cause of the patient's clinical symptoms has not been discovered.**

Notes

- Secondary findings were issued in a separate report.
- Please note this assay is not intended to confirm previously detected copy number variants.
- Genetic counseling is a recommended option for all patients undergoing genetic testing.

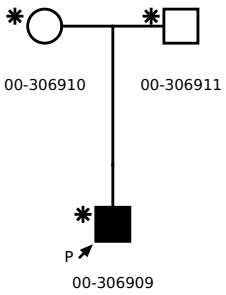
Order Summary: The following products were included in the test order for this individual. Please Note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- ExomeNext®-Trio (Product Code 9995)

Electronically Signed By {This value is automatically generated when clicking "Save & Sign"}

All content hereafter is supplemental information to the preceding report.

Family Pedigree



✱ Exome Sequencing Performed
Affected Proband
Affected or Partially/Possibly Affected Family Member
Presence of accession number means sample received

Analyses Performed

i) Full exome sequencing, bioinformatics, filtering and manual review based on autosomal and X-linked dominant and recessive and Y-linked inheritance models of the proband, mother, and father was performed. Medical review of characterized genetic etiologies did not reveal any alterations with likely clinical relevance.

ii) Medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings did not reveal any alterations with likely clinical relevance.

*Uncharacterized genes are not currently established to underlie Mendelian genetic conditions. An uncharacterized gene will be classified as a “candidate” when sufficient evidence, based on Ambry’s comprehensive, rule-based scoring criteria, is available (Farwell Hagman, 2017).

Raw Data

A table with additional variant filtering details can be found with the raw data filtered variant list (if requested). This list includes clinically irrelevant characterized genes and uncharacterized genes which could not be ruled out (if analyzed); these alterations are not systematically confirmed via Sanger sequencing. The filtered variant list can be requested via this form (www.ambrygen.com/file/material/view/1262/Raw_Sequence_Data_Consent_0619_final.pdf).

Metrics and Coverage

The values below represent metrics from the family's exome sequencing. Complete coverage data for this proband can be e-mailed or made available for download through AmbryPort by request.

	Depth of coverage	
Relationship	**% Bases ≥ 10x**	**% Bases ≥ 20x**
Proband	98.5	98.2
Mother	98.4	98
Father	98.5	98.2

Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

- 1000 Genomes [Internet]: 1000 Genomes Project Consortium (2010) *Nature* **467**(7319):1061-1073. Available from: http://www.1000genomes.org.
- Ambry Clinical Validity Assessment: Smith ED, *et al.* (2017) *Hum Mutat.* **38**(5):600-608.
- BayesDel [Internet]: Feng BJ. (2017) *Hum Mutat* **38**(3):243-251.
- Berkeley Drosophila Genome Project [Internet]: Reese MG, *et al.* (1997) *J Comp Biol* **4**(3), 311-23. http://www.fruitfly.org/seq_tools/splice.html.
- ClinGen Clinical Validity Classifications [Internet]: https://www.clinicalgenome.org/knowledge-curation/gene-curation/clinical-validity-classifications; Rehm HL, *et al.* (2015) *N Engl J Med* **372**(23):2235-2242.
- Clinical Genomic Database [Internet]: Solomon BD, *et al.* (2013) *Proc Natl Acad Sci U S A.* **110**(24):9851-5. Available from: http://research.nhgri.nih.gov/CGD.
- Combined Annotation Dependent Depletion (CADD) [Internet]: Kircher M, *et al.* (2014) *Nat Genet.* **46**(3):310-5. Available from: http://cadd.gs.washington.edu.
- Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]: Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 135). Available from: http://www.ncbi.nlm.nih.gov/projects/SNP.
- DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. Firth, H.V. *et al.*, 2009. *Am.J.Hum.Genet* **84**:524-533 (DOI: dx.doi.org/10/1016/j.ajhg.2009.03.010)
- ESEfinder [Internet]: Smith PJ, *et al.* (2006) *Hum Mol Genet* **15**(16):2490-2508 and Cartegni L, *et al.* (2003) *Nucleic Acid Res* **31**(13):3568-3571. Available from: http://cb.utdallas.edu/tools/ESE
- Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA (URL: http://exac.broadinstitute.org). (Lek M, *et al* 2016: see below)
- Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet]: Seattle, WA. Available from: http://evs.gs.washington.edu/EVS.
- Expression Atlas: Differential and Baseline Expression [Internet]: Petryszak, R. *et al.* (2013) *Nucleic Acids Res* **10**.1093/nar/gkt1270. Available from: http://www.ebi.ac.uk/gxa/home.
- Farwell Hagman KD, *et al.* (2016) *Genet Med* **19**(2):224-235.
- GeneMANIA [Internet]: Warde-Farley D, *et al.* (2010) *Nucleic Acids Res* **38**(Web Server issue):W214-20. Available from: http://genemania.org.
- GeneReviews [Internet]: Pagon RA, *et al.* editors. (1993-) Seattle, WA: University of Washington, Seattle. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1116.
- Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org/ (Lek M, *et al* 2016; Karczewski KJ, *et al.* 2020: see below)
- Grantham prediction: Grantham R. (1974) *Science* **185**(4151):862-864.
- Green RC, *et* al. (2013) *Genet Med* **15**(7):565-74.
- HGMD® [Internet]: Stenson PD, *et al.* (2014) *Hum Genet.* **133**(1):1-9. Available from: http://www.hgmd.cf.ac.uk.
- Integrative Genomics Viewer (IGV): Thorvaldsdóttir H, *et al.* (2012) *Brief Bioinform* **14**(2):178-192.
- Kalia SS, *et al.* (2016) *Genet Med* **19**(2):249-255.
- Karczewski KJ, *et al.* (2020) *Nature* **581**(7809):434-443
- Kyoto Encyclopedia of Genes and Genomes (KEGG) [Internet]: Kanehisa M, *et al.* (2014) *Nucleic Acids Res* **42**. http://www.genome.jp/kegg.
- Lek M, *et al* (2016) Nature 536(7616):285-91.
- Miller DT, *et al.* (2021) *Genet Med* **23**(8):1391-98.
- Miller DT, *et al.* (2022) *Genet Med* **24**(7):1407-14.
- Miller DT, *et al.* (2023) *Genet Med* 2023 Jun 15;100866. doi: 10.1016/j.gim.2023.100866.
- Mouse Gene Expression Database (GXD): Finger JH, *et al.* (2011): *Nucleic Acids Res* **39**(suppl 1):D835-D841. Available from: http://www.informatics.jax.org.
- Mouse Genome Database (MGD) [Internet]: Eppig JT, *et al.* (2012) *Nucleic Acids Res* **40**(1):D881-86 Available from: http://www.informatics.jax.org.
- Mutation Assessor (functional impact of protein mutations) [Internet]: Reva BA *et al.* (2011) *Nucleic Acids Re*s **39**(17):e118. Available from: http://mutationassessor.org.
- NeXtProt [Internet]: Lane L, *et al.* (2012) neXtProt: a knowledge platform for human proteins. *Nucleic Acids Res* **40**(D1): D76-D83. Available from: http://www.nextprot.org.
- Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol* 2004 **5**(2):89-99.
- OMIM (Online Inheritance in Man) [Internet]: Copyright© 1966-2012 Johns Hopkins University. Available from: http://www.omim.org.
- PolyPhen [Internet]: Adzhubei IA, *et al.* (2010) *Nat Methods* **7**(4):248-249. Available from: http://genetics.bwh.harvard.edu/pph2.
- PROVEAN: Choi Y, *et al.* (2012) *PLoS One* **7**(10):e46688.
- RefSeq: The NCBI handbook [Internet]: Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project. Available from: http://www.ncbi.nlm.nih.gov/refseq.
- Richards, *et al.* On behalf of the ACMG Laboratory Quality Assurance Committee (2015) Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, **17**(5), 405-424.
- SIFT [Internet]: Kumar P *et al.* (2009) *Nat Protoc.* **4**(7):1073-81. http://sift.jcvi.org.
- Splicing Prediction: Jaganathan K, *et al.* (2019) *Cell* **176**(3):535-548.e24.

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 mutations. Whole-exome sequencing has been successfully applied to identify both inherited and de novo mutations 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 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 mutation(s) likely to factor into the patient's current clinical presentation are always reported. 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, 2016; Miller, 2023). 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 mutations 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, efficiency of capture, 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 mutations: gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, trinucleotide repeat sequences, mutations involved in tri-allelic inheritance, certain mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, and X-linked recessive mutations 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 mutation. 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), the Single Nucleotide Polymorphism database (dbSNP), gnomAD, ESP, 1000 genomes, 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 mutations 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, alteration 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 6 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.