| 2.04.81 | Genetic Testing for Rett Syndrome | | |
|---|---|---|---|
| **Original Policy Date:** | March 30, 2015 | **Effective Date:** | August 1, 2023 |
| **Section:** | 2.0 Medicine | **Page:** | Page 1 of 18 |

## Policy Statement

I. Genetic testing for Rett syndrome–associated genes (e.g., *MECP2*, *FOXG1*, or *CDKL5*) may be considered **medically necessary** to establish a genetic diagnosis of Rett syndrome in a child with developmental delay and signs/symptoms of Rett syndrome, when a definitive diagnosis cannot be made without genetic testing.

II. Targeted genetic testing for a known familial Rett syndrome–associated variant may be considered **medically necessary** to determine carrier status of a mother or a sister of an individual with Rett syndrome.

III. All other indications for genetic testing for Rett syndrome–associated genes (e.g., *MECP2*, *FOXG1*, or *CDKL5*) are considered **investigational**, including **either** of the following:
   A. Routine carrier testing (preconception or prenatal) in persons with negative family history
   B. Testing of asymptomatic family members to determine future risk of disease

**NOTE**: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

## Policy Guidelines

### Genetics Nomenclature Update

The Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the HUman Genome Organization (HUGO), and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

**Table PG1. Nomenclature to Report on Variants Found in DNA**

| Previous | Updated | Definition |
|---|---|---|
| **Mutation** | Disease-associated variant | Disease-associated change in the DNA sequence |
| | Variant | Change in the DNA sequence |
| | Familial variant | Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives |

**Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification**

| Variant Classification | Definition |
|---|---|
| **Pathogenic** | Disease-causing change in the DNA sequence |
| **Likely pathogenic** | Likely disease-causing change in the DNA sequence |
| **Variant of uncertain significance** | Change in DNA sequence with uncertain effects on disease |
| **Likely benign** | Likely benign change in the DNA sequence |

| Variant Classification | Definition |
|---|---|
| **Benign** | Benign change in the DNA sequence |

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

### Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

### Coding

The following CPT coding describes genetic testing for Rett syndrome:

- **81302:** *MECP2 (methyl CpG binding protein 2)* (e.g., Rett syndrome) gene analysis; full sequence analysis
- **81303:** *MECP2 (methyl CpG binding protein 2)* (e.g., Rett syndrome) gene analysis; known familial variant
- **81304:** *MECP2 (methyl CpG binding protein 2)* (e.g., Rett syndrome) gene analysis; duplication/deletion variants

CPT code 81404 includes the following testing for *FOXG1*:

- **81404:** Molecular Pathology Procedure Level 5 *(*includes *FOXG1 [forkhead box G1]* [e.g., Rett syndrome], full gene sequence)

CPT code 81405 includes the testing for *CDKL5:*

- **81405**: Molecular Pathology Procedure Level 6, (includes *CDKL5 [cyclin-dependent kinase-like 5] [e.g., early infantile epileptic encephalopathy], duplication/deletion analysis)*

CPT code 81406 includes the following testing for *CDKL5*:

- **81406:** Molecular Pathology Procedure Level 7, (includes *CDKL5 [cyclin-dependent kinase-like 5]* [e.g., early infantile epileptic encephalopathy], full gene sequence)

## Description

Rett syndrome (RTT), a neurodevelopmental disorder, is usually caused by pathogenic variants in the methyl-CpG-binding protein 2 (*MECP2*)gene. Genetic testing is available to determine whether a pathogenic variant exists in RTT-associated genes (e.g., *MECP2*, *FOXG1*, or *CDLK5*) in a patient with clinical features of RTT or a patient's family member.

## Related Policies

- N/A

## Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

## Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Genetic testing for Rett syndrome is available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

## Rationale

### Background

#### Rett Syndrome

Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting girls, with an incidence of 1 in 10,000 female births, making it among the most common genetic causes of intellectual disability in girls.[1,] In its typical form, RTT is characterized by apparently normal development for the first 6 to 18 months of life, followed by regression of intellectual functioning, acquired fine and gross motor skills, and social skills. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, such as hand-wringing.[1,] Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation, and gait apraxia.[2,]

There is wide variability in the rate of progression and severity of the disease. In addition to the typical (or classic) form of RTT, there are recognized atypical variants. Three distinct atypical variants have been described: preserved speech, early seizure, and congenital variants. RTT occurring in males is also considered a variant type and is associated with somatic mosaicism or Klinefelter (XXY) syndrome. A small number of RTT cases in males arising from the *MECP2* exon 1 variant have been reported. Diagnostic criteria for typical (or classic) RTT and atypical (or variant) RTT have been established.[1,2,3,] For typical RTT, a period of regression followed by recovery or stabilization and fulfillment of all the main criteria are required to meet the diagnostic criteria for classic RTT. For atypical RTT, a period of regression followed by recovery or stabilization, at least 2 of the 4 main criteria, plus 5 of 11 supportive are required to meet the diagnostic criteria of variant RTT.

