Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

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(See policy history boxes for previous effective dates)

Title: Genetic Testing for Rett Syndrome

Description/Background

Rett Syndrome

Rett syndrome (RTT), a neurodevelopmental disorder, is usually caused by mutations in the *MECP2* gene. Genetic testing is available to determine whether a pathogenic mutation exists in a patient with clinical features of Rett syndrome, or in a patient's family member.

Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting girls with an incidence of 1:10,000 female births, making it one of the most common genetic causes of intellectual disability in girls.[1] RTT is characterized by apparent normal development for the first 6-18 months of life, followed by the loss of intellectual functioning, loss of acquired fine and gross motor skills and the ability to engage in social interaction. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, sometimes described as handwringing.[2] 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 classical form of RTT, there are a number of recognized atypical variants. Variants of RTT may appear with a severe or a milder form. The severe variant has no normal developmental period; individuals with a milder phenotype experience less dramatic regression and milder expression of the characteristics of classical RTT. The required diagnostic criteria for typical (or classic) RTT and atypical (or variant) RTT have been established for the diagnosis of classic and variant RTT.[1-3] The RTT Diagnostic Criteria 2010[3] defines the required criteria for typical (or classic) RTT and atypical (or variant) RTT. For typical RTT, a period of regression followed by recovery or stabilization and fulfillment of all main criteria without the presence of any exclusion 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 out of the 4 main criteria plus 5 out of 11 supportive are required to meet the diagnostic criteria of variant RTT.

Treatment

Management is mainly symptomatic and individualized, focusing on optimizing each patient's abilities.[1] A multidisciplinary approach is usually used, with specialist input from dietitians, physiotherapists, occupational therapists, speech therapists and music therapists. Regular monitoring for scoliosis and possible heart abnormalities may be recommended. The development of scoliosis (seen in about 87% of patients by age 25 years) and the development of spasticity can have a major impact on mobility, and the development of effective communication strategies. Occupational therapy can help children develop skills needed for performing self-directed activities (such as dressing, feeding, and practicing arts and crafts).

Trofinetide (Daybue) is the first and only approved drug for Rett syndrome in adults and pediatric patients ≥2 years of age.[6] In the pivotal 12-week LAVENDAR trial comparing trofinetide (n=93) to placebo (n=94) in female patients with Rett syndrome, statistically significant improvements were observed in the Rett Syndrome Behavior Questionnaire (RSBQ) score and Clinical Global Impression-Improvement (CGI-I) score.[7] However, the clinical significance of these findings is uncertain.

Pharmacological approaches to managing problems associated with RTT include melatonin for sleep disturbances and several agents for the control of 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 mutated *MECP2* has demonstrated reversibility of the genetic defect.[4,5]

In a mouse model of RTT, genetic manipulation of the *MECP2* gene has demonstrated reversibility of the genetic defect.[8,9]

Genetics

RTT is an X-linked dominant genetic disorder. Mutations in *MECP2* (methyl-CpG-binding protein 2), which is thought to control expression of several genes including some involved in brain development, were first reported in 1999. Subsequent screening of RTT patients has shown that over 80% of classical RTT have pathogenic mutations in the *MECP2* gene. More than 200 mutations in *MECP2* have been associated with RTT.[10] However, 8 of the most commonly occurring missense and nonsense mutations account for almost 70% of all cases, small C-terminal deletions account for approximately 10%, and large deletions, 8% to 10%.[7] *MECP2* mutation type is associated with disease severity.[11] Whole duplications of the *MECP2* gene have been associated with severe X-linked mental retardation 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.[12,13]

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* mutation, which arise 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 mental retardation). In the case of a carrier mother, the recurrence risk of RTT is 50%. If a mutation is not identified in leukocytes of the mother, the risk to a sibling of the proband is below 0.5% (since germline mosaicism in either parent cannot be excluded).

Identification of a mutation in *MECP2* does not necessarily equate to a diagnosis of RTT. Rare cases of *MECP2* mutations have also been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked mental retardation, PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders [most commonly bipolar disorder], parkinsonism, and mental retardation), autism and neonatal encephalopathy.[1,10,14] Recent studies have revealed that different classes of genetic variants in MECP2 result in variable clinical phenotypes and overlap with other neurodevelopmental disorders.[15-17]

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

Regulatory Status

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

Medical Policy Statement

Testing for Rett syndrome is established. It may be considered a useful diagnostic option when criteria are met.

Inclusionary and Exclusionary Guidelines

Inclusions:

- When testing is performed to confirm a diagnosis of Rett syndrome in a child with developmental delay and signs/symptoms of Rett syndrome, but when there is uncertainty in the clinical diagnosis (e.g., *MECP2, FOXG1, or CDKL5*).
- Targeted genetic testing for a known familial Rett syndrome-associated variant to determine carrier status of first-degree female relatives of an individual with Rett syndrome.

