GenomeNext™ Sample Report

Ambry Genetics
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Patient
Legal Name **Sample Patient**
Sex Assigned at Birth **Female**
Birthdate **01/01/2000**
MRN **000000**
Indication **Diagnostic**

Provider
Ordering Provider **Sample Doctor**
Client **Sample Facility**

Test Information
Accession # **00-000000**
Family # **000000**
Specimen **Blood EDTA (Purple top)**
Portal Order # **000000**
Collected **07/01/2026**
Received **07/01/2026**
Test Started **07/01/2026**

⚠ Positive

Clinically Relevant Variant(s) Detected

Results

Sequence Variants

Gene(s) (RefSeq ID)	Relevant Associated Condition (Mode of Inheritance)	Variant (Zygosity)	Genomic Coordinates (GRCh38)	Variant Classification
RNU4-2 (NR_003137.2) Characterized	**ReNU syndrome** (Autosomal dominant)	**n.64_65insT** (Heterozygous)	**chr12:g.120291839_120291840insA**	**Pathogenic**

Indication for Testing:
Growth restriction, microcephaly, skeletal dysplasia, cognitive impairment

Interpretation

- **Overall, the evidence suggests that the identified *RNU4-2* alteration is the cause of the patient's clinical symptoms.**
- Clinical correlation is recommended, and genetic testing results should be interpreted in the context of the patient's clinical and family history.

Considerations

- If secondary findings were requested, results were issued in a separate report.
- 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.

All content hereafter is supplemental information to the preceding report.

General Gene-Disease Information

Gene(s) (RefSeq ID)	Genomic Coordinates (GRCh38)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
RNU4-2 (NR_003137.2)	chr12:120291762-120291903	142	1	N/A	0

The *RNU4-2* gene is located on chromosome 12q24.31 and encodes a small nuclear RNA (snRNA). Pathogenic variants in this gene are known to cause ReNU syndrome, which is an autosomal dominant condition that generally occurs *de novo*, and *RNU4-2* related neurodevelopmental disorder, which is inherited in an autosomal recessive manner. ReNU syndrome is characterized by global developmental delay, moderate to severe intellectual disability, epilepsy, microcephaly, hypotonia, and brain anomalies (thin corpus callosum, ventriculomegaly). Variable features include short stature, autism spectrum disorder, skeletal abnormalities, and dysmorphic facial features (deep set eyes, epicanthal folds, tented mouth, thick lips). Variable expressivity is observed (Chen, 2024; Kuroda, 2025). Variants affecting the T-loop and Stem III regions of the U4/U6 snRNA duplex have been reported as disease-causing for ReNU syndrome. Autosomal recessive *RNU4-2*-related neurodevelopmental disorder is characterized by global developmental delay, moderate to severe intellectual disability, hypotonia, and structural brain anomalies (white matter changes, dilated perivascular spaces). Variable features include seizures, dysmorphic features, behavioral abnormalities, microcephaly, absent speech, and spasticity. Variable expressivity is observed (Hayashi, 2026; Rius, 2026). The mechanism of disease is unknown for autosomal recessive *RNU4-2*-related neurodevelopmental disorder.

Variant Details

Pathogenic n.64_65insT variant in the *RNU4-2* gene

Alteration description: The n.64_65insT variant results in an insertion of one nucleotide in the *RNU4-2* gene.

Affected individuals: This variant was determined to be *de novo* in at least one individual with features consistent with ReNU syndrome (Greene, 2024; Schot, 2024).

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

Based on the available evidence, this variant is classified as pathogenic.

