ExomeNext®

Ambry Genetics®

PATIENT
Legal Name: **PROD Exome, Duo-Proband**
Accession #: 00-300942
DOB: 01/01/2001
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: 05/26/2024
Received date: 05/27/2024
Test Started: 05/27/2024
Final Report:

MEDICAL PROFESSIONAL
Sample Doctor, MD
Sample Facility

ADDITIONAL RECIPIENTS
Sample Genetic Counselor, GC

UNCERTAIN: Alteration(s) of Uncertain Clinical Relevance Detected

Reportable Findings		
Single gene alterations	**Contiguous gene deletions/duplications**	**Mitochondrial genome**
1(1)*	**None**	**Not Ordered**

* genes(alterations)

Indication for Testing
Seizures, global developmental delay

Results

Gene (RefSeq ID)	Characterized/ Uncharacterized Gene	Relevant Associated Syndrome	Mode of Inheritance	Genotype	Alteration	Alteration Classification
USP7 (NM_003470)	Characterized	*USP7*-related neurodevelopmental disorder	Autosomal dominant	Heterozygous, not detected in mother	c.611+1G>A	Variant of Uncertain Significance

Interpretation

- **Overall, the evidence suggests it is uncertain if the identified *USP7* alteration is the cause of the patient's clinical symptoms.** Clinical correlation is recommended.
- RNA analysis of the *USP7* c.611+1G>A variant is in progress. When this analysis is completed, an additional report will be issued.

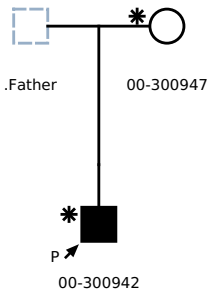
Notes

- 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.
- Any tests on hold, previously reported, and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) have not been included in this report. For additional information, please contact Ambry Genetics.

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, and mother was performed. Medical review of characterized genetic etiologies revealed an alteration with uncertain clinical relevance.

ii) Because fewer than three informative individuals in this family were available for sequencing and a characterized finding was identified, medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings was not performed.

*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	98.7	98.5
Mother	98.6	98.4

USP7 Gene Details

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
USP7	NM_003470	chr16:8985951-9057341	71391	31	31	1102 aa

The *USP7* gene is located on chromosome 16p13.2 and encodes mitochondrial ubiquitin carboxyl-terminal hydrolase 7. Pathogenic variants in this gene are known to cause *USP7*-related neurodevelopmental disorder, which is an autosomal dominant condition that generally occurs *de novo*. *USP7*-related neurodevelopmental disorder is characterized by developmental delay/intellectual disability (DD/ID), autism spectrum disorder (ASD), speech delays, hypotonia, eye anomalies, feeding difficulties, gastroesophageal reflux disease, behavioral issues, variable dysmorphic facial features, and abnormal brain magnetic resonance image findings including white matter changes and thinning of the corpus callosum. Other features seen in a minority of patients include seizures, hypogonadism, asthma, abnormal gait, sleep apnea, chronic constipation, short stature, and scoliosis or kyphosis (Hao, 2015; Fountain, 2019). Loss of function has been reported as the mechanism of disease for *USP7*-related neurodevelopmental disorder.

USP7 c.611+1G>A

Alteration description:
The c.611+1G>A intronic alteration results from a G to A substitution one nucleotide after coding exon 5 of the *USP7* gene. Alterations that disrupt the canonical splice site are expected to cause aberrant splicing, resulting in an abnormal protein or a transcript that is subject to nonsense-mediated mRNA decay.

Population frequency:
This variant was not reported in population-based cohorts in the Genome Aggregation Database (gnomAD).

Conservation:
This nucleotide position is highly conserved in available vertebrate species.

***In silico*:**
In silico splice site analysis predicts that this alteration will weaken the native splice donor site and will result in the creation or strengthening of a novel splice donor site.