Exclusions:

- All other indications for genetic testing for Rett syndrome-associated genes (e.g., *MECP2, FOXG1 or CDKL5*), including carrier testing (preconception or prenatal), and testing of asymptomatic family members to determine future risk of disease.

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81302 81303 81304 81404 81405 81406

Other codes (investigational, not medically necessary, etc.):

0234U

Rationale

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.

TESTING INDIVIDUALS 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, predict potential disease severity, initiate surveillance for potential disease complications (e.g., musculoskeletal deformities, autonomic dysfunction), and direct treatments.

The following **PICOs** were used to select literature to inform this review.

Populations

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

Interventions

The relevant intervention of interest is genetic testing for RTT-associated genes.

The primary settings would be in pediatric neurology, developmental pediatrics, or genetics outpatient offices.

Comparators

The relevant comparator of interest is 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/kyphosis). False-negative test results can lead to lack of appropriate surveillance and treatments.

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).

Huppke et al analyzed the *MECP2* gene in 31 female patients diagnosed clinically with RTT.[18] Sequencing revealed mutations in 24 of the 31 patients (77%). Of the 7 patients in whom no mutations were found, 5 fulfilled the criteria for classical RTT. In this study, 17 different mutations were detected, 11 of which had not been previously described. Several females carrying the same mutation displayed different phenotypes, suggesting that factors other than the type or position of mutations influence the severity of RTT.

Cheadle et al (2000) analyzed mutations in 48 females with classical sporadic RTT, 7 families with possible familial RTT, and 5 sporadic females with features suggestive, but not diagnostic, of RTT.[19] The entire *MECP2* gene was sequenced in all cases. Mutations were identified in 44/55 (80%) of unrelated classical sporadic and familial RTT patients. Only 1 out of 5 (20%) sporadic cases with suggestive but non-diagnostic features of RTT had mutations identified. Twenty-one different mutations were identified (12 missense, 4 nonsense, and 5 frame-shift mutations); 14 of the mutations identified were novel. Significantly milder disease was noted in patients carrying missense mutations as compared to those with truncating mutations.

The 2 studies previously outlined were included in a summary of 6 articles by Lotan et al (2006), who attempted to disclose a genotype-phenotype correlation.[3] The authors found that these studies have yielded inconsistent results and that further controlled studies are needed before valid conclusions can be drawn about the effect of mutation type on phenotypic expression. Two subsequent studies[20,21] used the InterRett database to examine genotype and RTT severity. Of 357 girls with epilepsy who had *MECP2* genotype recorded, those with large deletions were more likely than those with 10 other common mutations to have active epilepsy (odds ratio [OR]: 3.71 (95% confidence interval [CI]: 1.13, 12.17); p=0.03) 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 (OR: 0.42 (95% CI: 0.22, 0.79), p=0.007). Of 260 girls with classic RTT enrolled in the multicenter RTT Natural History study (NCT00299312), those with the *R133C* substitution mutation had clinically less severe disease, assessed by the Clinical Severity, Motor Behavior Analysis, and Physician Summary scales.[22] Fabio et al (2014) reported similar genotype-phenotype correlations among 144 patients with RTT in Italy.[23]

Halbach et al (2016) analyzed a cohort of a group of 132 well-defined RTT females aged between 2 and 43 years with extended clinical, molecular, and neurophysiological assessment.[24] Genotype-phenotype analyses of clinical features and cardiorespiratory data were performed after grouping mutations by the same type and localization or having the same putative biological effect on the MeCP2 protein, and subsequently on eight single recurrent pathogenic variants. A less severe phenotype was seen in females with CTS, p.R133C, and p.R294X mutations. Autonomic disturbances were present in all females and not restricted to nor influenced by one specific group or any single recurrent mutation. The objective information from non-invasive neurophysiological evaluation of the disturbed central autonomic control is of great importance in helping to organize the lifelong care for females with RTT. The study concluded that further research 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* (methyl-CpG-binding protein 2) gene.[25] 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 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 mutations. On categorical variables as reported by parents at the time of initial evaluation, patients with R133C and R294X were most likely to have hand use, those with R133C, R294X, R306C and small deletions were most likely to be ambulatory, and those with R133C were most likely to be verbal.