### Treatment

Currently, there are no specific treatments that halt or reverse disease progression, and there are no known medical interventions that will change the outcome of patients with RTT. Management is mainly symptomatic and individualized, focusing on optimizing each patient's abilities.[1,] A multidisciplinary approach is usually applied, with specialist input from dietitians, physical therapists, occupational therapists, speech therapists, and music therapists. Regular monitoring for scoliosis (seen in ~87% of patients by age 25 years) and possible heart abnormalities, particularly cardiac conduction abnormalities, may be recommended. Spasticity can have a major impact on mobility; physical therapy and hydrotherapy may prolong mobility. Occupational therapy can help children develop communication strategies and skills needed for performing self-directed activities (e.g., dressing, feeding, practicing arts and crafts).

Pharmacologic approaches to managing problems associated with RTT include melatonin for sleep disturbances and several agents to control breathing disturbances, seizures, and stereotypic

movements. RTT patients have an increased risk of life-threatening arrhythmias associated with a prolonged QT interval, and avoidance of a number of drugs is recommended, including prokinetic agents, antipsychotics, tricyclic antidepressants, antiarrhythmics, anesthetic agents, and certain antibiotics.

In a mouse model of RTT, genetic manipulation of the *MECP2* gene has demonstrated reversibility of the genetic defect.[4,5,]

## Genetics

RTT is an X-linked dominant genetic disorder. Pathogenic variants in the *MECP2* gene, which is thought to control expression of several genes, including some involved in brain development, were first reported in 1999. Subsequent screening has shown that over 80% of patients with classic RTT have pathogenic variants in the *MECP2* gene. More than 200 pathogenic variants in *MECP2* have been associated with RTT.[6] However, 8 of the most commonly occurring missense and nonsense variants account for almost 70% of all cases; small C-terminal deletions account for approximately 10%; and large deletions, 8% to 10%.[7] *MECP2* variant type is associated with disease severity.[6,] Whole duplications of the *MECP2* gene have been associated with a severe X-linked intellectual disability with progressive spasticity, no or poor speech acquisition, and acquired microcephaly. Additionally, the pattern of X-chromosome inactivation influences the severity of the clinical disease in females.[7,8,]

Because the spectrum of clinical phenotypes is broad, to facilitate genotype-phenotype correlation analyses, the International Rett Syndrome Association has established a locus- specific MECP2 variation database (RettBASE) and a phenotype database (InterRett).

Approximately 99.5% of cases of RTT are sporadic, resulting from a de novo variant, which arises almost exclusively on the paternally derived X chromosome. The remaining 0.5% of cases are familial and usually explained by germline mosaicism or favorably skewed X-chromosome inactivation in the carrier mother that results in her being unaffected or only slightly affected (mild intellectual disability). In the case of a carrier mother, the recurrence risk of RTT is 50%. If a variant is not identified in leukocytes of the mother, the risk to a sibling of the proband is below 0.5% (because germline mosaicism in either parent cannot be excluded).

Identification of a variant in *MECP2* does not necessarily equate to a diagnosis of RTT. Rare cases of *MECP2* variants also have been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders [most commonly bipolar disorder], parkinsonism, and intellectual disability), autism, and neonatal encephalopathy.[1,9,10,] Recent studies have revealed that different classes of genetic variants in *MECP2* result in variable clinical phenotypes and overlap with other neurodevelopmental disorders.[11,12,13,]

A proportion of patients with a clinical diagnosis of RTT do not appear to have pathogenic variants in the *MECP2* gene. Two other genes (*CDKL5*, *FOXG1*) have been shown to be associated with atypical variants.

## Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical

reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations

## Testing Individual With Signs or Symptoms of Rett Syndrome

### Clinical Context and Test Purpose

The purpose of genetic testing of individuals with signs or symptoms of Rett syndrome (RTT) is to determine the underlying pathogenic variant, to predict potential disease severity, to initiate surveillance for potential disease complications (e.g., musculoskeletal deformities, autonomic dysfunction), and to direct treatments.

The question addressed in this evidence review is: Does genetic testing for RTT-associated genes in individuals with suspected but unconfirmed RTT lead to improved health outcomes?
The following PICO was used to select literature to inform this review.

#### *Populations*

The relevant population of interest is individuals with signs or symptoms of RTT.

#### *Interventions*

The test being considered is genetic testing for RTT-associated genes.

#### *Comparators*

The following practice is currently being used: standard clinical management without genetic testing.

#### *Outcomes*

The potential beneficial outcomes of primary interest are establishing a genetic diagnosis for RTT and predicting potential disease severity and course to initiate surveillance and treatments for disease complications. Some genetic variants may be associated with prolonged QT syndrome, which would require periodic screening and avoidance of certain medications.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary surveillance (e.g., musculoskeletal or autonomic dysfunction) and treatments (e.g., spinal fusion for scoliosis or kyphosis). False-negative test results can lead to lack of appropriate surveillance and treatments.

