# ExomeNext® Report

Ambry Genetics

**FINAL REPORT - 2/20/2023**

**PATIENT**

Legal Name: Last, First

Chosen Name
Sex at Birth: F
Gender

Accession #: 22-000000
Birthdate: 01/01/1980
MRN #: 000000
Indication: Diagnostic

**TEST INFORMATION**

Specimen #: 44-55-66
Specimen: Blood EDTA
AP2 Order #: 00000
Collected: 12/30/2022
Received: 12/31/2022
Test Started: 12/31/2022

**ORDERING PROVIDER**

Ordering Provider:
Sample Doctor, MD

Client:
Sample Facility

**ADDITIONAL RECIPIENTS**

Sample GC, CGC

## UNCERTAIN: Variant(s) of Uncertain Significance Detected

## REPORTABLE FINDINGS

| SINGLE GENE ALTERATIONS | CONTIGUOUS GENE DELETIONS/DUPLICATIONS | MITOCHONDRIAL GENOME |
|---|---|---|
| 1(2)* | None | Not Ordered |

* genes(alterations)

**Indication for Testing**
Global developmental delay, congenital heart defect, microsomia, dysmorphic features, suspected right hemiparesis, oral aversion

## RESULTS

| Gene | Relevant Associated Syndrome (inheritance) | Variant | Classification | Interpretation |
|---|---|---|---|---|
| ***CEP57*** Characterized | *CEP57*-related mosaic variegated aneuploidy syndrome (Autosomal Recessive) | c.191G>A (p.S64N) Heterozygous, paternal | Variant of Uncertain Significance | Uncertain if the identified *CEP57* alterations are the cause of the patient's clinical symptoms |
| | | c.974G>C (p.R325P) Heterozygous, maternal | Variant of Uncertain Significance | |

## RECOMMENDATIONS

- Clinical correlation is recommended, and genetic testing results should be interpreted in the context of the patient's clinical and family history.
- Genetic testing for variants of uncertain significance (VUSs) in family members may be pursued to help clarify VUS significance, but cannot be used to identify at-risk individuals at this time. For information about Ambry's complimentary VUS Resolution Program (VRP), please visit www.ambrygen.com/science/family-studies.
- Genetic counseling is recommended and can assist with additional testing, evaluation, medical management, appropriate testing of family members, and/or family planning.

**Notes**

- Secondary findings were issued in a separate report.
- Any previously detected single gene variants not included on this test report were either not detected or did not meet our exome reporting criteria. Please note this assay is not intended to confirm previously detected copy number variants.

## 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.

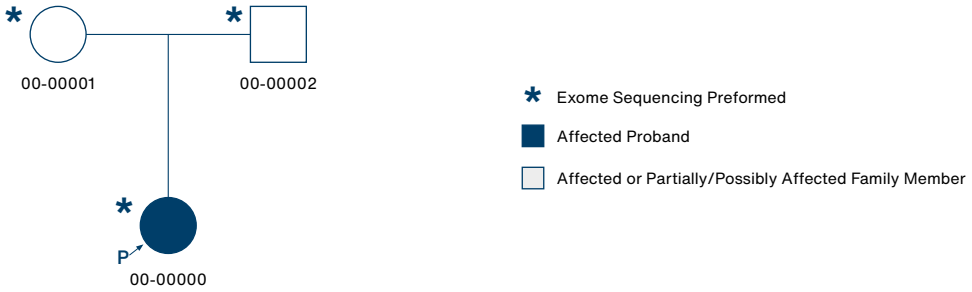
- ExomeNext -Trio (Product Code 9995)
- ExomeNext ACMG Secondary Findings (Product Code 9920)

**Electronically Signed By**

All content hereafter is supplemental information to the preceding report.

## FAMILY PEDIGREE

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 inheritance models of the proband, mother, and father was performed. Medical review of characterized genetic etiologies revealed an alteration with likely clinical relevance.

ii. Because 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" or "suspected candidate" when sufficient evidence, based on Ambry's comprehensive, rule-based scoring criteria, is available (Farwell Hagman, 2017) (http://www.ambrygen.com/candidate-gene-reporting).