Sajan et al (2017) analyzed 22 RTT patients without apparent *MECP2*, *CDKL5*, and *FOXG1* pathogenic variants were subjected to both whole-exome sequencing and single-nucleotide polymorphism array-based copy-number variant (CNV) analyses.[26] 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 CNVs (10 patients had 2 or more). Interestingly, 13 patients had variants in a gene/region previously reported in other neurodevelopmental disorders (NDDs), 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

NDDs, and complicated by a high variant burden. Dysregulation of chromatin structure and abnormal excitatory synaptic signaling may form two common pathological bases of RTT.

Vidal et al (2017) investigated the utility of next-generation sequencing and its ability to identify an affected person genetically.[27] 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 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 indicates that the clinical sensitivity of genetic testing for classical RTT is reasonably high, in the range of 75-80%. However, the sensitivity may be lower when classic features of RTT are absent. Clinical specificity is unknown but is also likely to be high, as only rare cases of *MECP2* mutations have been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked mental retardation, PPM-X syndrome, autism and neonatal encephalopathy. Recent studies have indicated that specific classes, types or burden of pathogenic variants in genes associated with Rett syndrome affect severity of disease (e.g., degree of autonomic dysfunction, functional outcomes and degree of neurodevelopment 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. No studies were identified that demonstrated direct evidence of clinical utility.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. There is no specific treatment for RTT; however, identification of the pathogenic variant leading to Rett syndrome has been found to correlate with disease severity and predict potential complications of 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: Clinical Utility
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).

TARTGETED FAMILIAL VARIANT TESTING OF SISTERS OF INDIVIDUALS WITH RETT SYNDROME

Clinical Context and Test Purpose

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

The following **PICOs** were used to select literature to inform this review.

Populations

The relevant population of interest includes asymptomatic sisters of individuals with Rett syndrome.

Interventions

Targeted genetic testing for a known familial variant.

Comparators

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 reproductive-age females. A negative genetic test result would eliminate the need for surveillance to detect development of symptoms and disease. A positive genetic test result would confirm a need for active surveillance and potentially reproductive decision-making, in

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/kyphosis). False-negative test results can lead to lack of appropriate surveillance and inaccurate risk assessment to determine likelihood of an affected offspring.

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).

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 CONSIDERING FURTHER CHILDBEARING

Clinical Context and Test Purpose
The purpose of targeted familial variant testing of females with a child with Rett syndrome is to determine carrier status and aid in reproductive planning.

The following **PICOs** were used to select literature to inform this review.

Populations
The relevant population of interest includes females with a child with Rett syndrome.

Interventions
Targeted genetic testing for a known familial variant.

Comparators
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 Rett syndrome 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 affect offspring, 50% chance of a hemizygous affected male or 50% chance of a heterozygous affect 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 affect offspring. False-negative test results can lead to lack of appropriate preimplantation genetic diagnosis and inaccurate risk assessment to determine likelihood of an affected offspring.

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.[16] In heterozygous females the variable phenotypic severity is modulated by non-random 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 biological effect of specific pathogenic variant. A wide range of phenotypic/clinical severity, ranging from neonatal encephalopathy to mild psychiatric abnormalities was observed consistent functional/molecular results. Overall, clinical severity showed a direct correlation with the functional impairment of *MECP2*.

Zahorakova et al (2016) analyzed RTT patients with *MECP2* pathogenic variants and X-chromosome inactivation (XCI).[15] Skewed XCI (ratio >75%) was found in 19.3% of the girls, but no gross divergence in clinical severity was observed. Findings confirm 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 of the phenotypic differences in RTT were noted.

Zhang et al (2017) investigated familial cases with RTT or X-linked mental retardation.[28] 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 females are confounded by both random X-chromosome inactivation (XCI) and the class or type of pathogenic variant present. In heterozygous females, the clinical sensitivity correlates with variant type and variable effects of skewed XCI. In contrast, for hemizygous males, the phenotypic/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.[29]

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.

SUMMARY OF EVIDENCE
For individuals who have signs and/or symptoms of Rett syndrome (RTT), the evidence for genetic testing for Rett-syndrome-associated genes includes case series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, health status measures, and quality of life. *MECP2* variants are found in most patients with RTT, particularly those who present with classical clinical features of RTT. The diagnostic accuracy of genetic testing for RTT cannot be determined with absolute certainty given variable clinical presentations of typical versus atypical RTT, but testing appears to have high sensitivity and specificity. Genetic testing has clinical utility when signs and symptoms of Rett syndrome are present to establish a specific genetic diagnosis. Identification of a specific class or type of pathogenic variant may alter some aspects of management and may eliminate or necessitate surveillance for different clinical manifestations of disease. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic sisters of a child with RTT with a known pathogenic variant, the evidence for targeted familial variant testing includes case series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, changes in reproductive decision making, symptoms, and symptoms. 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 and clinical severity based on the type of pathogenic variant present. In reproductive-age sisters, determination of carrier status can eliminate or necessitate prenatal testing and inform reproductive decision making. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who are women with a child with RTT and who are considering future childbearing, the evidence for targeted genetic testing for a familial Rett syndrome-associated variant includes cases series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, and changes in reproductive decision making. Targeted familial variant testing of a woman with a child with RTT to determine carrier status may inform prenatal testing and reproductive decision making. In the rare situation where the mother carries a pathogenic variant, all future offspring have a 50% of being affected with males typically presenting with more severe disease. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