The time frame for outcome measures varies from the short-term development of a severe neurodevelopmental disorder to long-term complications such as autonomic dysfunction, scoliosis or kyphosis, and growth retardation.

### Study Selection Criteria

Below are selection criteria for studies to assess whether a test is clinically valid.

- The study population represents the population of interest. Eligibility and selection are described.
- The test is compared with a credible reference standard.
- If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.

- Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (eg, ROC, AUROC, c-statistic, likelihood ratios) may be included but are less informative.
- Studies should also report reclassification of diagnostic or risk category.

## Review of Evidence

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Huppke et al (2000) analyzed the methyl-CpG-binding protein 2 (*MECP2*)gene in 31 females diagnosed clinically with RTT.[15,] Sequencing revealed variants in 24 (77%) of the 31 patients. Of the 7 patients in whom no variants were found, 5 fulfilled criteria for classic RTT. In this study, 17 different variants were detected, 11 of which had not been previously described. Several females carrying the same variant displayed different phenotypes, suggesting that factors other than the type or position of variants influenced the severity of RTT.

Cheadle et al (2000) analyzed variants in 48 females with classic sporadic RTT, 7 families with possible familial RTT, and 5 sporadic females with features suggestive, but not diagnostic, of RTT.[16,] The entire *MECP2* gene was sequenced in all cases. Variants were identified in 44 (80%) of 55 unrelated classic sporadic and familial RTT patients. Only 1 (20%) of 5 sporadic cases with suggestive but nondiagnostic features of RTT had variants identified. Twenty-one different variants were identified (12 missense, 4 nonsense, and 5 frame-shift variants); 14 of the variants identified were novel. Significantly milder disease was noted in patients carrying missense variants compared with those with truncating variants.

Lotan and Ben-Zeev (2006) included the 2 studies previously discussed in a summary of 6 articles that attempted to elicit a genotype-phenotype correlation.[3,] They found that these studies yielded inconsistent results and that more controlled studies were needed before valid conclusions could be drawn about the effect of variant type on phenotypic expression. Two subsequent studies used the InterRett database to examine genotype and RTT severity.[17,18,] Of 357 girls with epilepsy who had *MECP2* genotype recorded, those with large deletions were more likely than those with 10 other common variants to have active epilepsy (odds ratio, 3.71; 95% CI, 1.13 to 12.17; p=.03) and had the earliest median age at epilepsy onset (3 years 5 months). Among all girls in the database, those with large deletions were more likely to have never walked (odds ratio, 0.42; 95% CI, 0.22 to 0.79; p=.007). Of 260 girls with classic RTT enrolled in the multicenter RTT Natural History study (NCT00299312), those with the R133C substitution variant had clinically less severe disease, as assessed by the Clinical Severity, Motor Behavior Analysis, and Physician Summary scales.[19,] Fabio et al (2014) reported similar genotype-phenotype correlations among 144 patients with RTT in Italy.[20,]

Halbach et al (2016) analyzed a cohort from a group of 132 females between 2 and 43 years of age with well-defined RTT with extended clinical, molecular, and neurophysiological assessments.[21,] Genotype -phenotype analyses of clinical features and cardiorespiratory data were performed after grouping variants by the same type and localization or having the same putative biologic effect on the MeCP2 protein, and subsequently on 8 single recurrent pathogenic variants. A less severe phenotype was seen in females with a C-terminal segment of *MECP2* (p.R133C and p.R294X variants). Autonomic disturbances were present in all females and not restricted to or influenced by one specific group or any single recurrent pathogenic variant. The objective information from noninvasive neurophysiological evaluation of the disturbed central autonomic control is of great importance for organizing the lifelong care for females with RTT. The study concluded that greater clarity is needed to provide insights into the pathogenesis of autonomic dysfunction and to develop evidence-based management in RTT.

Pidock et al (2016) identified 96 RTT patients with pathogenic variants in the *MECP2* gene.[22,] Among 11 pathogenic variant groups, a statistically significant group effect of variant type was observed for self-care, upper-extremity function, and mobility on standardized measures administered by occupational and physical therapists. Patients with R133C and uncommon variants tended to perform best on upper-extremity and self-care items, whereas patients with R133C, R306C, and R294X variants had the highest scores on the mobility items. The worst performers on upper-extremity and self-care items were patients with large deletions (R255X, R168X, and T158M variants). The lowest scores for mobility were found in patients with T158M, R255X, R168X, and R270X variants. For categorical variables as reported by parents at the time of initial evaluation, patients with R133C and R294X variants were most likely to have hand use; those with R133C, R294X, R306C, and small deletions were most likely to be ambulatory; and those with the R133C variant were most likely to be verbal.