Report References

- Greene D *et al.* | Nat Med | 2024 | PMID: 38821540
  Mutations in the U4 snRNA gene RNU4-2 cause one of the most prevalent monogenic neurodevelopmental disorders.
- Schot R *et al.* | Clin Genet | 2024 | PMID: 38859706
  Re-analysis of whole genome sequencing ends a diagnostic odyssey: Case report of an RNU4-2 related neurodevelopmental disorder.
- Rius R *et al.* | Nat Genet | 2026 | PMID: 41951959
  Biallelic variants in the noncoding RNA gene RNU4-2 cause a recessive neurodevelopmental syndrome with distinct white matter changes.
- Hayashi Y *et al.* | J Hum Genet | 2025 | PMID: 41408479
  Monoallelic and biallelic RNU4-2 variants in neurodevelopmental disorders.
- Kuroda Y *et al.* | J Med Genet | 2025 | PMID: 40413032
  Genotype-phenotype correlations and phenotypic expansion in a case series of ReNU syndrome associated with RNU4-2 variants.
- Chen Y *et al.* | Nature | 2024 | PMID: 38991538
  De novo variants in the RNU4-2 snRNA cause a frequent neurodevelopmental syndrome.
- Benoit-Pilven C *et al.* | PLoS One | 2020 | PMID: 32628740
  Clinical interpretation of variants identified in RNU4ATAC, a non-coding spliceosomal gene.

Samples Received, Metrics, and Coverage

Family Member (Accession #)	Affected	Depth of Coverage	
		% Bases ≥ 10x	% Bases ≥ 20x
Proband* (00-000000)	**AFFECTED**	**98.5**	**96.6**

*Genome sequencing performed.
All family members with samples received are represented in this table. Complete coverage data for the proband can be e-mailed or made available for download through AmbryPort by request.

Analyses Performed

- Genome sequencing, bioinformatics, filtering and manual review based on dominant and recessive inheritance models was performed. Medical review of characterized genetic etiologies revealed an alteration with clinical relevance.
- Medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings was not performed because both parents were not available for sequencing and a characterized finding was identified.
- Additional analyses were performed as described in the methodology. Clinically significant variants with relevance to the patient phenotype, if identified by these analyses, are included on this report.

*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 list of variants prioritized by our filtering pipeline is available upon request via this form (www.ambrygen.com/file/material/view/1262/Raw_Sequence_Data_Consent_0619_final.pdf). This list includes variants in characterized and uncharacterized genes that were determined not to be clinically relevant. Additional variant filtering details are provided. These variants are not systematically confirmed via orthogonal methods.

Assay Information

General Information	Ambry's GenomeNext™ is a comprehensive genome sequencing assay designed to increase diagnostic yield for suspected genetic disorders that have remained unsolved using traditional approaches. The genome includes both protein-coding and non-coding regions, and genome sequencing has been successfully applied to identify inherited and de novo variants across autosomal dominant, autosomal recessive, and X-linked disorders. This assay includes analysis of single nucleotide variants (SNVs); small insertions and deletions (indels); copy number variants (CNVs); mitochondrial genome SNVs and indels; repeat expansions in the *DMPK*, *FMR1*, and *PHOX2B* genes; *SMN1* deletions; uniparental disomy (UPD); and aneuploidy. In addition to the primary analysis focused on the patient's reported clinical presentation, genome testing may also detect secondary findings (pathogenic/likely pathogenic variants in select genes) unrelated to the indication for testing.
Methodology	Genomic DNA is isolated from the patient's specimen using standardized technology. PCR-free libraries are prepared and sequenced on Illumina platforms using paired-end sequencing chemistry with an average mean sequencing coverage of at least 30x. Initial data processing (base calling, alignment, and variant calling) is performed using validated bioinformatics tools and aligned to the GRCh38/hg38 human reference genome assembly. Variants are annotated and prioritized using the clinical and family information provided by the ordering provider, population databases, published literature, sequence level quality metrics, and established variant classification guidelines. Copy number changes are assessed using coverage and/or breakpoint evidence from NGS data. Reportable findings not satisfying established quality metrics are verified with an appropriate orthogonal method as needed. Screening for repeat expansions in *DMPK*, *FMR1*, and *PHOX2B* is performed via sequencing. For reported *DMPK* and *FMR1* expansions, repeat size is determined by repeat-primed PCR. *SMN1* deletions are assessed by sequencing and are confirmed by multiplex ligation-dependent probe amplification (MLPA) prior to reporting. Co-segregation studies may be performed when family members are available. 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.
Result Reports	A primary clinical report is generated only for the proband; family member information will be reported if applicable. Only variants relevant to the proband's current phenotype are included in the primary report. Relevant pathogenic and likely pathogenic variants are routinely reported; selected variants of uncertain significance may also be reported. Variants that increase a statistical risk for disease are not routinely reported. Incidental findings, as well as benign or likely benign variants, are not reported. Pathogenic and likely pathogenic secondary findings within the ACMG-recommended gene list are reported separately unless opted out.
Test Limitations and Disclaimers	This test was developed and its performance characteristics determined by Ambry Genetics. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity clinical laboratory testing. Results should be interpreted in the context of the patient's clinical and family history and do not constitute medical advice; a negative result does not rule out a genetic condition. Detectable variant types include nucleotide substitutions and small insertions/deletions <50 bp, copy number variants and select structural variants, and mitochondrial variants with ≥5% heteroplasmy. Sensitivity may be reduced for copy number variants between 1kb and 10kb. Uniparental disomy of clinically relevant regions will only be reported when testing is run as a trio analysis. Trinucleotide repeat screening by this assay is limited to the *DMPK*, *FMR1*, and *PHOX2B* genes. Repeat