Clinical Input Received From Physician Specialty Societies and Academic Medical Centers

In response to requests, BCBSA received input related to the use mutation testing for Rett syndrome in June 2012 from 3 academic medical centers and 2 specialty medical societies (3 reviewers), for a total of 6 reviewers. 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.

There was consensus/near total consensus supporting the use of mutation testing for the diagnosis of Rett syndrome in a girl in whom the clinical differential diagnosis includes Rett syndrome, especially when the clinical diagnosis is uncertain. Support for testing sisters of individuals with Rett syndrome and for prenatal screening was mixed.

PRACTICE GUIDELINES AND POSITION STATEMENTS

American Academy of Neurology/Child Neurology Society

In 2011, a quality standards subcommittee of the American Academy of Neurology (AAN) and the Practice Committee of the Child Neurology Society issued an evidence report on the genetic and metabolic testing of children with global developmental delay.[30] The American Academy of Neurology recommends considering *MECP2* mutation testing for all girls with unexplained moderate to severe developmental delay.

American Academy of Pediatrics

A 2007 policy statement from the American Academy of Pediatrics, reaffirmed in 2014 and 2019,[31,32] recommended *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”[33]

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 revised its evidence-based guideline for clinical genetics evaluation of autism spectrum disorders.[34] Testing for *MECP2* mutations is recommended as part of the diagnostic workup of females who present with an autistic phenotype. Routine *MECP2* testing in males with autistic spectrum disorders is not recommended.

International Rett Syndrome Foundation

The "Consensus Guidelines on Managing Rett Syndrome Across the Lifespan" published in 2020 by the International Rett Syndrome Foundation make the following statement regarding genetic testing:[5] "For suspicion of RTT, *MECP2* gene sequencing and MLPA [multiplex ligation-dependent probe amplification] testing is recommended. MLPA testing is needed to detect deletions otherwise missed by sequencing; this test is necessary if no abnormalities are found by sequencing."

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			
Unpublished			
NCT02153723	Pharmacological treatment of Rett syndrome with Glatiramer Acetate (Copaxone)	20	June 2015 (unknown)
NCT01777542	Pharmacological treatment of Rett syndrome by stimulation of synaptic maturation with recombinant human IGF-1 (Mecasermin [rDNA] injection)	30	Nov 2016 (completed)
NCT01520363	Placebo controlled trial of dextromethorphan in Rett syndrome	60	Dec 2017
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

NCT: national clinical trial

Government Regulations
National/ Local:

There is no national or local coverage determination specifically addressing genetic testing for Rett syndrome. No fees listed for 81302-4.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

Genetic Testing and Counseling

References

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through March 2025, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
3/1/14	12/10/13	1/6/14	Joint policy established
7/1/15	4/24/15	5/8/15	Routine maintenance. No change in policy status.
7/1/16	4/19/16	4/19/16	Routine policy maintenance. Updated references and rationale. Add codes 81404 & 81406.
7/1/17	4/18/17	4/18/17	Routine maintenance. No change in policy status.
7/1/18	4/17/18	4/17/18	Routine maintenance. Updated rationale, added reference #12, 13, 14, 21, 22, and 23.
7/1/19	4/16/19		Routine policy maintenance, added references 23 and 24. No change in policy status.
7/1/20	4/14/20		Routine policy maintenance, no added references. No change in policy status.
7/1/21	4/20/21		Routine policy maintenance added code 0234U as E/I, effective 1/1/21. No change in policy status.
7/1/22	4/19/22		Routine policy maintenance, no change in policy status.
7/1/23	4/26/23		Removed “female” inclusion section. Added “first-degree female relatives” in place of mother or sister. Added code 81405 as established. Vendor managed: N/A. (ds)
7/1/24	4/16/24		Routine policy maintenance, no change in policy status. Vendor managed: N/A (ds)
7/1/25	4/15/25		Routine policy maintenance, Added reference #6 &7. No change in policy status. Vendor managed: N/A (ds)

Next Review Date: 2nd Qtr. 2026

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING FOR RETT SYNDROME

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered; criteria apply.
BCNA (Medicare Advantage)	See government section.
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.