Sajan et al (2017) analyzed 22 RTT patients without apparent *MECP2*, *CDKL5*, and *FOXG1* pathogenic variants were had both whole-exome sequencing and single-nucleotide variant array-based copy-number variant analyses.[23,] Three patients had *MECP2* variants initially missed by clinical testing. Of the remaining 19, 17 (89.5%) had 29 other likely pathogenic intragenic variants and/or copy-number variants (10 patients had ≥2). Thirteen patients had variants in a gene or region previously reported in other neurodevelopmental disorders, thereby providing a potential diagnostic yield of 68.4%. The genetic etiology of RTT without *MECP2*, *CDKL5*, and *FOXG1* variants is heterogeneous, overlaps with other neurodevelopmental disorders, and is complicated by a high variant burden. Dysregulation of chromatin structure and abnormal excitatory synaptic signaling may form common pathologic bases of RTT.

Vidal et al (2017) investigated the utility of next-generation sequencing and its ability to identify an affected person genetically.[24,]For next-generation sequencing, several different techniques were employed, such as Sanger sequencing and whole-exome sequencing. This study included 1577 patients who exhibited signs of having RTT but no formal diagnosis. Using Sanger sequencing, 1341 patients were evaluated, and 26% had RTT genes variants identified. Two hundred forty-two patients were assessed using the Haloplex Custom Panel, and 22% were diagnosed genetically. Fifty-one patients were evaluated using the TruSight One panel, and 15 (29%) patients were diagnosed genetically; 25 patients were studied by whole-exome sequencing, and it was diagnosed genetically; diagnosed genetically; discovered that 5 variants occurred in genes previously associated with neurodevelopmental disorders with features similar to those of RTT.

### Section Summary: Clinically Valid

Evidence from several small studies has indicated that the clinical sensitivity of genetic testing for classic RTT is reasonably high, in the range of 75% to 80%. However, sensitivity may be lower when classic RTT features are absent. Clinical specificity is unknown but also is likely to be high, because only rare cases of *MECP2* variants have been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, PPM-X syndrome, autism, and neonatal encephalopathy. Recent studies have indicated that specific classes, types, or burden of pathogenic variants in genes associated with RTT affect the severity of disease (e.g., the degree of autonomic dysfunction, functional outcomes, the degree of neurodevelopmental disorder).

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No studies were identified that provided direct evidence of clinical utility.

### Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

There is no specific treatment for RTT; however, identification of the pathogenic variant leading to RTT has been found to correlate with disease severity and predict potential complications of the disease (e.g., autonomic dysfunction and functional outcomes such as mobility). Increased surveillance for clinical manifestations, such as scoliosis or cardiac arrhythmia, and tailoring of ancillary treatments, such as occupational or physical therapy, may be performed.

### Section Summary: Clinically Useful

There are no studies that report direct evidence on the clinical utility of genetic testing for RTT. Thus, the clinical utility of genetic testing for RTT relies on whether a strong chain of evidence exists. For individuals with suspected RTT, identification of a pathogenic variant may alter patient management via increased surveillance of clinical manifestations such as scoliosis, cardiac arrhythmia, or autonomic dysfunction. The class or type of pathogenic may also impact disease severity, allowing for tailoring of ancillary treatments (e.g., occupational therapy) to maintain or improve functional outcomes (e.g., extremity mobility, ambulation).

## Targeted Familial Variant Testing of Asymptomatic Sisters of Individuals With Rett Syndrome

### Clinical Context and Test Purpose

The purpose of targeted familial variant testing of asymptomatic sisters of individuals with RTT is to predict the potential development of symptoms to determine the need for surveillance in young females and to aid in reproductive planning in females of reproductive age.

The question addressed in this evidence review is: Does targeted familial variant testing of asymptomatic sisters of individuals with RTT lead to improved net health outcomes, including changes in surveillance, preimplantation genetic testing to determine the likelihood of an affected offspring, or to inform reproductive planning decisions?

The following PICO was used to select literature to inform this review.

#### *Populations*

The relevant population of interest is asymptomatic sisters of individuals with RTT.

#### *Interventions*

The test being considered is targeted genetic testing for a known familial variant.

#### *Comparators*

The following practice is currently being used: standard management without genetic screening.

#### *Outcomes*

The potential beneficial outcomes of primary interest would be confirming or excluding the need for surveillance in young females or changes in reproductive decision making in females of reproductive age. A negative genetic test result would eliminate the need for surveillance to detect the development of symptoms and disease. A positive genetic test result has the potential to confirm a

need for active surveillance and may inform reproductive decision making in reproductive age individuals.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary surveillance (e.g., musculoskeletal or autonomic dysfunction) and treatments (e.g., spinal fusion for scoliosis or kyphosis). False-negative test results can lead to lack of appropriate surveillance and inaccurate risk assessment to determine the likelihood of an affected offspring.

