Genetic Testing for Rett Syndrome

Policy Number: AHS – M2088 – Genetic Testing for Rett Syndrome
Initial Presentation Date: 1/01/2020
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Policy Description

Rett syndrome (RTS) is a rare X-linked neurodevelopmental disorder usually caused by mutations in the Methyl CpG binding protein 2 (MECP2) gene (Amir et al., 1999). Affecting girls almost exclusively, it is characterized by normal early growth and development followed by regressions in development, walking, language, and purposeful use of the hands, along with slowed brain and head growth, distinctive hand movements, seizures, and intellectual disability (Colvin et al., 2004; Hagberg, Aicardi, Dias, & Ramos, 1983; Leonard, Cobb, & Downs, 2017; Naidu, Murphy, Moser, & Rett, 1986; Neul et al., 2010; Rett, 1966).

Related Policies

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Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request

1. Genetic testing for MECP2, CDKL5 and/or FOXG1 mutation on the X chromosome of a child with developmental delay/intellectual disability and signs/symptoms of Rett syndrome MEETS COVERAGE CRITERIA to confirm a diagnosis when there is uncertainty in the clinical diagnosis.

   The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient’s illness.

2. All other indications for mutation testing for Rett syndrome, including prenatal screening and testing of family members IS CONSIDERED INVESTIGATIONAL.

Scientific Background

Rett syndrome (RTS) is a severe neurodevelopmental disorder which affects approximately 1:10,000 live female births in the United States annually (Hagberg, 1985; NORD, 2019). It is a prominent cause of severe intellectual disability in women, accounting for up to 10% of cases inherited genetically (Armstrong, 1997). Originally thought to be lethal in males (Amir et al., 1999; Chahil, Yelam, & Bolli, 2018; Franco & Ballabio, 2006), RTS has been identified in up to 1.3% of male patients with mental retardation (Villard, 2007) and can be associated with a more severe phenotype (Q. Zhang et al., 2017). Reichow, George-Puskar, Lutz, Smith, and
Volkmar (2015) claim to have published the first review of male RTS data in 2015, and they were only identified a total of 57 published cases.

RTS can be inherited as an X-linked dominant disorder; however, more than 99% of cases result from a de novo pathogenic mutation in the Methyl CpG binding protein 2 (MECP2) gene (Amir et al., 1999; Christodoulou & Ho, 1993), a transcriptional regulator located on the X chromosome. More than 200 mutations in MECP2 have been associated with RTS (Suter, Treadwell-Deering, Zoghbi, Glaze, & Neul, 2014). Analysis of parental origin of the mutated MECP2 gene in sporadic cases of RTS showed that 94.4% of mutations were from paternal origin, 90.6% of which were point mutations; further, 5.6% of mutations were from maternal origin (X. Zhang et al., 2012). This may explain the high occurrence of RTS in female gender.

MECP2 is a multifunctional protein which interprets DNA methylation and regulates chromatin architecture, gene transcription, and RNA splicing (Sun et al., 2018). The complex upstream and downstream pathways of MECP2 involve microRNAs and neurotrophic factors, such as GABA and BDNF (Kang, Kim, Johnston, & Kadam, 2014). Transcriptome level analysis in tissues derived from RTS patients report dysregulations in dendritic connectivity and synapse maturation, mitochondrial dysfunction, and glial cell activity (Shovlin & Tropea, 2018).

Researchers have recently identified two individuals with an RTS diagnosis who lacked a mutation in the MECP2 gene but had a mutation in other genes previously unassociated with RTS: CTNNB1 and WDR45 (Percy et al., 2018).

MECP2 is critical for neuronal maturation (Fukuda, Itoh, Ichikawa, Washiyama, & Goto, 2005; Smrt et al., 2007), and its deficiency results in impaired dendritic morphogenesis and reduced dendritic spine numbers (Chapleau et al., 2009; Kishi & Macklis, 2010). This results in dysfunctional synaptic transmission and neural network activity (Sun et al., 2018), affecting successive stages of brain development, including prenatal neurogenesis, postnatal development of synaptic connections and function, experience-dependent synaptic plasticity, and maintenance of adult neural function, including sensory integration (Feldman, Banerjee, & Sur, 2016).

