Rett syndrome (RTT), a neurodevelopmental disorder affecting almost exclusively females, is usually caused by mutations in the \textit{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.

**MEDICAL POLICY CRITERIA**

I. Genetic testing for one or any combination of the following: \textit{MECP2}, \textit{FOXG1}, and \textit{CDKL5}, for Rett syndrome may be considered \textbf{medically necessary} when all of the following criteria are met:

   A. To confirm a diagnosis of Rett syndrome in a child with developmental delay and signs/symptoms of Rett syndrome; AND

   B. 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 \textbf{medically necessary} to determine carrier status for a mother or a sister of an individual with Rett syndrome.
III. All other indications for genetic testing for Rett syndrome, including but not limited to prenatal screening, testing of other family members, and panel testing including genes other than MECP2, FOXP1 and/or CDKL5 are considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   o History and physical exam including any relevant diagnoses related to the genetic testing
   o Conventional testing and outcomes
   o Conservative treatments, if any

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

RETT SYNDROME

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. 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 hand-wringing. Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation, and gait apraxia.

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 diagnosis of RTT remains a clinical one, using diagnostic clinical criteria that have been established for the diagnosis of classic and variant Rett syndrome.
TREATMENT OF RETT SYNDROME

There are currently no specific treatments that halt or reverse the progression of the disease, 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 generally 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), while physical therapy and hydrotherapy may prolong mobility.

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.[4,5]

GENETICS OF RETT SYNDROME

Classic RTT results from an X-linked dominant condition. 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 described. However, eight 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, 8–10%.[6] MECP2 mutation type is associated with disease severity.[7] Whole duplications of the MECP2 gene have been associated with severe X-linked intellectual disability with progressive spasticity, no or poor speech acquisition, and acquired microcephaly. In addition, the pattern of X-chromosome inactivation influences the severity of the clinical disease in females.

As 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).[8]

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 intellectual disability). 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).

The 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 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]

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 of RTT. Mutations in CDKL5 are associated with a variant of RTT observed in females with apparently classic Rett syndrome in whom the presentation is dominated by seizures and onset is before age six months.[9] Mutations in FOXG1 are associated with a variant of RTT referred to as congenital or precocious RTT, in which regression is never clearly identified but the clinical picture is otherwise classic.[10]

REGULATORY STATUS

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

EVIDENCE SUMMARY

Validation of the clinical use of any genetic test focuses on 3 main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;

2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and

3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence related to the ability of test results to:

• Guide decisions in the clinical setting related to either treatment, management, or prevention, and

• Improve health outcomes as a result of those decisions.

ANALYTIC VALIDITY

In 2015 the Agency for Healthcare Research and Quality (AHRQ) published results a Technical Brief that addressed Genetic Testing for Developmental Disabilities, Intellectual Disability, and Autism Spectrum Disorder.[11] The report summarizes information on genetic tests clinically available in the U.S. to detect genetic markers that predispose patients to developmental disabilities (DD). Input was sought from nine Key Informants to identify important clinical, technology, and policy issues from different perspectives. The National Center for Biotechnology Information's Genetic Testing Registry (GTR) was searched to identify genetic tests. The authors' search of the GTR database identified 672 laboratory-
developed tests offered by 63 providers in 29 States. The authors found a limited number of studies reporting on the analytic validity of the DD genetic testing, but did identify one study citing analytic validity for RTT.[12]

In 2014, Kalman et al published results from a study that was conducted by the Centers for Disease Control and Prevention's Genetic Testing Reference Material Coordination Program, in collaboration with the genetic testing community and the Coriell Cell Repositories.[12] In this study the authors' established 27 new cell lines and characterized the MECP2 mutations in these and in eight previously available cell lines. DNA samples from the 35 cell lines were tested by eight clinical genetic testing laboratories using DNA sequence analysis and methods to assess copy number (multiplex ligation-dependent probe amplification, semiquantitative PCR, or array-based comparative genomic hybridization). The eight common point mutations known to cause approximately 60% of Rett syndrome cases were identified, as were other MECP2 variants, including deletions, duplications, and frame shift and splice-site mutations.

