Ambry Genetics®

SAMPLE REPORT

FINAL REPORT - 06/15/2025

Ordered By
Medical Professional: Sample Clinician, MD
Client: Sample Organization

Patient Legal Name: **Sample Patient**
Accession #: **25-000000**
AP2 Order #: 0000000

Birthdate: 08/01/2020
MRN #: N/A
Indication: Diagnostic

Specimen #: N/A
Specimen: Swab
Sex Assigned at Birth: F
Collected: 05/13/2025
Received: 05/17/2025
Test Started: 05/20/2025

AutismNext®: Analyses of 72 Genes Associated with Autism Spectrum Disorders and/or Intellectual Disability

SUMMARY

POSITIVE: Pathogenic Mutation Detected

RESULTS

Gene	Inheritance	Alteration	Proband
MECP2 **(NM_004992)**	**X-linked dominant, X-linked recessive**	**Pathogenic Mutation: c.880C>T (p.R294*)**	**Heterozygous**

INTERPRETATION

- This individual is heterozygous for the **c.880C>T (p.R294*)** pathogenic mutation in the *MECP2* gene.
- This result is consistent with a diagnosis of Rett syndrome.
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for pathogenic and/or likely pathogenic variants in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations or gross deletions or duplications were detected. Variants of uncertain significance were not requested in this order.

MECP2 Additional Information

GENE INFORMATION:

Gene (RefSeq ID)	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
MECP2 (NM_004992)	chrX:153287264-153363188	75925	4	3	486 aa

The *MECP2* gene is located on chromosome Xq28 and encodes the methyl-CpG-binding protein 2. Pathogenic variants in this gene are known to cause a spectrum of disorders including typical Rett syndrome (RTT), atypical/variant RTT, and neonatal severe encephalopathy, which are X-linked dominant conditions that generally occur *de novo*, and *MECP2*-related neurodevelopmental disorder and *MECP2* duplication syndrome (MDS), which are inherited in an X-linked recessive fashion. In heterozygous individuals, typical RTT is characterized by developmental regression, following a period (6-18 months) of apparently normal development, with rapid loss of acquired motor skills, particularly of the hand and language abilities, development of distinctive hand stereotypies, deceleration of head growth and microcephaly, seizures, and gait abnormalities. Additional features include spasticity, scoliosis, growth retardation, diminished response to pain, abnormal muscle tone, impaired sleep, distinctive behavioral traits such as autism and bruxism, and breathing dysrhythmia. RTT-associated pathogenic *MECP2* variants can also manifest in hemizygous individuals as neonatal severe encephalopathy, which is characterized by severe developmental delay, progressive microcephaly, breathing abnormalities, seizures, involuntary movements, and death typically prior to the age of 2 years. Atypical RTT presents with fewer features than those seen in typical RTT and can vary in terms of age of onset and severity of symptoms. Hemizygous individuals with atypical RTT tend to be more severely affected and have less distinctive features of RTT than heterozygotes with atypical RTT (Schanen, 2004; Kankirawatana, 2006; Villard, 2007; Neul, 2010; Halbach, 2016; Neul, 2019). Loss of function has been reported as the mechanism of disease for typical/atypical RTT and neonatal severe encephalopathy. *MECP2*-related neurodevelopmental disorder in hemizygous individuals is characterized by moderate to severe intellectual disability, gait abnormalities, moderate to profound speech delay, spasticity that may be progressive, extrapyramidal movements, episodic psychosis, and macroorchidism. Heterozygous individuals have either no symptoms or mild symptoms due to skewed X-chromosome inactivation. Variable expressivity has been reported (Meloni, 2000; Orrico, 2000; Kaur, 2019). Hypomorphic loss of function has been reported as the mechanism of disease for *MECP2*-related neurodevelopmental disorder. Neonatal severe encephalopathy, typical RTT, atypical RTT, and *MECP2*-related neurodevelopmental disorder are a continuum of phenotypes based on various factors including variant type, genotype, pattern of X-inactivation, and mosaicism. MDS, also known as syndromic Lubs type intellectual disability, in hemizygous individuals is characterized by intellectual disability, hypotonia, and absent speech. Additional features include recurrent infections, autism, seizures, spasticity, ataxia, dysmorphic facial features including large ears, and abnormal brain MRI findings including ventricular dilatation. Heterozygous individuals have either no symptoms or mild symptoms due to skewed X-chromosome inactivation (Ramocki, 2010; Van Esch, 2012). Overexpression has been reported as the mechanism of disease for MDS.

