DISCLAIMER

Our medical policies are designed for informational purposes only and are not an authorization, explanation of benefits or a contract. Receipt of benefits is subject to satisfaction of all terms and conditions of the coverage. Medical technology is constantly changing, and we reserve the right to review and update our policies periodically.

POLICY

Genetic testing for Rett syndrome–associated genes (eg, 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.

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.

All other indications for genetic testing for Rett syndrome–associated genes (eg, MECP2, FOXG1, or CDKL5), including routine carrier testing (preconception or prenatal) in persons with negative family history, and testing of asymptomatic family members to determine future risk of disease, are considered investigational.

POLICY GUIDELINES

GENETICS NOMENCLATURE UPDATE

The Human Genome Variation Society 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, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology 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

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA</td>
<td>sequence</td>
</tr>
<tr>
<td>Familial</td>
<td>Disease-associated</td>
<td>variant identified in a proband for use in subsequent targeted genetic</td>
</tr>
<tr>
<td></td>
<td>variant</td>
<td>testing in first-degree relatives</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

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
There are CPT codes for this testing:

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

CPT code 81404 includes the following testing for FOXG1:

FOXG1 (forkhead box G1) (eg, Rett syndrome), full gene sequence.

CPT code 81405 includes the testing for CDKL5:

CDKL5 (cyclin-dependent kinase-like 5) (eg, early infantile epileptic encephalopathy), duplication/deletion analysis.

CPT code 81406 includes the following testing for CDKL5:

CDKL5 (cyclin-dependent kinase-like 5) (eg, early infantile epileptic encephalopathy), full gene sequence.

BENEFIT APPLICATION

BLUE CARD/NATIONAL ACCOUNT ISSUES
Some Plans may have contract or benefit exclusions for genetic testing.
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.\(^2\) 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 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.\(^1-3\) 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 criteria 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.\(^3\) 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 \(\approx87\)% 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 (eg, 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.\(^5\) 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%.\textsuperscript{7} \textit{MECP2} variant type is associated with disease severity.\textsuperscript{8} Whole duplications of the \textit{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.\textsuperscript{9,10}

Because the spectrum of clinical phenotypes is broad, to facilitate genotype-phenotype correlation analyses, the International Rett Syndrome Association has established a locus-specific \textit{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 \textit{MECP2} does not necessarily equate to a diagnosis of RTT. Rare cases of \textit{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.\textsuperscript{1,6,11} Recent studies have revealed that different classes of genetic variants in \textit{MECP2} result in variable clinical phenotypes and overlap with other neurodevelopmental disorders.\textsuperscript{12-14}

A proportion of patients with a clinical diagnosis of RTT do not appear to have pathogenic variants in the \textit{MECP2} gene. Two other genes (\textit{CDKL5}, \textit{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 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**

This evidence review was created in July 2012 and has been updated regularly with searches of the MEDLINE database. The current literature update was performed through April 5, 2018.

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

The relevant 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 PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest includes 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.

Timing
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.

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

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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. 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. 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.

The 2 studies previously discussed were included in a summary of 6 articles by Lotan and Ben-Zeev (2006) who attempted to elicit a genotype-phenotype correlation. 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. 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% confidence interval, 1.13 to 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 (odds ratio, 0.42; 95% confidence interval, 0.22 to 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 variant had clinically less severe disease, as assessed by the Clinical Severity, Motor Behavior Analysis, and Physician Summary scales. Fabio et al (2014) reported similar genotype-phenotype correlations among 144 patients with RTT in Italy.

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. 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 1 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. 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. 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. 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 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 (eg, 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 (eg, occupational therapy) to maintain or improve functional outcomes (eg, extremity mobility, ambulation).

**TARGETED FAMILIAL VARIANT TESTING OF ASYMPTOMATIC SISTERS OF INDIVIDUALS WITH RTT**

**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 relevant question addressed in this evidence review is: Does targeted familial variant testing of asymptomatic sisters of individuals with RTT lead to improved 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 PICOTS were used to select literature to inform this review.

**Patients**

The relevant population of interest includes 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 patients.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary surveillance (eg, musculoskeletal or autonomic dysfunction) and treatments (eg, 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.
**Timing**
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.

**Setting**
The primary setting would be in pediatric neurology or genetics outpatient offices for young female patients and in obstetrics, general practice, or genetics outpatient offices for female patients of reproductive age.

**Technically Reliable**
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

**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 RTT 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 health outcomes, including preimplantation genetic testing to determine the likelihood of an affected offspring, or alter reproductive planning decisions?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest includes female patients 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.

Timing
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.

Setting
The primary setting would be in obstetrics, genetics, or general practitioners outpatient offices.
Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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. 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. 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. 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 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.

**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 RTT who receive genetic testing for RTT-associated genes, the evidence 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 in those who present with classic clinical features of RTT. The diagnostic accuracy of genetic testing for RTT cannot be determined with absolute certainty given variable clinical presentations of typical vs atypical RTT, but testing appears to have high sensitivity and specificity. Genetic testing has clinical utility when signs and symptoms of RTT 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 the disease. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic sisters of an individual with RTT who receive targeted genetic testing for a known familial RTT-associated variant, the evidence 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 sisters of reproductive age, determination of carrier status can eliminate or necessitate prenatal testing and inform reproductive decision making. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are females with a child with RTT who are considering future childbearing who receive targeted genetic testing for a known familial RTT-associated variant, the evidence 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 that the technology results in a meaningful improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

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 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

American Academy of Neurology and Child Neurology Society

American Academy of Pediatrics
A 2007 policy statement from the American Academy of Pediatrics, reaffirmed in 2014, recommended MECP2 testing to confirm a diagnosis of suspected Rett syndrome (RTT), especially when the diagnosis was unclear from symptoms alone.

