GenomeNext™ Sample Report

Ambry Genetics
A TEMPUS COMPANY

Patient
Legal Name **Sample Patient**
Sex Assigned at Birth **Male**
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**

Novel Candidate

Candidate: Alterations of Potential Clinical Relevance Detected

Results

Sequence Variants

Gene(s) (RefSeq ID)	Relevant Associated Condition (Mode of Inheritance)	Variant (Zygosity)	Genomic Coordinates (GRCh38)	Variant Classification
NRXN2 (NM_015080.4) Uncharacterized	***NRXN2*-related neurodevelopmental disorder** (N/A)	**c.3073C>T (p.Gln1025*)** (Heterozygous)	**chr11:g.64650484G>A**	**Uncertain Significance**

Indication for Testing:
Developmental delay, autism

Interpretation

- **Overall, the evidence suggests it is uncertain if the identified *NRXN2* 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
NRXN2 *(NM_015080.4)*	chr11:64606174-64723197	117024	23	22	1713

Lines of evidence to evaluate the involvement of *NRXN2* in the patient's clinical phenotype:

***NRXN2* is an uncharacterized gene:**
The *NRXN2* gene currently has limited evidence for involvement in human Mendelian disease based on the ClinGen clinical validity assessment criteria (aka "uncharacterized") (Rehm, 2015). The functional consequences of presumably deleterious alterations within uncharacterized genes are typically unknown, including whether mutation mechanism is gain-of-function or dominant negative versus loss-of-function or whether a phenotype is produced in a dominant or recessive manner. While evidence may support the involvement with a patient's phenotype, not all alterations in uncharacterized genes can be definitively stated as disease-causing until further functional studies and multiple case reports have proven their clinical significance. As part of an ongoing process to better understand the clinical significance of the reported candidate gene finding in your patient and to characterize novel candidate disease genes, Ambry participates in data sharing and follow-up research collaborations with physicians, clinics, and researchers. Please let us know if you would like us to enroll your patient in a research collaboration if one is/becomes available by e-mailing ClinicalAssistants@ambrygen.com.

Supportive evidence:

Gene function:
The *NRXN2* gene is located on chromosome 11q13.1 and encodes the neurexin-2-beta protein (*NRXN2*) which belongs to the neurexin family of synaptic proteins involved in Ca2+-dependent neurotransmitter release during synaptic transmission at neuronal cell surfaces. The *NRXN2* gene encodes two major protein isoforms which are transcribed from distinct promoters. The promoter for the longer α-*NRXN2* isoform is located upstream of exon 1, and that for the shorter β-*NRXN2* is located downstream of exon 17. The α isoform contains a signal peptide, followed by six laminin/*NRXN*/sex-hormone binding globulin (LNS) domains, three epidermal growth factor (EGF)-like domains, a transmembrane domain, and a short cytoplasmic region, while the β isoform has the same transmembrane and cytoplasmic domains but only the sixth LNS domain (reviewed in Tromp, 2021).

Expression profile:
NRXN2 is predominantly expressed in the brain. Expression analysis of human cortical tissue samples, prior to extensive synapse formation, showed consistently higher levels of expression of *NRXN2* colocalizing with other presynaptic proteins such as synaptophysin in neurites of the cortical synaptogenic presubplate, and with GAP43 and CASK in the growing axons of the intermediate zone (reviewed in Harkin, 2017). Results of this analysis indicates that *NRXN2* may be involved in early cortical synaptogenesis, axon guidance, and intercellular communication in neurons.

Previously reported patients:
Copy number variants (CNVs) and intragenic truncating variants in the *NRXN2* gene have been implicated in multiple individuals affected with autism spectrum disorders (ASD) and other neurodevelopmental disorders (NDDs). In a male individual diagnosed with autism, Gauthier et al. (2011) identified a frameshift variant, NM_138732.2 c.2733delT (p.D912Mfs*36), in exon 12 of the *NRXN2* gene, inherited from his father who had severe language delay. Functional analysis of the homologous variant in the mouse demonstrated a loss of synaptogenic activity in a neuron coculture assay as well as failure to bind either of its postsynaptic binding partners. A second truncating variant in the *NRXN2* gene, NM_138732.2 c.809dupG (p.N271Qfs*2), was reported to be *de novo* in an unrelated individual with ASD (Wu, 2018). A few *de novo* missense variants in *NRXN2* have also been identified in individuals affected with ASD and/or NDDs (Deciphering Developmental Disorders Study, 2017; Wu, 2018). Deletions which encompass the entire *NRXN2* gene along with additional genes have been reported in a few individuals presenting with autistic behaviors, developmental delay, and intellectual disability (reviewed in Yuan, 2018).

Protein family, co-localization, or interaction:
Pathogenic variants in another member of the neurexin gene family, NRXN1, have been reported in patients with neurodevelopmental disorders. NRXN1-related neurodevelopmental disorder is characterized by moderate-to-severe intellectual disability, autism spectrum disorder, epilepsy, and delays in receptive and/or expressive language development. Additionally, NRXN1 is associated with autosomal recessive Pitt-Hopkins-like syndrome, which is characterized by severe intellectual disability, moderate-to-severe developmental delay, hypotonia, speech impairment, and autism spectrum disorder or autistic features (reviewed in Castranovo, 2020).

Mutational mechanism:
Notably, the gnomAD database reports a lower-than-expected number of truncating variants (probability of loss-of-function intolerance score, pLI = 1) for the *NRXN2* gene, indicating that this gene is highly intolerant to loss-of-function variants.

Variant Details

Uncertain Significance c.3073C>T (p.Gln1025*) variant in the *NRXN2* gene

Alteration description: The c.3073C>T (p.Gln1025*) variant in the *NRXN2* gene is predicted to result in a novel stop codon. This variant is expected to result in loss of function by premature protein truncation or nonsense-mediated mRNA decay.

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

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

Report References

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  Genomic landscapes of Chinese sporadic autism spectrum disorders revealed by whole-genome sequencing.
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  Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia.
- Born G *et al.* | Front Synaptic Neurosci | 2015 | PMID: 25745399
  Genetic targeting of NRXN2 in mice unveils role in excitatory cortical synapse function and social behaviors.
- Castronovo P *et al.* | Clin Genet | 2019 | PMID: 30873608
  Phenotypic spectrum of NRXN1 mono- and bi-allelic deficiency: A systematic review.
- Harkin LF *et al.* | Cereb Cortex | 2016 | PMID: 28013231
  Neurexins 1-3 Each Have a Distinct Pattern of Expression in the Early Developing Human Cerebral Cortex.
- undefined | Nature | 2017 *et al.* | PMID: 28135719
  Prevalence and architecture of de novo mutations in developmental disorders.
- Tromp A *et al.* | Mol Psychiatry | 2020 | PMID: 33191396
  Neurexins in autism and schizophrenia-a review of patient mutations, mouse models and potential future directions.
- Wu J *et al.* | J Genet Genomics | 2018 | PMID: 30392784
  Genomic landscapes of Chinese sporadic autism spectrum disorders revealed by whole-genome sequencing.
- Yuan H *et al.* | Eur J Med Genet | 2018 | PMID: 29654904
  A de novo 921 Kb microdeletion at 11q13.1 including neurexin 2 in a boy with developmental delay, deficits in speech and language without autistic behaviors.

Samples Received, Metrics, and Coverage

Family Member (Accession #)	Affected	Depth of Coverage	
		% Bases ≥ 10x	% Bases ≥ 20x
Proband* (00-000000)	**AFFECTED**	**98.1**	**91.1**

*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 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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