VARIANT DETAILS:

- The c.880C>T (p.R294*) alteration, located in exon 4 (coding exon 3) of the *MECP2* gene, consists of a C to T substitution at nucleotide position 880. This changes the amino acid from an arginine (R) to a stop codon at amino acid position 294. This alteration occurs at the 3' terminus of the *MECP2* gene, is not expected to trigger nonsense-mediated mRNA decay, and impacts the last 38% of the protein. However, premature stop codons are typically deleterious in nature and the impacted region is critical for protein function (Ambry internal data).
- This alteration has been detected in many females with classic Rett syndrome. In addition, several studies show that this pathogenic variant is associated with a milder phenotype including, but not limited to: a later age at diagnosis (5-6 years of age), delayed onset of regression, later onset of stereotypical behaviors, more retention of words and hand function, and ambulatory ability (Cheadle, 2000; Fieremans, 2016; Pidcock, 2016; Wen, 2017; Colvin, 2004; Fehr, 2010).
- Functional analysis demonstrated that the p.R294* alteration retains DNA binding capabilities at levels comparable to those of the wild-type protein, but failed to repress DNA transcription (Yusufzai, 2000).
- The p.R294* amino acid is located within the transcription repression domain, which normally binds methylated DNA in the context of chromatin, leading to long-term transcriptional repression (Hite, 2009).
- Based on the available evidence, the *MECP2* c.880C>T (p.R294*) alteration is classified as pathogenic.

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.

- AutismNext® (Product Code 6863)

Electronically Signed By Sample Director, on 06/15/2025 at 0:00:00 PM

All content hereafter is supplemental information to the preceding report.

Report References

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- Wen Z, *et al*. (2017) *Mol Autism* **8**:43. **PMID:**28785396
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Genes Analyzed

(72 total): ***ACSL4, ADNP, AFF2, ANK2, ASH1L, BRWD3, CAMK2A, CAMK2B, CC2D1A, CELF4, CHAMP1, CHD2, CHD3, CHD8, CIC, CREBBP, CTNNB1, CTNND2, DLL1, DYRK1A, EHMT1, EIF3F, ELP2, FMR1, FOXP1, FOXP2, FRMPD4, GABRB3, GRIA2, GRIA3, GRIN2B, HECW2, KDM5C, KMT2C, KMT5B, MAGEL2, MAOA, MECP2, MED12, MED13, MEF2C, NLGN3, NLGN4X, OPHN1, PAK3, PHIP, POGZ, PTCHD1, PTEN, RAB39B, RAI1, RORB, SETBP1, SETD2, SETD5, SHANK2, SHANK3, SYN1, TANC2, TBR1, TCF20, TCF7L2, TRIP12, TSC1, TSC2, UBE2A, UPF3B, WDFY3, YY1, ZDHHC9, ZMIZ1 and ZNF292.***

Metrics and Coverage

Complete coverage data for this proband is available for download through AmbryPort or can be e-mailed by request.

The following genes (coverage)* did not achieve 100% coverage at 10X for all nucleotides in the coding regions:

CHD3 (99.42%), ***FOXP1*** (99.31%), ***HECW2*** (94.83%), ***SHANK2*** (85.3%), ***SHANK3*** (98.63%)

*percentage of the coding region covered at ≥10X

Assay Information

General Information: Autism spectrum disorder (ASD), which affects 1-2% of children in the United States, is a neurodevelopmental disability that can cause behavioral, social and communication difficulties that begin in early childhood. Genetic testing is recommended for all children with ASD and can be a critical step in providing accurate diagnosis, treatment, prognosis, and genetic counseling. **AutismNext®** is a focused panel analyzing genes primarily associated with non-syndromic presentations of ASD.