Family inheritance:

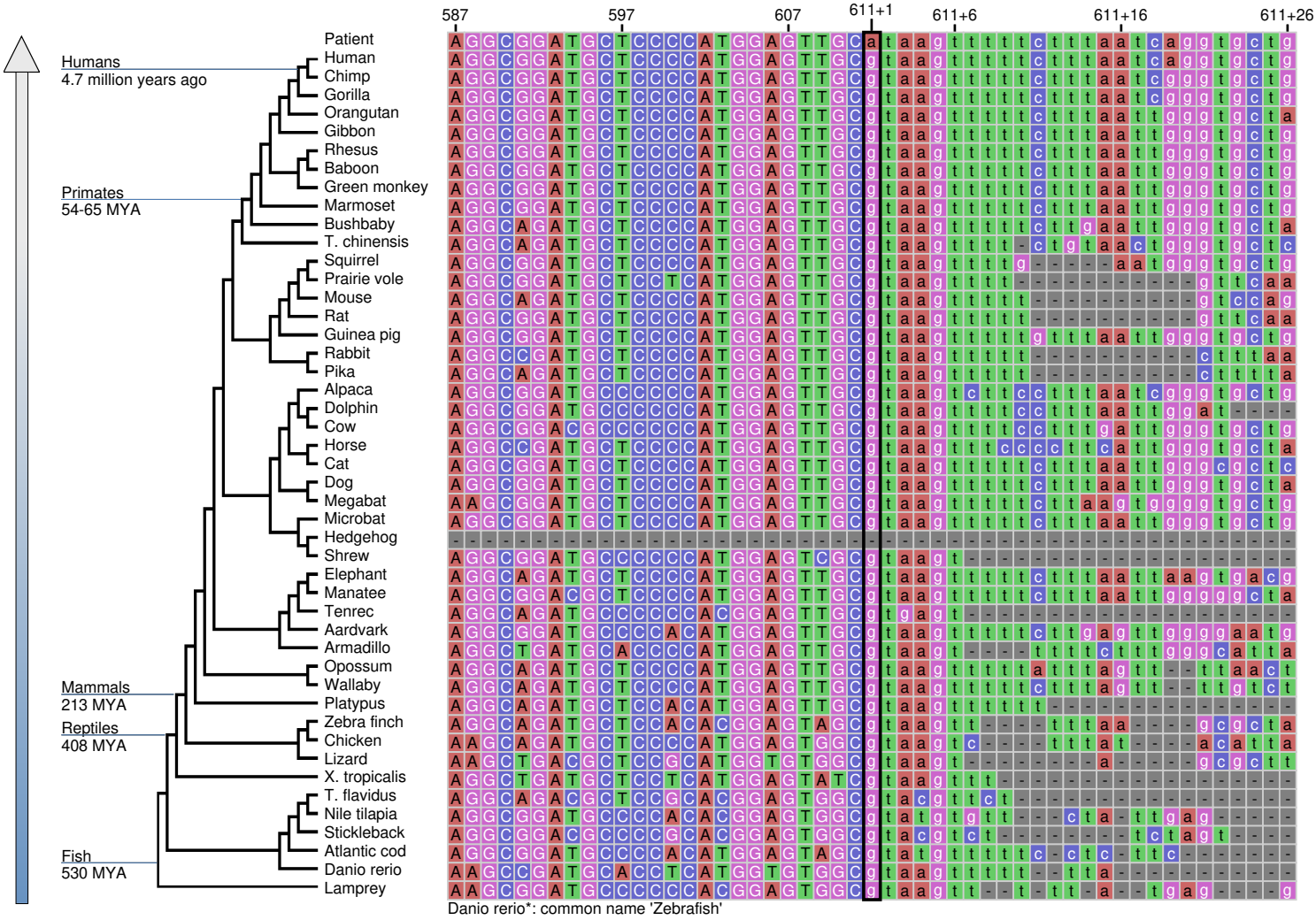
Gene (RefSeq ID)	Alteration	Exon	Proband (00-300942)	Mother (00-300947)	Inheritance
USP7[#] (NM_003470)	c.611+1G>A	Intron 5	Heterozygous	Negative	Not Detected In Mother

[#]Alteration(s) detected via exome sequencing with Q-score and read depth above established confidence thresholds. Confirmation by automated fluorescence dideoxy sequencing (aka "Sanger") sequencing not performed.