sizes are reported with an accuracy of +/- 2 repeats for alleles with 1-54 repeats and an accuracy of +/- 5 repeats for alleles with 55-200 repeats. Exact repeat size cannot be determined for alleles with >200 repeats.

The overall coverage of each genomic region varies and each individual may have slightly different coverage yield; regions with shared homology/repetitive sequence and other sequence-context limitations may reduce sensitivity and specificity (including for CNVs/structural variation). This test is not intended to analyze certain variant types, including but not limited to: gross/balanced rearrangements, portions of genes with highly homologous pseudogenes, mitochondrial genome deletions/duplications, epigenetic effects, oligogenic inheritance, X-linked recessive variants in females who manifest disease due to skewed X-inactivation, or variants involved in tri-allelic inheritance. Some variants may not be detected due to technical limitations such as but not limited to low coverage, repetitive/highly homologous regions, certain structural rearrangements, or low-level mosaicism. Although molecular tests are highly accurate, rare diagnostic errors may occur (e.g., sample mix-up, erroneous paternity identification, technical, clerical, or genotyping errors; trace contamination; rare variants that may interfere with analysis; or other sources of error).

Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

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Understanding Your Positive Exome or Genome Sequencing Test Result

INFORMATION FOR PATIENTS WITH ONE OR MORE POSITIVE RESULTS

Chromosomes and Genes	Chromosomes are packages of DNA. They are made up of genes that provide instructions for how our bodies develop. Almost everyone has two copies of each chromosome, one from each parent. Variants in our genes or chromosomes can cause genetic conditions. These genetic variants may be passed down in families or occur for the first time in the person who has a genetic condition. Even if there is no history of the specific condition in your family, it can still be caused by a variant in a person's genes or chromosomes.
Exome and Genome Sequencing	Exome and genome sequencing tests are designed to look for genetic variants in genes that may be the cause of an existing medical condition. Exome sequencing is a test that analyzes all genes known to cause medical conditions. Genome sequencing is another test that analyzes all genes known to cause medical conditions; it can also detect rare types of genetic variants that can not be detected by exome sequencing.
Result	This testing found one or more pathogenic or likely pathogenic variants in one or more genes that are known to be associated with your existing medical condition.
Cause	The test result confirms a genetic cause of your medical condition. Sometimes, genetic conditions can put a person at increased risk of other medical problems later in life. Talk with your healthcare provider to learn more about whether additional medical screening may be considered.
Patient for Life	As part of Ambry's Patient for Life program, we keep your results on file. Future genetic discoveries may provide enough information to update your result. We will notify your healthcare provider if any other clinically significant results are identified in the future.
Management Options	Management and treatment options vary by condition and other factors. Knowing the genetic cause of your medical condition may also help to avoid some tests or procedures. Talk to your healthcare provider about which management options may be right for you.
Family Members	Many people with a genetic condition are the first person in their family to have it. Often, genetic testing can find a genetic variant in someone even if the variant was not found in other family members and was not passed down from a parent. In other families, genetic variants can be passed down from parent to child. Talk to your healthcare provider about how the specific genetic condition may run in your family and what this means for the rest of your family. It is recommended that you share this information with your family members so they can learn more and discuss with their healthcare providers.

Please discuss this information with your healthcare provider. The field of genetics is continuously changing, so updates related to your genetic testing result, medical recommendations, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

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