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

RettSearch Consortium
In 2010, RettSearch, a consortium of international clinical RTT specialists, suggested that patients who are negative for MECP2 variants and have a strong clinical diagnosis of RTT should be considered for further screening for the CDKL5 gene if there are early-onset seizures, or for the FOXG1 gene if there are congenital features (eg, severe postnatal microcephaly).

American College of Medical Genetics and Genomics
The American College of Medical Genetics and Genomics (2013) revised its evidence-based guidelines for clinical genetics evaluation of autism spectrum disorders. 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

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ongoing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02061137</td>
<td>A Phase 1 Clinical Study to Assess Safety and Efficacy of Oral Fingolimod (FTY720) in Children With Rett Syndrome.</td>
<td>6</td>
<td>Jul 2018</td>
</tr>
<tr>
<td>NCT02171104</td>
<td>MT2013-31: Allogeneic Hematopoietic Cell Transplantation for Inherited Metabolic Disorders and Severe Osteopetrosis Following Conditioning With Busulfan (Therapeutic Drug Monitoring), Fludarabine +/- ATG</td>
<td>100</td>
<td>Sep 2019</td>
</tr>
<tr>
<td><strong>Unpublished</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02023424</td>
<td>An Open Label, Exploratory Study to Investigate the Treatment Effect of Glatiramer Acetate (Copaxone ®) on Girls With Rett Syndrome</td>
<td>10</td>
<td>Feb 2015 (unknown)</td>
</tr>
<tr>
<td>NCT02153723</td>
<td>Pharmacological Treatment of Rett Syndrome With Glatiramer Acetate (Copaxone)</td>
<td>20</td>
<td>Jun 2015 (unknown)</td>
</tr>
<tr>
<td>NCT01777542</td>
<td>Pharmacological Treatment of Rett Syndrome by Stimulation of Synaptic Maturation With Recombinant Human IGF-1(Mecasermin [rDNA] Injection)</td>
<td>30</td>
<td>Nov 2016 (completed)</td>
</tr>
<tr>
<td>NCT01520363</td>
<td>Placebo Controlled Trial of Dextromethorphan in Rett Syndrome</td>
<td>60</td>
<td>Dec 2017 (completed)</td>
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</table>

NCT: national clinical trial.

REFERENCES


**CODES**

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>81302</td>
<td>MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td></td>
<td>81303</td>
<td>MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; known familial variant</td>
</tr>
<tr>
<td></td>
<td>81304</td>
<td>MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; duplication/deletion variants</td>
</tr>
<tr>
<td>ICD-10-CM</td>
<td>F84.2</td>
<td>Rett’s syndrome</td>
</tr>
<tr>
<td></td>
<td>F88</td>
<td>Other disorders of psychological development</td>
</tr>
<tr>
<td></td>
<td>Z13.4</td>
<td>Encounter for screening for certain developmental disorders in childhood</td>
</tr>
<tr>
<td>ICD-10-PCS</td>
<td>Not applicable. ICD-10-PCS codes are only used for inpatient services. There are no ICD procedure codes for laboratory tests.</td>
<td></td>
</tr>
</tbody>
</table>

**Type of service** Laboratory/Pathology

**Place of service** Laboratory/Reference Laboratory

**POLICY HISTORY**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>09/11/14</td>
<td>Replace policy</td>
<td>Policy updated with literature review through August 18, 2014; references 6, 8-11, 17, and 19-22 added; reference 12 updated. No change to policy statements.</td>
</tr>
<tr>
<td>11/12/15</td>
<td>Replace policy</td>
<td>Policy updated with literature review through October 31, 2015; no references added. Policy statement edited for clarity, no change to intent of policy statement.</td>
</tr>
<tr>
<td>08/11/16</td>
<td>Replace policy – coding only</td>
<td>ICD-10-CM code F88 added to Codes table</td>
</tr>
<tr>
<td>02/24/17</td>
<td>Replace policy</td>
<td>Blue Cross of Idaho annual review; no change to policy.</td>
</tr>
</tbody>
</table>
| 06/01/17 | Replace policy              | Policy updated with literature review through March 23, 2017; references 12-14 and 21-23. The policy is revised with updated genetics nomenclature. “Mutations” changed to “variants” in policy statements. Policy rewritten with new PICOs for indications 2 and 3 to limit populations to sisters of child with Rett syndrome (indication 2) or females with a child with Rett syndrome (indication 3) with the
<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Description</th>
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<tbody>
<tr>
<td>05/30/18</td>
<td>Replace</td>
<td>Blue Cross of Idaho adopted changes as noted. Policy updated with literature review through April 5, 2018; references 23-24 added. Edits made to the investigational policy statement; statements otherwise unchanged.</td>
</tr>
</tbody>
</table>

intervention revised to “targeted genetic testing for a known familial variant.” Policy statements updated to define “genetic testing for Rett syndrome– associated genes (e.g., MECP2, FOXG1, or CDKL5)”; Removed “female” requirement of child for testing; Added 2 new medical necessity statements for “targeted genetic testing for a known familial variant” in a sister of a child with Rett syndrome or a female with a child with Rett syndrome.