The time frame for outcome measures varies from the short-term development of a neurodevelopmental disorder in young females to long-term complications such as autonomic dysfunction, scoliosis or kyphosis, and growth retardation. In women of reproductive age, outcomes vary from short-term identification of subclinical or mild cognitive disorders to long-term birth of an affected offspring.

### Study Selection Criteria

Below are selection criteria for studies to assess whether a test is clinically valid.

- The study population represents the population of interest. Eligibility and selection are described.
- The test is compared with a credible reference standard.
- If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
- Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g., ROC, AUROC, c-statistic, likelihood ratios) may be included but are less informative.
- Studies should also report reclassification of diagnostic or risk category.

### Review of Evidence

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

See the discussion of clinical validity in the Testing Individuals with Signs or Symptoms of Rett Syndrome section.

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence of the clinical utility for targeted genetic testing of a known familial variant in asymptomatic sisters is lacking.

### Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A chain of evidence can be constructed for targeted genetic testing to determine if sisters of an affected child are asymptomatic or subclinical carriers of the known familial variant. The variable penetrance of disease due to random X inactivation in females as well as different classes or types of pathogenic variants leading to different disease severity suggest that targeted testing for a familial variant has potential clinical utility. In young sisters of an affected child, targeted testing for the known familial variant has potential clinical utility in identifying subclinical manifestations and eliminating or necessitating the need for surveillance of clinical manifestations of the disease. In sisters of reproductive age, targeted testing can guide whether prenatal testing may be indicated and potentially alter reproductive decisions.

### Section Summary: Clinically Useful

Targeted familial variant testing of asymptomatic sisters can eliminate or necessitate surveillance given the variability of clinical presentation in girls due to X-chromosome inactivation (XCI) and clinical severity based on the type of pathogenic variant present. In sisters of reproductive age, determination of carrier status can eliminate or necessitate prenatal testing and inform reproductive decision making.

## Targeted Testing of Females With a Child With Rett Syndrome Who Are Considering Further Childbearing

### Clinical Context and Test Purpose

The purpose of targeted familial variant testing of females with a child with RTT who are considering having additional children is to determine carrier status and to aid in reproductive planning.

The relevant question addressed in this evidence review is: Does targeted familial variant testing of females with a child who has RTT who are considering having additional children lead to improved net health outcomes, including preimplantation genetic testing to determine the likelihood of an affected offspring, or alter reproductive planning decisions?

The following PICO was used to select literature to inform this review.

#### *Populations*

The relevant population of interest is female individuals who have a child with RTT.

#### *Interventions*

The test being considered is targeted genetic testing for a known familial variant.

#### *Comparators*

The following practice is currently being used: reproductive planning without genetic testing.

#### *Outcomes*

The potential beneficial outcomes of primary interest would be to determine carrier status to aid in reproductive decision making. A negative genetic test result would exclude a maternal inheritance of RTT and predict a low likelihood of an affected offspring derived from paternal inheritance. A positive genetic test result would predict a high likelihood of an affected offspring-a 50% chance of a hemizygous affected male or a 50% chance of a heterozygous affected female.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to reproductive decisions based on an incorrectly high prediction for an affected offspring. False-negative test results can lead to lack of appropriate preimplantation genetic diagnosis and inaccurate risk assessment to determine the likelihood of an affected offspring.

The time frame for outcome measures varies from short-term (ie, months) in the case of identification of seizures or subclinical or mild cognitive disorders, to long-term (ie, decades), in the case of decision-making about childbearing.

### Study Selection Criteria

Below are selection criteria for studies to assess whether a test is clinically valid.

- The study population represents the population of interest. Eligibility and selection are described.
- The test is compared with a credible reference standard.
- If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
- Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g., ROC, AUROC, c-statistic, likelihood ratios) may be included but are less informative.
- Studies should also report reclassification of diagnostic or risk category.

### Review of Evidence

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Sheikh et al (2016) analyzed pathogenic variants in hemizygous males.[13,] In heterozygous females, the variable phenotypic severity is modulated by nonrandom X inactivation, thus making genotype-phenotype comparisons unreliable. However, genotype-phenotype correlations in males with hemizygous *MECP2* pathogenic variants can provide more accurate insights into the true biologic effect of specific pathogenic variant. A wide selection of phenotypic and clinical severity was observed, ranging from neonatal encephalopathy to mild psychiatric abnormalities, with correlating functional and molecular results. Overall, clinical severity showed a direct correlation with the functional impairment of the MeCP2 protein.

Zahorakova et al (2016) analyzed RTT patients with *MECP2* pathogenic variants, and XCI.[12,] Skewed XCI (ratio, >75%) was found in 19.3% of the girls, but no gross divergence in clinical severity was observed. Findings confirmed a high pathogenic variant frequency in classic RTT (92%) and a correlation between the *MECP2* variant type and clinical severity. Additionally, limitations of XCI in explaining all phenotypic differences in RTT were noted.