## 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).

## Uncertain Variants Identified

### UNCERTAIN c.191G>A (p.S64N) VARIANT IN THE *CEP57* GENE

VARIANT DETAILS

- The c.191G>A (p.S64N) alteration is located in exon 2 (coding exon 2) of the *CEP57* gene. This alteration results from a G to A substitution at nucleotide position 191, causing the serine (S) at amino acid position 64 to be replaced by an asparagine (N).
- Based on data from gnomAD, the A allele has an overall frequency of 0.002% (4/250856) total alleles studied. The highest observed frequency was 0.012% (4/34574) of Latino alleles.
- This amino acid position is highly conserved in available vertebrate species.
- This alteration is predicted to be tolerated by *in silico* analysis.
- Based on the available evidence, the clinical significance of the *CEP57* c.191G>A (p.S64N) alteration is uncertain.

### UNCERTAIN c.974G>C (p.R325P) VARIANT IN THE *CEP57* GENE

VARIANT DETAILS

- The c.974G>C (p.R325P) alteration is located in exon 9 (coding exon 9) of the *CEP57* gene. This alteration results from a G to C substitution at nucleotide position 974, causing the arginine (R) at amino acid position 325 to be replaced by a proline (P).
- This variant was not reported in population-based cohorts in the Genome Aggregation Database (gnomAD).
- This amino acid position is highly conserved in available vertebrate species.
- The *in silico* prediction for this alteration is inconclusive.
- Based on the available evidence, the clinical significance of the *CEP57* c.974G>C (p.R325P) alteration is uncertain.

### FAMILY INHERITANCE:

| Gene (RefSeq ID) | Alteration | Proband (22-00000) | Mother (22-00001) | Father (22-00002) | Inheritance |
|---|---|---|---|---|---|
| *CEP57*^(NM_014679) | c.191G>A (p.S64N) | Heterozygous | Negative | Heterozygous | Paternal |
| | c.974G>C (p.R325P) | Heterozygous | Heterozygous | Negative | Maternal |

^ 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.

### GENE-DISEASE DETAILS

| Gene | RefSeq ID | Genomic Coordinates (GRCh37) | Genomic Size (bp) | Total Exons | Coding Exons | Number of Amino Acids |
|---|---|---|---|---|---|---|
| *CEP57* | NM_014679 | chr11:95523642-95565854 | 42213 | 11 | 11 | 500 |

The *CEP57* gene is located on chromosome 11q21 and encodes the centrosomal protein of 57 kDa. Pathogenic variants in this gene are known to cause *CEP57*-related mosaic variegated aneuploidy syndrome (MVA), which is inherited in an autosomal recessive fashion. *CEP57*-related MVA is characterized by intrauterine and postnatal growth restriction, skeletal abnormalities including rhizomelic limb shortening and variable digit abnormalities, mild developmental delay and intellectual disability, variable congenital heart defects, variable dysmorphic facial features, and mosaic aneuploidy observed on chromosome analysis. Other features seen in a minority of patients include microcephaly or macrocephaly and endocrine abnormalities including hypothyroidism and growth hormone deficiency. Pituitary hypoplasia or atrophy was reported in patients with growth hormone deficiency. No childhood malignancies have been reported in patients with *CEP57*-related MVA in contrast to MVA associated with other genes (Santos-Simarro, 2021; Feng, 2022). Biallelic loss of function has been reported as the mechanism of disease for *CEP57*-related MVA.

## REPORTS REFERENCED


- Feng B, *et al.* (2022) *Mol Genet Genomic Med* **10**(6):e1951. **PMID:**35434947
- Santos-Simarro F, *et al.* (2021) *Eur J Med Genet* **64**(11):104338. **PMID:**34500087


## 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 | 98.5 | 98.4 |
| Father | 98.6 | 98.3 |

## 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 virtually 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.

**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 condition 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.

**Expected (Normal) Value:** Diagnostic: 0, 1, or more mutation(s) detected.

**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 and small indels. Exome sequencing is not intended to analyze the following types of mutations: gross deletions/duplications, 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 K, et al., Genet Med., 2014 and Farwell Hagman K, et al., Genet Med., 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 whole blood. 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 GRCh 37/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), ExAC, 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 with the exception of gross deletions/duplications, which are confirmed in the proband only.. 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 trio exome sequencing with 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 E, et al., Hum Mutat, 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 uncharacterzied 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.

**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 (eg. 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 mean depth of coverage for targeted mitochondrial bases is greater than 1,000X.

**Resources:** The following references are used in variant analysis and classification when applicable for observed genetic alterations,

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