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Corporate Medical Policy

Genetic Testing for Rett Syndrome AHS – M2088

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Description of Procedure or Service

Description

Rett syndrome (RTS) is a rare X-linked neurodevelopmental disorder that occurs almost exclusively in girls by mutations in the Methyl CpG binding protein 2 (*MECP2*) gene (Amir et al., 1999). 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
Testing for Autism Spectrum Disorder and Developmental Delay AHS – M2176

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for genetic testing for Rett syndrome when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Genetic Testing for Rett Syndrome is covered

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 is covered to confirm a diagnosis when there is uncertainty in the clinical diagnosis.

When Genetic Testing for Rett Syndrome is not covered

All other indications for mutations testing for Rett syndrome, including prenatal screening and testing of family members are not covered.

Policy Guidelines

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, & Bollu, 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). These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the *MECP2* variant. Reichow, George-Puskar, Lutz, Smith, and Volkmar (2015) claim to have published the first review of male RTS data in 2015, and they 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).

Fu et al, 2020 published a set of "consensus guidelines" with input from several clinical sites, Rett Syndrome-focused centers, two patient advocacy groups, and Rett Syndrome clinical specialists. Although this guideline focuses on "management" of Rett Syndrome, the guideline does comment on the genetics of Rett Syndrome. The guideline remarks that "nearly" all individuals with Rett Syndrome (RTT) have a loss-of-function mutation on the *MECP2* and that these mutations are "almost always" *de novo* (and thereby not expected to recur in families). Two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT, although the guideline does not note any specific treatments based on type of mutation. However, the guideline states that "Alterations in *MECP2*, *CDKL5* and *FOXG1* should be considered in all individuals, male and female, with developmental delays and intellectual disability (Fu et al., 2020)."

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).

Seventy-two classical Rett syndrome (RTT) female patients were included in a cohort study by Khajuria et al. (2020) to analyze exons 2-4 of *MECP2* gene by Sanger sequencing for sequence

variations followed by deletion/duplication analysis using Multiplex Ligation-dependent Probe Amplification (MLPA). Patients were defined as classical when they showed signs of partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language, gait abnormalities, impaired or absence of ability to walk, and stereotypic hand movements. Through Sanger Sequencing, *MECP2* sequence variations where identified in 90.3% of patients. With further evaluation using MLPA, large deletions of *MECP2* were identified in 9.7% of the patients, which were negative on DNA sequencing. MLPA analysis increased the detection rate of *MECP2* sequence variants identified in patients from 90.3% to 98.6%. The authors emphasize that "MLPA analysis of *MECP2* is crucial and needs to be performed in classical RTT patients. Large deletions can be missed using DNA sequencing and reaffirms the view that large *MECP2* deletions are an important cause of classical RTT (Khajuria et al., 2020)."

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).

This report was reaffirmed on August 9, 2014 (AAN, 2014).

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) (Hyman, Levy, & Myers, 2020; 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).

The above guideline was reaffirmed in 2019 (AAP, 2019).

The AAP also published a guideline focusing on children with autism spectrum disorder (ASD). In it, they note that other disorders may meet certain criteria for ASD. However, the AAP notes that these disorders should prompt the "appropriate targeted testing" (or referral to a specialist). The AAP lists an example of Rett Syndrome, stating that "for example, a girl with significant

developmental delays, deceleration in head growth velocity, and characteristic midline hand movements should prompt genetic testing for a mutation or deletion or duplication of *MECP2,* the gene implicated in Rett syndrome". In Supplemental Table 13, they list the following findings as representative of Rett Syndrome: "Deceleration of head growth velocity, acquired microcephaly, loss of purposeful hand use, prominent hand stereotypies (especially hand wringing or clasping), apraxia, hyperventilation or breath-holding, seizures" (Hyman et al., 2020).

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).

Applicable Federal Regulations

A search of the FDA database on 11/08/2020 using the term "genotyping" yielded 24 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.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 81302, 81303, 81304, 81404, 81405, 81406, 0234U

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

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Policy Implementation/Update Information

1/1/2019	BCBSNC will provide coverage for genetic testing for Rett syndrome when it is determined to be medically necessary because criteria and guidelines are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (jd)
4/1/2019	Description section, policy guidelines and references updated. Medical Director review 4/2019. (jd)
10/29/19	Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (hb)
2/11/20	Annual review by Avalon 4th Quarter 2019 CAB. No revisions and no change to policy intent. Medical Director review 12/2019. (jd)
3/31/20	Specialty Matched Consultant Advisory Panel review 3/2020. Medical Director review 3/2020. (jd)
2/9/21	Annual review by Avalon 4th Quarter 2020 CAB. Related Policies added to description section. Policy guidelines and references updated. Added PLA code 0234U to the Billing/Coding section, effective 4/1/21. Medical Director review 1/2021. (jd)

3/31/21	Specialty Matched Consultant Advisory Panel review 3/2021. Medical Director review 3/2021. (jd)