In addition to the study by Kalman et al. discussed in the above AHRQ review, a large reference laboratory reports MECP2 testing for RTT has an analytical sensitivity for sequencing of 99% and for MLPA, 90%; analytic specificity is 99% for sequencing and for MLPA, 98%.[13]

CLINICAL VALIDITY

The AHRQ report, as discussed above, reported they identified just one study that addressed clinical validity, and it was not specific to RTT; however, there have been several small studies that were not included in the AHRQ report that discussed the clinical validity of genetic testing for RTT.[11]

In 2013, Maortua and colleagues evaluated the presence of MECP2 mutations (sequencing of four exons and rearrangements) in 120 female patients with suspected Rett syndrome, 120 female patients with intellectual disability of unknown origin and 861 (519 females and 342 males) controls.[14] Eighteen different pathological mutations were identified in both patients suspected of Rett syndrome and in those without a specific diagnosis. Authors concluded, "MECP2 must be studied not only in patients with classical/atypical Rett syndrome but also in patients with other phenotypes related to Rett syndrome."

Two studies published in 2013 and 2012 respectively[15,16] 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). Among 260 girls with classic RTT enrolled in the multicenter RTT Natural History study, those with the R133C substitution mutation had clinically less severe disease, assessed by the Clinical Severity, Motor Behavior Analysis, and Physician Summary scales.[6] Fabio et al reported similar genotype-phenotype correlations among 144 patients with RTT in Italy.[17]

In 2009, 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 seven patients in whom no mutations were found, five 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.

In 2006, Lotan et al. reviewed and summarized six articles that attempted to disclose a genotype-phenotype correlation, which included the two studies outlined above.[2] 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.

In 2000, Cheadle et al. analyzed mutations in 48 females with classical sporadic RTT, seven families with possible familial RTT, and five 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.

Section Summary

Although the AHRQ report reported finding no studies on clinical validity for RTT, there is 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 not present. The 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 intellectual disability, PPM-X syndrome, autism and neonatal encephalopathy.

CLINICAL UTILITY

The AHRQ report found that the majority of the clinical studies identified for RTT were for indirect assessment of clinical utility as “most of the genetic tests relevant to this report are intended to establish an etiologic diagnosis and rarely used in isolation to confirm a clinical diagnosis”. [11] Finally, no studies were identified that directly assessed the impact of genetic testing on health outcomes.

However, the clinical utility of genetic testing can be considered in the following clinical situations: 1) individuals with suspected RTT, 2) family members of individuals with RTT, and 3) prenatal testing for mothers with a previous RTT child. These situations are discussed separately below.

Individuals with Suspected RTT

The clinical utility for these patients depends on the ability of genetic testing to make a definitive diagnosis and for that diagnosis to lead to management changes that improve outcomes. No studies were identified that described how a molecular diagnosis of RTT changed patient management. Therefore, there is no direct evidence for the clinical utility of genetic testing in these patients.

Given that there is no specific treatment for RTT, making a definitive diagnosis will not lead to treatment that alters the natural history of the disorder. However, there are several potential
ways in which adjunctive management might be changed following genetic testing after 
confirmation of the diagnosis:

- Further diagnostic testing may be avoided
- Referral to a specialist(s) may be made
- Heightened surveillance for Rett-associated clinical manifestations, such as scoliosis or 
cardiac arrhythmias may be performed
- More appropriate tailoring of ancillary treatments such as occupational therapy may be 
possible

Therefore, genetic testing for RTT syndrome in developmentally delayed female children, 
without a clear diagnosis, may offer some surveillance benefits as well as help to avoid 
unnecessary additional diagnostic testing.