Zhang et al (2017) investigated familial cases with RTT or X-linked mental retardation.[25,] For this study, 429 children were recruited from 427 Chinese families. Each child either had RTT or X-linked mental retardation. All patients provided genomic DNA samples. Of the 427 families, 3 girls and 5 boys (from 6 families) were identified as having the *MECP2* variant. The 3 girls met the diagnostic criteria for RTT; the 5 boys were X-linked mental retardation. The *MECP2* gene was sequenced, and authors observed a random XCI pattern in all girls and 2 mothers. A skewed XCI was seen in the other 4 mothers. In all *MECP2* variant cases, the variant was confirmed as an identical variant inherited from the mother. No variants were inherited from the father. This study adds to the sparse literature on familial cases with *MECP2* variants, with evidence for maternal inheritance of *MECP2* variants.

### Section Summary: Clinically Valid

Genotype-phenotype correlations in heterozygous individuals who are femaleare confounded by both random XCI and the class or type of pathogenic variant present. In heterozygous females, clinical sensitivity correlates with variant type and variable effects of skewed XCI. In contrast, for hemizygous males, the phenotypic and clinical severity of a particular pathogenic variant manifest completely.

### Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

### Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence of clinical utility for targeted genetic testing of a known familial variant in females with a child who has RTT is lacking.

### Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A chain of evidence can be constructed for targeted genetic testing of a known familial variant to determine carrier status. The variable penetrance of disease due to random XCI in females as well as different classes or types of pathogenic variants leads to unpredictable disease severity. Although most cases of RTT are due to de novo pathogenic variants in RTT-associated genes, determination of carrier status in a female with a child with RTT eliminates or necessitates prenatal testing and informs reproductive decision making. If a female tests negative for a known familial variant, future offspring are not at increased risk for RTT. In the rare situation where the mother carries a pathogenic variant, all future offspring have a 50% chance of being affected, with males typically presenting with more severe disease.[26,]

### Section Summary: Clinically Useful
Most cases of RTT are due to de novo pathogenic variants in RTT-associated genes. Maternally-inherited RTT is rare but has been documented. In several cases, a mild form of RTT was also identified in the mother. Determination of carrier status in a female with a child with RTT eliminates or necessitates prenatal testing and informs reproductive decision making.

### Supplemental Information
The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

### Clinical Input From Physician Specialty Societies and Academic Medical Centers
While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

In response to requests, input on the use of variant testing for Rett syndrome (RTT) was received from 2 specialty medical societies (3 reviewers) and 3 academic medical centers, for a total of 6 reviewers, while this policy was under review in 2012. There was consensus or near consensus supporting the use of variant testing for the diagnosis of RTT in a girl in whom the clinical differential diagnosis includes RTT, especially when clinical diagnosis is uncertain. Support for testing sisters of individuals with RTT and prenatal screening was mixed.

### Practice Guidelines and Position Statements
Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to

guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

### American Academy of Neurology and Child Neurology Society

In 2011, the American Academy of Neurology and the Child Neurology Society issued an evidence report on genetic and metabolic testing of children with global developmental delay.[27,] The 2 societies recommended considering methyl-CpG-binding protein 2 (*MECP2*) genetic testing for all girls with unexplained moderate-to-severe developmental delay.

### American Academy of Pediatrics

In 2007, the American Academy of Pediatrics (AAP) issued a policy statement (reaffirmed in 2014 and 2019)[28,29,] recommending *MECP2* testing to confirm a diagnosis of suspected Rett syndrome (RTT), especially when the diagnosis was unclear from symptoms alone.

In 2020, the AAP published Clinical Report Guidance on the identification, evaluation, and management of children with autism spectrum disorder which stated that "if patient is a girl, consider evaluation for Rett syndrome, MECP2 testing."[30,]

Neither the American Academy of Neurology nor the American Academy of Pediatrics has provided recommendations on when to use *CDKL5* or *FOXG1* testing.

### American College of Medical Genetics and Genomics

In 2013, the American College of Medical Genetics and Genomics revised its evidence-based guidelines for clinical genetics evaluation of autism spectrum disorders.[31,] Testing for *MECP2* genetic variants was recommended as part of the diagnostic workup of females who present with an autistic phenotype. Routine *MECP2* testing in males with autism spectrum disorders was not recommended.

### U.S. Preventive Services Task Force Recommendations

Not applicable.

### Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

### Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 1.