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 (Integrated DNA Technologies). 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 (analyzed after early 2022; previous NextSeq or HiSeq). Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools. Data is annotated with the Ambry Variant Analyzer tool (AVA), including, but not limited to, the following information: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. The following sites are used to search for previously described gene mutations and polymorphisms: The Human Gene Mutation Database (HGMD), the Online Mendelian Inheritance in Man (OMIM), the genome aggregation database (gnomAD), HapMap data, and online search engines (e.g., PubMed). Variants are filtered further by the bioinformatics pipeline based on likelihood of pathogenicity (Farwell, 2015). For example, alterations in the following categories are typically filtered out unless otherwise protected: non-coding changes, synonymous changes, and alterations with a high population allele frequency (>1%). Additional manual screening is performed by licensed genetic counselors using criteria obtained from Ambry's General Variant Classification Scheme (https://www.ambrygen.com/science/variant-classification) to further filter alterations that are unlikely to be classified as disease-causing. The exome is targeted and sequenced, but analysis is limited to only the set of genes listed in this report. All reportable findings undergo manual review by molecular geneticists using integrated genomics software (IGV) and undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via coverage and alternate read ratios above established confidence thresholds (heterozygous calls with 40-65% variant allele frequency and >35x coverage (analyzed after early 2022; previous >40x coverage), hemizygous and homozygous calls with 100% variant allele frequency and >35x coverage (analyzed after early 2022; previous >40x coverage)). Additionally, gross deletions/duplications are confirmed by SNP Microarray (Affymetrix® CytoScan™ HD Array), in-house targeted array, MLPA, or Sanger sequencing. When familial samples are received, co-segregation analysis of potentially informative alterations will be performed, except for gross deletions/duplications which are confirmed in the proband only. Co-segregation results may be confounded by many factors which cannot be completely ruled out including reduced penetrance, age-of-onset, and/or variable expressivity. In most cases, phase cannot be determined.

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. Coverage is sufficient to detect >98% and up to 99.7% of disease-causing mutations (LaDuca H, et al. (2017) *PLoS ONE* 12(2):e0170843). Coding exons plus at least 6 bases into the 5' and 3' ends of all the introns are analyzed and reported. Gross deletions and duplications are only assessed for the proband for the genes included on the ordered panel using a custom pipeline based on coverage (>4 exons in size) and/or breakpoint analysis from NGS data and confirmed by targeted chromosomal microarray, SNP array or MLPA when applicable. Gross deletions and duplications detected by NGS pipeline for which no orthogonal method of confirmation is available will not be reported.

Result Reports: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation:** Alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Likely Pathogenic (VLP):** alterations with strong evidence in favor of pathogenicity. Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Unknown Significance (VUS): only if requested**, alterations with limited and/or conflicting evidence regarding pathogenicity will be reported.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) and gross deletions and duplications classified as VUS are not included on results reports. These include findings classified as "likely benign" and "benign" alterations. A clinical report will only be generated for the proband, even when familial samples are received. Default reporting does not include variants of unknown significance (VUS), however clinicians may opt-in. If clinicians do not opt-in to receiving VUSs, family member samples will not be used for co-segregation analysis for alterations without the potential to be upgraded to VLP/Pathogenic. Gender identity (if provided) is not used in the interpretation of results, and sex assigned at birth is used in the interpretation of results only when necessary.

Test Limitations and Disclaimer: 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 background samples. Therefore, the specificity and sensitivity of gross deletion and duplication detection by exome sequencing maybe reduced. This assay is not intended to systematically detect and analyze gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, repeat expansions, mutations involved in tri-allelic inheritance, mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, or 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 mutation in the undetectable region. 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 maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, germline or somatic mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, active hematologic disease, a history of allogeneic bone marrow or peripheral stem cell transplant, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data. The clinical sensitivity of the test may vary widely according to the specific clinical and family history. Mutations in other genes or regions not analyzed by this panel can also give rise to similar clinical conditions.

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

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