The clinical picture of RTS is characterized by a broad clinical spectrum of signs and symptoms (Pini et al., 2016) and a distinctive course of apparent normal development for the first 6 to 18 months of life, followed by characteristic developmental stagnation and loss of acquired skills, including loss of intellectual functioning, loss of acquired fine and gross motor skills and communication (Colvin et al., 2004; Dolce, Ben-Zeev, Naidu, & Kossoff, 2013; Hagberg et al., 1983; Leonard et al., 2017; Naidu et al., 1986; Neul et al., 2010; Rett, 1966). Purposeful use of the hands is often replaced by repetitive stereotypical hand movements (Dy et al., 2017; Elian & de, 1996; Goldman & Temudo, 2012). Other clinical observations include deceleration of head growth, seizures, disturbed breathing patterns, scoliosis, growth retardation, and gait apraxia (Cianfaglione et al., 2015).

Despite this period of apparently normal early development, these profound neurological regressions have been found to result from MECP2-related defects in the establishment and refinement of early neural circuits and, later, cortical plasticity (Feldman et al., 2016). Subtle signs, such as hypotonia, jerkiness in limb movement, and limited social interaction, can be present during early infancy (Ip, Mellios, & Sur, 2018).

The severity and rate of progression of this disease can vary greatly with several recognized atypical variants. The milder forms (Zappella) present with less severe regression and milder expression of the clinical characteristics of RTS. In the most severe forms, there is no normal development period (Neul et al., 2010). Both genetic and clinical variants of RTS are associated with distinct electrophysiological profiles reflecting how genetic dysregulation of synapse formation results in differences in neuronal network architecture (Sun et al., 2018) and varying clinical phenotypes (Keogh et al., 2018). The pattern of X-chromosome inactivation can also influence the severity of the clinical disease (Archer et al., 2007; Weaving et al., 2003).

Mutations in the upstream cyclin-dependent kinase-like 5 (CDKL5) gene cause an early seizure (Hanefield) variant of the RTS phenotype (Bahi-Buisson et al., 2008), and mutations in the
forkhead box G1 (FOXG1) gene have been found in the congenital variant (Rolando) (Ariani et al., 2008). Two cases of females with pathogenic de novo mutations in SCN1A, which usually leads to Dravet syndrome, but fulfill the diagnostic criteria for classic RTS have also been reported (Henriksen, Ravn, Paus, von Tetzchner, & Skjeldal, 2018). In males, MECP2 duplication phenotypically presents with infantile hypotonia, recurrent respiratory infections, and severe mental retardation (Villard, 2007).

Clinical Validity

Lallar et al. (2018) used Sanger sequencing to diagnose suspected RTS cases; participants were divided into two groups. Group 1 was comprised of girls with symptoms of classical and atypical RTS, and Group 2 was comprised of girls with other “Rett like features” that did not fit into the first category. MECP2 mutations were identified in 74% of girls in Group 1 and in 0% of girls in Group 2; girls in Group 1 with classical RTS had a mutation detection rate of 93% (Lallar et al., 2018). This shows that Sanger sequencing is efficient in detecting RTS in patients with the classical form of the disease.

Recently, brain-enriched microRNAs (miRNAs) were utilized to identify miRNA biomarkers of RTS; for this study, 30 patients with RTS were matched with 30 healthy controls of similar age (Sheinerman, Djukic, Tsivinsky, & Umansky, 2019). Results showed that miRNAs identified RTS patients with 85-100% sensitivity when compared to controls; further, the researchers determined that “the dynamics in levels of miRNAs appear to be associated with disease development (involvement of liver, muscle and lipid metabolism in the pathology)” (Sheinerman et al., 2019). These results may suggest that circulating miRNAs could be used to measure RTS disease progression or individual response to treatment.

Clinical Utility

Confirmation of the genetic diagnosis can improve the medical management of the patient, end the diagnostic odyssey, provide a general idea of prognosis for the patient, and/or provide closure to the family (Mroch, Flanagan, & Stein, 2012). Complex neurodevelopmental disorders need multi-disciplinary treatment approaches for optimal care. The clinical effectiveness of treatments is limited in patients with rare genetic syndromes and multisystem morbidity such as RTS; single drug strategies may not be sufficient due to the multiple overlapping physiological systems affected (Singh & Santosh, 2018).

