Genetic Testing for Rett Syndrome

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I. Policy Description

Rett syndrome (RTS) is a rare X-linked neurodevelopmental disorder that occurs almost exclusively in girls usually caused by mutations in the Methyl CpG binding Protein 2 (*MECP2*) gene. 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.

II. 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* (Cyclin-Dependent Kinase-Like 5) and/or *FOXG1* (Forkhead Box G1) 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, **DO NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Rett syndrome (RTS) is a severe neurodevelopmental disorder which affects approximately 1:10,000 live female births in the United States annually. It is a prominent cause of severe intellectual disability in women, accounting for up to 10% of cases inherited genetically. Originally thought to be lethal in males, RTS has been identified in up to 1.3% of male patients with mental retardation and can be associated with a more severe phenotype. These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the *MECP2* variant. Reichow et al. (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, a transcriptional regulator located on the X chromosome. More than 200 mutations in *MECP2* have been associated with RTS. 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. 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. The complex upstream and downstream pathways of *MECP2* involve microRNAs and neurotrophic factors, such as GABA and BDNF. Transcriptome level analysis in tissues derived from RTS patients report dysregulations in dendritic connectivity and synapse maturation, mitochondrial dysfunction, and glial cell activity. 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*.

MECP2 is critical for neuronal maturation, and its deficiency results in impaired dendritic morphogenesis and reduced dendritic spine numbers. This results in dysfunctional synaptic transmission and neural network activity, 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.

The clinical picture of RTS is characterized by a broad clinical spectrum of signs and symptoms 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. Purposeful use of the hands is often replaced by repetitive stereotypical hand movements. Other clinical observations include deceleration of head growth, seizures, disturbed breathing patterns, scoliosis, growth retardation, and gait apraxia.

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. Subtle signs, such as hypotonia, jerkiness in limb movement, and limited social interaction, can be present during early infancy.

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. 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 and varying clinical phenotypes. The pattern of X-chromosome inactivation can also influence the severity of the clinical disease.

Mutations in the upstream cyclin-dependent kinase-like 5 (*CDKL5*) gene cause an early seizure (Hanefield) variant of the RTS phenotype, and mutations in the forkhead box G1 (*FOXG1*) gene have been found in the congenital variant (Rolando). 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. In males, *MECP2* duplication phenotypically presents with infantile hypotonia, recurrent respiratory infections, and severe mental retardation.

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 Theguideline does not note any specific treatments based on type of mutation,

though two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT. 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." This consensus guideline also notes there is hope for disease-modifying therapy as reversing symptoms in mice has occurred in a clinical research context. Banerjee et al. (2019) published a paper titled "Towards a Better Diagnosis and Treatment of Rett Syndrome: A Model" summarizing the developments in the diagnosis and treatment of Rett syndrome over the past 50 years. They note that the first gene therapy trial for *MECP2* "was modelled after the successful (i.e. improved survival and motor functions) single dose intravenous adeno-associated virus serotype 9 delivery of complementary DNA." Even with promising gene therapy techniques, the authors note that the field has challenges. "The Rett syndrome field is experiencing the same challenges as other neurodevelopmental disorders pursuing neurobiologically based treatments: inadequate outcomes and measures of response." The authors also comment on the complexity of the *MECP2* mutation's role in the syndrome, "*MECP2* mutations is supportive, but not confirmatory because of the limited genotype-phenotype correlations in Rett syndrome."

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%. This shows that Sanger sequencing is efficient in detecting RTS in patients with the classical form of the disease.

Recently, Sheinerman et al. (2019) used brain-enriched microRNAs (miRNAs) to identify miRNA biomarkers of RTS; for this study, 30 patients with RTS were matched with 30 healthy controls of similar age. 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)." 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. It can also end the diagnostic odyssey, provide a general idea of prognosis for the patient, and/or provide closure to the family. 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.

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.

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. 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.

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.

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.

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.

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. (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.

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 were 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.” Xiol et al. (2021)

performed a clinical review of technological advances in RTT genetics. The authors review summarizes that our understanding of Rett syndrome has evolved "towards a spectrum of overlapping phenotypes with great genetic heterogeneity." The authors note that advances in genetic diagnosis have been greatly impacted by the rise in next generation sequencing (NGS) and whole genome sequencing. Of particular note are the "90 causative genes" and "significantly overlapping phenotypes" involved in RTT spectrum disorders. To achieve an accurate and quick diagnosis of Rett syndrome, the authors strongly recommend simultaneous multiple gene testing and thorough phenotypic characterization.Bassuk (2021) published a paper concerning methyl-CpG-binding protein 2 (*MECP2*) and its encoding of an epigenetic reader, MeCP2. The author notes that loss of function of the epigenetic reader may be a factor in RTT, but also that "locus duplications also cause a severe neurodevelopmental disorder, *MECP2* duplication syndrome (MDS)." This suggests that MeCP2 (the protein) could be what is called a "Goldilocks protein," that is, one that requires an activity level that is precise. Using the re-expression of the MeCP2 protein in mouse models, the author presents a case for the development of therapeutic interventions in people and the restoration of the desirable phenotypes. However, gene therapy must be approached with caution, as restoring function to the protein still carries the risk of "MDS overexpression phenotypes."

IV. Guidelines and Recommendations

Practice Guidelines and Position Statements from the American Academy of Neurology (AAN) and Child Neurology Society (CNS)

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.

This report was reaffirmed on August 9, 2014.

Canadian Pediatric Society (CPS)

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."

American Academy of Pediatrics (AAP)

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.

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.

The above guideline was reaffirmed in 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."

RettSearch

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." 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)

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.

V. State and Federal Regulations, as applicable

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coveragedatabase/overview-and-quick-search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

A search of the FDA database on 12/14/2021 using the term "genotyping" yielded 49 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.

VI. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4), or for QUEST Integration members under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status. HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

VII. Evidence-based Scientific References

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VIII. Policy History

Policy approved by Medical Directors	9/20/2022
Policy approved at UMC	12/16/2022
Policy effective	6/1/2023

Updated Lines of Business	12/18/2023