GenomeNext™ Sample Report

Ambry Genetics
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Patient
Legal Name **Sample Patient**
Sex Assigned at Birth **Male**
Birthdate **01/01/2020**
MRN **000000**
Indication **Diagnostic**

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

Additional Recipients
Sample Genetic Counselor

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**

Uncertain

Variant(s) of Uncertain Clinical Relevance Detected

Results

Sequence Variants

Gene(s) (RefSeq ID)	Relevant Associated Condition (Mode of Inheritance)	Variant (Zygosity)	Genomic Coordinates (GRCh38)	Variant Classification
NSD1 (NM_022455.5) Characterized	**Sotos syndrome** (Autosomal dominant)	**c.4931A>G (p.His1644Arg)** (Heterozygous)	**chr5:g.177257116A>G**	**Uncertain Significance**

Indication for Testing:
Developmental delay, macrocephaly, hypotonia

Interpretation

- **Overall, the evidence suggests it is uncertain if the identified *NSD1* 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
NSD1 (NM_022455.5)	chr5:177133773-177300213	166441	23	22	2697

The *NSD1* gene is located on chromosome 5q35.2q35.3 and encodes the histone-lysine N-methyltransferase, H3 lysine-36 specific protein. Pathogenic variants in this gene are known to cause Sotos syndrome, which is an autosomal dominant condition that generally occurs *de novo*. Inheritance from an affected parent has also been reported. Sotos syndrome is characterized by developmental delay, learning disability ranging from mild to severe, dysmorphic features including frontal bossing, high hairline, downslanting palpebral fissures, and pointed chin, and childhood overgrowth including height and/or head circumference greater than the 98th percentile, large hands and feet, and advanced bone age. Additional features seen in the majority of patients include neonatal complications such as hypotonia, poor feeding, and jaundice. Other features seen in a minority of patients include dilated ventricles on neuroimaging, seizures, scoliosis, pes planus, and cardiac anomalies (de Boer, 2004; Tatton-Brown, 2005; Waggoner, 2005). Loss of function has been reported as the mechanism of disease for Sotos syndrome. When comparing patients with intragenic mutations versus deletions, patients with intragenic mutations were significantly taller and patients with deletions had more severe learning disability. While penetrance is complete, expressivity can be highly variable even within the same family or among unrelated patients with the same recurrent mutation (Ocansey, 2004; Tatton-Brown, 2005).

Variant Details

Uncertain Significance c.4931A>G (p.His1644Arg) variant in the *NSD1* gene

Alteration description: The c.4931A>G (p.His1644Arg) variant in the *NSD1* gene is predicted to result in an amino acid substitution.

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

In silico: The *in silico* prediction for this variant is inconclusive.

Based on the available evidence, the clinical significance of this variant is uncertain.

Report References

- Ocansey S *et al.* | GeneReviews | 2004 [updated 2025] | PMID: 20301652
  Sotos Syndrome
- Waggoner DJ *et al.* | Genet Med | 2005 | PMID: 16247291
  NSD1 analysis for Sotos syndrome: insights and perspectives from the clinical laboratory.
- Tatton-Brown K *et al.* | Am J Hum Genet | 2005 | PMID: 15942875
  Genotype-phenotype associations in Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations.
- de Boer L *et al.* | Horm Res | 2004 | PMID: 15452385
  Genotype-phenotype correlation in patients suspected of having Sotos syndrome.

Samples Received, Metrics, and Coverage

Family Member (Accession #)	Affected	Depth of Coverage	
		% Bases ≥ 10x	% Bases ≥ 20x
Proband* 00-000000	**AFFECTED**	**98.8**	**98.5**

*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 possible 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 Uncertain Exome or Genome Sequencing Test Result

INFORMATION FOR PATIENTS WITH ONE OR MORE UNCERTAIN 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 variants in one or more genes, but it is not clear if this test result is the cause for your existing medical condition.
Diagnosis	This test result does not change your diagnosis. If you have been diagnosed with a specific condition, that remains the same.
Reclassification	Collecting information about an uncertain result is an ongoing process. It is possible that your result may be better understood in the future. The healthcare provider that ordered your test will be notified if new information becomes available about your uncertain result.
Patient for Life	As part of Ambry's Patient for Life™ program, we keep your results on file. While we did not find a genetic cause of your medical condition today, future genetic discoveries may provide enough information to update your result. We will notify your healthcare provider if any clinically significant results are identified in the future.
Family Members	Your report will indicate if testing family members may help us learn more about your specific result.
Management Options	Management options vary by condition and other factors. Talk to your healthcare provider about which may be right for you.

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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