Functional performance for self-care, upper extremity function, and mobility in RTS patients may relate to the type of mutation. Knowledge of these relationships is useful for developing appropriate rehabilitation strategies and prognosis (Pidcock et al., 2016).

Of the clinical criteria for RTS, loss of hand skills was the most significant clinical predictor of a positive genetic test for mutations of a MECP gene in girls. Gait abnormalities and stereotypic hand movements were also strong predictors of a positive genetic test for mutations of MECP. Language delay is the least specific of the major criteria (Knight, Horn, Gilbert, & Standridge, 2016). A reliable and single multidimensional questionnaire, the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire, is being developed to combine physiological aspects of the disease obtained using wearable sensor technology, along with genetic and psychosocial data to stratify patients and streamline the care pathway (Santosh, Lievesley, Fiori, & Singh, 2017).

In at least 95% of Rett syndrome cases, the cause is a de novo mutation in the child; MECP2 variants are rarely inherited from a carrier mother with a germline mutation in MECP2, in whom favorable skewing of X-chromosome inactivation results in minimal to no clinical findings. When the mother is a known carrier, inheritance follows an X-linked dominant pattern with a 50% risk to her offspring of inheriting the MECP2 variant (Christodoulou & Ho, 1993).

A mutation in MECP2 does not necessarily equate to a clinical diagnosis of RTS. MECP2 mutations have also been reported in other clinical phenotypes, including individuals
with an Angelman-like picture, nonsyndromic X-linked intellectual disability, autism, in males as PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders, parkinsonism, and intellectual disability), and most commonly as neonatal encephalopathy (Liyanage & Rastegar, 2014; Suter et al., 2014; Williamson & Christodoulou, 2006).

Recent expert opinion in the UK concluded that genetic testing for all children with unexplained global developmental delay (GDD) should be first-line if an exogenous cause is not already established. All patients, irrespective of severity of GDD, should have investigations for treatable conditions. The yield for treatable conditions is higher than previously thought and that investigations for these conditions should be considered as first-line. Additional second-line investigations can be led by history, examination, and developmental trajectories (Mithyantha, Kneen, McCann, & Gladstone, 2017).

Vidal et al. (2017) have utilized next generation sequencing (NGS) in a total of 1577 patients with RTS-like clinical diagnoses or patients with potential RTS genetic mutations as determined previously by Sanger Sequencing. Of the 1577 patients with RTS-like clinical diagnoses, the NGS method was able to confirm the RTS diagnosis in 477 patients (about 30%). Further, “Positive results were found in 30% by Sanger sequencing, 23% with a custom panel, 24% with a commercial panel and 32% with whole exome sequencing,” suggesting that NGS is a competitive diagnostic RTS tool compared to the aforementioned methods (Vidal et al., 2017).

Vidal et al. (2019) used multiplex ligation-dependent probe amplification (MLPA) in the MECP2 gene of 21 RTS patients to identify deletions of varying sizes; these researchers identified both total or partial deletions of the MECP2 gene in each patient, with identified partial deletions ranging from 1,235 bp to 85 kb. Breakpoints were delineated by DNA-qPCR; the results have allowed the researchers to “propose a genotype–phenotype correlation” which will assist in appropriate genetic counseling (Vidal et al., 2019).

Guidelines and Recommendations

**Practice Guidelines and Position Statements from the American Academy of Neurology (AAN) and Child Neurology Society (CNS) (Michelson et al., 2011)**

In 2011, a quality standards subcommittee of the AAN and the Practice Committee of the CNS issued an evidence report on the genetic and metabolic testing of children with global developmental delay. AAN recommended considering MECP2 mutation testing for all girls with unexplained moderate to severe developmental delay. Males with a history strongly suggestive of X-linked inheritance may be considered for testing of one or more individual X-linked intellectual disability (XLID) genes or for screening of the entire X chromosome (Michelson et al., 2011).