Based on the available evidence, the clinical significance of the *USP7* c.611+1G>A alteration is uncertain.

Ambry Genetics offers complimentary genetic studies for variants meeting specific criteria in appropriate family members. Review of clinical information is required. Additional information, application instructions and required forms, and patient education materials are available at http://ambrygen.com/family-studies-program. For additional information, please email us at clinicalassistants@ambrygen.com or call 949-900-5500 and ask to speak with a genetic counselor specializing in exome analysis.

USP7 c.611+1G>A



Danio rerio*: common name 'Zebrafish'

Report References

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Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

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ExomeNext® Assay Information

General Information: Ambry's ExomeNext® is a cost-effective, comprehensive, integrated exome sequencing assay designed to increase the diagnostic yield for genetic disorders that have eluded definitive delineation using traditional diagnostic approaches. The exome represents all the exons, which are the regions in the human genome that are translated into proteins. It is estimated that the protein-coding regions of the human genome contain about 85% of the 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 clinical diagnostic exome may also be utilized to provide secondary findings, which are pathogenic or likely pathogenic alterations 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 member's results based on the proband's report. Pathogenic mutation(s) likely to factor into the patient's current clinical presentation are always reported. Since 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. Pathogenic mutations or likely pathogenic variants identified within the ACMG secondary findings gene list are reported separately unless opted out (Kalia, 2016; Miller, 2023). Expanded childhood onset secondary findings are available in a separate report for prenatal exome testing orders.

Test Limitations: This test was developed and its performance characteristics were determined by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or physician skilled in evaluating the relevant medical literature. 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, small insertions, small indels, and gross deletions and 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 by exome sequencing is dependent on several factors such as inherent sequence properties of the targeted regions, including shared homology and exon size, depth-of-coverage, efficiency of capture, and degree of read depth variation in the selected reference samples. Therefore, the specificity and sensitivity of gross deletion and duplication detection by exome sequencing 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 and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutations in an undetectable region. Ambry's ExomeNext® detection rate is 30% for positive or likely positive relevant findings identified in established disease-gene associations, and an additional 7% for candidate gene findings (Farwell, 2015; Farwell Hagman, 2017). Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that may interfere with analysis, or from 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 the 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, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. Data analysis is focused on small insertions and deletions, canonical splice site alterations, and non-synonymous alterations. Gross deletion/duplication analysis is assessed for proband only for all 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 gene mutations and polymorphisms: 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 manual review by molecular geneticists using integrated genomics software (IGV) and/or undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via coverage and alternate read ratios above established confidence thresholds. Additionally, 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 haploinsufficiency, 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 not currently known or with insufficient evidence to be associated with a Mendelian genetic condition. Characterized genes are analyzed first. If no positive findings are identified, reflex analysis of uncharacterized genes occurs 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 alterations. Each alteration remaining after inheritance model filtering is analyzed and/or reviewed by board certified molecular geneticists to identify the most likely causative alteration(s). Interpretation is based on the clinical, family, and test information provided by the referring provider and the current knowledge of genes and alterations 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 mitochondrial DNA (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 impact 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, variants reported as secondary findings do not qualify for RNA analysis. For eligible variants, primers are designed to amplify the relevant region of the pertinent gene from reverse transcribed cDNA. The splicing patterns in variant carriers are then compared to those in 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 Alterations: The following lines of evidence are used to assess the pathogenic nature of an alteration: presence in affected and healthy populations, co-segregation information, functional studies, alteration type, conservation, *in silico* predictions, and presence in a functional protein domain. The absence of a particular line of evidence implies that no information was found or it does not apply for that alteration type (e.g. in silico for truncating alterations).

Analytical range: Approximately 75% of the bases are expected to have quality scores of Q30 or higher, which translates to an expected base-calling error rate of 1:1000, or an expected base-calling 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 greater than 1,000X.