**Table 1. Summary of Key Trials**

| NCT No. | Trial Name | Planned Enrollment | Completion Date |
|---|---|---|---|
| *Ongoing* | | | |
| **NCT02171104** | MT2013-31: Allogeneic Hematopoietic Cell Transplantation for Inherited Metabolic Disorders and Severe Osteopetrosis Following Conditioning With Busulfan (Therapeutic Drug Monitoring), Fludarabine +/- ATG | 100 | Dec 2022 |
| *Unpublished* | | | |
| **NCT02153723** | Pharmacological Treatment of Rett Syndrome With Glatiramer Acetate (Copaxone) | 20 | Jan 2016 (updated 11/05/2018) |
| **NCT01777542** | Pharmacological Treatment of Rett Syndrome by Stimulation of Synaptic Maturation With Recombinant Human IGF-1(Mecasermin [rDNA] Injection) | 30 | Nov 2016 (updated 03/26/2018) |
| **NCT01520363** | Placebo Controlled Trial of Dextromethorphan in Rett Syndrome | 60 | Oct 2016 (updated 12/04/2018) |

NCT: national clinical trial.

## References

1. Williamson SL, Christodoulou J. Rett syndrome: new clinical and molecular insights. Eur J Hum Genet. Aug 2006; 14(8): 896-903. PMID 16865103
2. Petriti U, Dudman DC, Scosyrev E, et al. Global prevalence of Rett syndrome: systematic review and meta-analysis. Syst Rev. Jan 16 2023; 12(1): 5. PMID 36642718
3. Lotan M, Ben-Zeev B. Rett syndrome. A review with emphasis on clinical characteristics and intervention. ScientificWorldJournal. Dec 06 2006; 6: 1517-41. PMID 17160339
4. Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: revised diagnostic criteria and nomenclature. Ann Neurol. Dec 2010; 68(6): 944-50. PMID 21154482
5. Guy J, Gan J, Selfridge J, et al. Reversal of neurological defects in a mouse model of Rett syndrome. Science. Feb 23 2007; 315(5815): 1143-7. PMID 17289941
6. Robinson L, Guy J, McKay L, et al. Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. Brain. Sep 2012; 135(Pt 9): 2699-710. PMID 22525157
7. Suter B, Treadwell-Deering D, Zoghbi HY, et al. Brief report: MECP2 mutations in people without Rett syndrome. J Autism Dev Disord. Mar 2014; 44(3): 703-11. PMID 23921973
8. Cuddapah VA, Pillai RB, Shekar KV, et al. Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett syndrome. J Med Genet. Mar 2014; 51(3): 152-8. PMID 24399845
9. Archer H, Evans J, Leonard H, et al. Correlation between clinical severity in patients with Rett syndrome with a p.R168X or p.T158M MECP2 mutation, and the direction and degree of skewing of X-chromosome inactivation. J Med Genet. Feb 2007; 44(2): 148-52. PMID 16905679
10. Weaving LS, Williamson SL, Bennetts B, et al. Effects of MECP2 mutation type, location and X-inactivation in modulating Rett syndrome phenotype. Am J Med Genet A. Apr 15 2003; 118A(2): 103-14. PMID 12655490
11. Liyanage VR, Rastegar M. Rett syndrome and MeCP2. Neuromolecular Med. Jun 2014; 16(2): 231-64. PMID 24615633
12. Zahorakova D, Lelkova P, Gregor V, et al. MECP2 mutations in Czech patients with Rett syndrome and Rett-like phenotypes: novel mutations, genotype-phenotype correlations and validation of high-resolution melting analysis for mutation scanning. J Hum Genet. Jul 2016; 61(7): 617-25. PMID 26984561
13. Sheikh TI, Ausió J, Faghfoury H, et al. From Function to Phenotype: Impaired DNA Binding and Clustering Correlates with Clinical Severity in Males with Missense Mutations in MECP2. Sci Rep. Dec 08 2016; 6: 38590. PMID 27929079
14. Schönewolf-Greulich B, Tejada MI, Stephens K, et al. The MECP2 variant c.925C T (p.Arg309Trp) causes intellectual disability in both males and females without classic features of Rett syndrome. Clin Genet. Jun 2016; 89(6): 733-8. PMID 26936630
15. Huppke P, Laccone F, Krämer N, et al. Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. Hum Mol Genet. May 22 2000; 9(9): 1369-75. PMID 10814718
16. Cheadle JP, Gill H, Fleming N, et al. Long-read sequence analysis of the MECP2 gene in Rett syndrome patients: correlation of disease severity with mutation type and location. Hum Mol Genet. Apr 12 2000; 9(7): 1119-29. PMID 10767337
17. Bao X, Downs J, Wong K, et al. Using a large international sample to investigate epilepsy in Rett syndrome. Dev Med Child Neurol. Jun 2013; 55(6): 553-8. PMID 23421866
18. Bebbington A, Downs J, Percy A, et al. The phenotype associated with a large deletion on MECP2. Eur J Hum Genet. Sep 2012; 20(9): 921-7. PMID 22473088
19. Lane JB, Lee HS, Smith LW, et al. Clinical severity and quality of life in children and adolescents with Rett syndrome. Neurology. Nov 15 2011; 77(20): 1812-8. PMID 22013176
20. Fabio RA, Colombo B, Russo S, et al. Recent insights into genotype-phenotype relationships in patients with Rett syndrome using a fine grain scale. Res Dev Disabil. Nov 2014; 35(11): 2976-86. PMID 25124696