Family Member and Prenatal RTT Testing

Genetic testing can be done in sisters of girls with RTT who have an identified MECP2 
mutation to determine if they are asymptomatic carriers of the disorder. However, this is an 
extremely rare possibility, since the disorder is nearly always sporadic. Testing of family 
members of individuals with RTT will therefore result in an extremely low yield. However, 
testing for a known familial Rett-syndrome associated variant may aid mothers and sisters of 
affected individuals in reproductive decision-making.

Similarly, in cases of prenatal testing the risk of a family having a second child with the 
disorder is less than 1%, except in the rare situation where the mother carries the mutation.[20] 
Therefore, for mothers without the Rett phenotype, it is extremely unlikely that prenatal testing 
will identify cases of RTT.

Section Summary

The clinical utility of genetic testing for RTT has not been established in the literature; however, 
genetic testing can confirm a diagnosis in patients with clinical signs and symptoms of Rett 
syndrome. A definitive diagnosis may help avoid further testing for other possible syndromes 
as well as alter surveillance and management of Rett associated conditions. In regards to 
family member and prenatal testing, the evidence clinical utility is lacking, however there may 
be some benefit in terms of reproductive decision making.

PRACTICE GUIDELINE SUMMARY

No evidence-based clinical practice guidelines were identified which gave recommendations 
on when to perform CDKL5 or FOXG1 testing. However, studies have suggested that patients 
who are negative for MECP2 mutations and who have a strong clinical diagnosis of RTT 
should be considered for further screening of the CDKL5 gene if there are early-onset 
seizures, or the FOXG1 gene if there are congenital features (e.g., severe postnatal 
microcephaly).[1-3]

AMERICAN ACADEMY OF NEUROLOGY AND THE PRACTICE COMMITTEE OF THE 
CHILD NEUROLOGY SOCIETY[21]

In 2011, a quality standards subcommittee of the American Academy of Neurology 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. The report concluded, “Girls with severe impairment may be appropriate for testing for MECP2 mutations, regardless of whether the specific clinical features of Rett syndrome are present.”

**AMERICAN ACADEMY OF PEDIATRICS**[22,23]

In 2014 the American Academy of Pediatrics (AAP) reaffirmed earlier their recommendation for MECP2 testing to confirm a diagnosis of suspected Rett syndrome in females, especially when the diagnosis is unclear from symptoms alone.

**AMERICAN COLLEGE OF MEDICAL GENETICS**

In 2013, ACMG updated their guideline for the genetic evaluation of autism spectrum disorders. Testing for MECP2 mutations is recommended as part of the diagnostic workup of females who present with an autistic phenotype.[24] Routine MECP2 testing in males with autistic spectrum disorders is not recommended.

**SUMMARY**

There is enough research to show that genetic testing for variants in MECP2, FOXG1 and/or CDKL5 may be useful in confirming or excluding the diagnosis of Rett syndrome (RTT). Although there is no effective treatment for RTT, a definitive diagnosis can end a diagnostic workup for other possible diagnoses and may alter some aspects of management. Therefore, genetic testing of the MECP2, FOXG1 and/or CDKL5 genes for RTT may be considered medically necessary in select patients who meet the policy criteria.

There is enough research to show that genetic testing for Rett syndrome (RTT) variants in asymptomatic sisters and mothers of patients with RTT may help with reproductive decision-making. Therefore, targeted genetic testing of known familial RTT variants may be considered medically necessary for these individuals.

Current research shows that the risk of a family having a second female child with Rett syndrome (RTT) is less than one percent, except in the rare situation where the mother carries the mutation. Also, MECP2, FOXG1 and CDKL5 are the only genes that have been shown to cause RTT. Therefore, genetic testing for Rett syndrome is considered investigational for all other indications, including but not limited to prenatal screening and panel testing that includes genes other than MECP2, FOXG1 and/or CDKL5.

**REFERENCES**


21. Michelson, DJ, Shevell, MI, Sherr, EH, Moeschler, JB, Gropman, AL, Ashwal, S. Evidence report: Genetic and metabolic testing on children with global developmental


### CODES

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*Date of Origin: May 2010*