**Canadian Pediatric Society (CPS) (Belanger & Caron, 2018)**

The CPS supports the guidelines mentioned above by the AAN and CNS. The CPS stated that “According to the AAP and the AAN, MECP2 molecular analysis should be ordered when characteristic symptomatology is present (i.e., initially normal development followed by loss of speech and purposeful hand use, stereotypical hand movement, gait abnormalities) or for moderately-to-severely affected girls (Belanger & Caron, 2018).”

**American Academy of Pediatrics (AAP) (Moeschler & Shevell, 2014)**

A 2014 policy statement from the AAP recommends MECP2 mutation analysis for girls with microcephaly or deceleration of head growth and other features of Rett syndrome, or who present with stereotypical hand-wringing movements and developmental regression. MECP2 gene mutations are extremely rare in males but may be considered in boys who present with
clinical features of Rett syndrome or severe developmental regression (Moeschler & Shevell, 2014).

Complete MECP2 deletion, duplication, and sequencing study is also recommended for females with intellectual disability or global developmental delay for whom the chromosomal microarray, specific metabolic testing, and fragile X genetic testing did not produce a diagnosis (Moeschler & Shevell, 2014).

**RettSearch (Neul et al., 2010)**

Neither AAN nor AAP have provided recommendations on when to use CDKL5 or FOXG1 testing. RettSearch members, representing the majority of the international clinical RTS specialists, “participated in an iterative process to come to a consensus on a revised and simplified clinical diagnostic criteria for RTS” (Neul et al., 2010). This group provided clarifications for diagnosis of classic or typical RTS and atypical RTS and provided guidelines for molecular evaluation of specific variant forms of RTS. The authors define RTS as a clinical diagnosis based on distinct clinical criteria, independent of molecular findings. Presence of a MECP2 mutation is not sufficient for the diagnosis of RTS. Neul et al. (2010) proposed three distinct criteria for diagnosis of variant forms of RTS: preserved speech variant (Zapella variant), early seizure variant (Hanefeld variant) and congenital variant (Rolando variant); identifying the molecular genetics of each variant was also recommended. In the Zapella variant, the molecular analysis for MECP2 was recommended. In Hanefeld and Rolando variants, recommended mutations for analysis were in the CDKL5 and FOXG1 genes respectively. Further, it was stated that patients found negative for MECP2 mutations and who have a strong clinical diagnosis of RTS should be considered for further screening for the CDKL5 gene if early onset seizures or FOXG1 gene congenital features (e.g., severe postnatal microcephaly) are present.

**American College of Medical Genetics (ACMG) (Schaefer & Mendelsohn, 2013)**

In 2013, the SCMG revised its evidence-based guidelines for clinical genetics 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. Routine MECP2 testing in males with autistic spectrum disorders is not recommended. However, when features of MECP2 duplications (e.g., drooling, recurrent respiratory infections, hypotonic facies) are present, MECP2 duplication testing in boys with autism and such features may be considered (Schaefer & Mendelsohn, 2013).

**State and Federal Regulations, as applicable**

A search of the FDA database on 11/04/2019 using the term “genotyping” yielded 23 results. Additional tests may be considered laboratory developed tests (LDTs); developed, validated and performed by individual laboratories. LDTs are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA’88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

**Applicable CPT/HCPCS Procedure Codes**

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<td>81304</td>
<td>MECP2 (methyl cpg binding protein 2) (eg, rett syndrome) gene analysis; duplication/deletion variants</td>
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| 81404       | Molecular pathology procedure, Level 5  
Gene: **FOXG1** (forkhead box G1) (e.g., Rett syndrome), full gene sequence |
| 81405       | Molecular pathology procedure, Level 6  
Gene: **CDKL5** full gene sequence (cyclin-dependent kinase-like 5) |
| 81406       | Molecular pathology procedure, Level 7  
Gene: **CDKL5** (cyclin-dependent kinase-like 5) (e.g., epileptic encephalopathy), full gene sequence |


Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

**Evidence-based Scientific References**


**Policy Implementation/Update Information**

1/1/20 New Policy
3/1/20 Updated background, guidelines, and evidence-based scientific references. Included the statement regarding lack of published literature for the old E&I CC. The literature review did not necessitate any other modifications to the CCs.

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