21. Halbach N, Smeets EE, Julu P, et al. Neurophysiology versus clinical genetics in Rett syndrome: A multicenter study. Am J Med Genet A. Sep 2016; 170(9): 2301-9. PMID 27354166
22. Pidcock FS, Salorio C, Bibat G, et al. Functional outcomes in Rett syndrome. Brain Dev. Jan 2016; 38(1): 76-81. PMID 26175308
23. Sajan SA, Jhangiani SN, Muzny DM, et al. Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in MECP2. Genet Med. Jan 2017; 19(1): 13-19. PMID 27171548
24. Vidal S, Brandi N, Pacheco P, et al. The utility of Next Generation Sequencing for molecular diagnostics in Rett syndrome. Sci Rep. Sep 25 2017; 7(1): 12288. PMID 28947817
25. Zhang Q, Zhao Y, Bao X, et al. Familial cases and male cases with MECP2 mutations. Am J Med Genet B Neuropsychiatr Genet. Jun 2017; 174(4): 451-457. PMID 28394482
26. Amir RE, Sutton VR, Van den Veyver IB. Newborn screening and prenatal diagnosis for Rett syndrome: implications for therapy. J Child Neurol. Sep 2005; 20(9): 779-83. PMID 16225835
27. Michelson DJ, Shevell MI, Sherr EH, et al. Evidence report: Genetic and metabolic testing on children with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. Oct 25 2011; 77(17): 1629-35. PMID 21956720
28. Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. Pediatrics. Nov 2007; 120(5): 1183-215. PMID 17967920
29. AAP Publications Retired and Reaffirmed. Pediatrics. Dec 2014;134(5):e1520.
30. Hyman SL, Levy SE, Myers SM, et al. Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. Pediatrics. Jan 2020; 145(1). PMID 31843864
31. Schaefer GB, Mendelsohn NJ. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. Genet Med. May 2013; 15(5): 399-407. PMID 23519317

## Documentation for Clinical Review

**Please provide the following documentation:**

- History and physical and/or consultation notes including:
- Reason for performing test
- Signs/symptoms/test results related to reason for genetic testing
- Family history if applicable
- Lab results documenting one/both partners carrier status or genetic disorder
- Name and description of genetic test
- CPT codes billed for the particular genetic test

**Post Service (in addition to the above, please include the following):**

- Procedure report(s)

## Coding

*This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.*

*The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.*

| Type | Code | Description |
|---|---|---|
| CPT® | 81302 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; full sequence analysis |
| | 81303 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; known familial variant |
| | 81304 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; duplication/deletion variants |
| | 81404 | Molecular pathology procedure Level 5 |
| | 81405 | Molecular Pathology Procedure Level 6 |
| | 81406 | Molecular pathology procedure Level 7 |
| HCPCS | None | |

## Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

| Effective Date | Action |
|---|---|
| 03/30/2015 | BCBSA medical policy adoption |
| 09/01/2016 | Policy revision without position change |
| 12/01/2017 | Policy revision without position change |
| 09/01/2018 | Policy revision without position change |
| 07/01/2019 | Policy revision without position change |
| 08/01/2023 | Policy reactivated. Previously archived from 07/01/2020 to 07/31/2023. |

## Definitions of Decision Determinations

**Medically Necessary:** Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

**Investigational/Experimental:** A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation:** Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

## Prior Authorization Requirements and Feedback (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

*Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.*

## Appendix A

| POLICY STATEMENT | |
|---|---|
| **BEFORE** | **AFTER**<br>**Blue font: Verbiage Changes/Additions** |
| **Reactivated Policy**<br><br>**Policy Statement:**<br>N/A | **Genetic Testing for Rett Syndrome 2.04.81**<br><br>**Policy Statement:**<br>I. Genetic testing for Rett syndrome–associated genes (e.g., *MECP2*, *FOXG1*, or *CDKL5*) may be considered **medically necessary** to establish a genetic diagnosis of Rett syndrome in a child with developmental delay and signs/symptoms of Rett syndrome, when a definitive diagnosis cannot be made without genetic testing.<br><br>II. Targeted genetic testing for a known familial Rett syndrome–associated variant may be considered **medically necessary** to determine carrier status of a mother or a sister of an individual with Rett syndrome.<br><br>III. All other indications for genetic testing for Rett syndrome–associated genes (e.g., *MECP2*, *FOXG1*, or *CDKL5*) are considered **investigational**, including **either** of the following:<br>A. Routine carrier testing (preconception or prenatal) in persons with negative family history<br>B. Testing of asymptomatic family members to determine future risk of disease |