



### Disease Information

*MECP2* mutations have been identified in patients with Rett syndrome, autism, X-linked mental retardation, females with mild learning disabilities, and males with neonatal encephalopathy.<sup>1</sup> Rett syndrome is an X-link dominant condition that is a severe, progressive neurologic disorder, and is the only known Autism Spectrum disorder with a known cause. It is characterized by normal birth development followed by rapid regression in intelligence, language and motor skills, autistic features, and development of stereotypic hand movements. There may also be seizures, hyperventilation, apnea, scoliosis, growth retardation and gait dyspraxia.<sup>2</sup> The prevalence of Rett syndrome in females is estimated to be 1:8,000,<sup>3</sup> but the prevalence of all *MECP2*-related disorders is unknown. It is possible for males to have Rett syndrome, but most have such severe encephalopathy that they do not live past infancy.<sup>1</sup> However, other *MECP2* mutations may account for 1.3-1.7% of mental retardation in male patients.<sup>4</sup> In addition, duplications in *MECP2* can cause infantile hypotonia, seizures, mental retardation, absence of speech, spasticity and recurrent respiratory infections in boys, which is also known as *MECP2* Duplication Syndrome.<sup>5</sup>

The *MECP2* gene encodes the MeCP2 protein which is thought to be involved in neuronal development and differentiation,<sup>6</sup> gene expression through CpG binding, and as a transcriptional repressor.<sup>7</sup> Variability in the phenotype is often seen due to the type of mutation and X-chromosome inactivation.<sup>1</sup> The majority of *MECP2*-related disorders result from a de novo mutation, though inheritance of the disease mutation can occur due to an affected mother with mild symptoms caused by favorably skewed X-inactivation, or a parent with a germ-line mosaicism.<sup>8</sup> *MECP2* analysis can detect mutations in 96% of classic Rett syndrome patients and 58 - 75% of atypical cases<sup>9,10</sup> with deletions accounting for 11.9% of mutations.<sup>11</sup> The lack of *MECP2* mutations in some clinically defined Rett syndrome cases suggests genetic heterogeneity, with the existence of at least one other locus, *CDKL5*. Rett syndrome, *MECP2* negative Rett, and autism spectrum disorder cases with seizures.<sup>12</sup> Out of the population with atypical Rett who do not have an *MECP2* mutation, approximately 12.9% will have a mutation in the *CDKL5* gene.<sup>16</sup>

For information about *CDKL5*-Related Infantile Spasms, please see our separate test information sheet and webpage.

### Testing Benefits & Indications

Testing is recommended for those with cognitive impairment<sup>13</sup> or symptoms of *MECP2*-related disorders. Because germ-line mosaicism cannot be excluded, prenatal diagnosis should be offered to those with a family history of a *MECP2*-related disorder regardless of whether the mutation has been found in a parent.<sup>9</sup>

Testing for *MECP2* mutations has also been recommended by the American College of Medical Genetics as part of their diagnostic evaluation for Autism Spectrum Disorder.<sup>14</sup>

### Testing Descriptions

The Ambry SEQUENCE: Rett Syndrome is our most comprehensive test for typical and atypical Rett. This is a three-step reflex testing pathway to detect, in order, *MECP2* sequence variants, *MECP2* deletions and duplications, and lastly, *CDKL5* sequence variants. If a causative mutation is detected in steps one or two of the SEQUENCE, the remaining steps are cancelled to avoid unnecessary testing and cost.

*MECP2* AMPLIFIED is also a stepwise analysis for *MECP2* sequence variants with reflex to *MECP2* deletion/duplication analysis. *CDKL5* analysis is not part of the testing pathway in *MECP2* AMPLIFIED.

## The Ambry Tests®: Rett Syndrome

Gene sequence analysis for *MECP2* or *CDKL5* and deletion/duplication analysis of *MECP2* may also be ordered separately or in any combination. *MECP2* deletion/duplication testing can be ordered alone for diagnosis of *MECP2* Duplication Syndrome. Specific mutation analysis for individual mutations known to be in the family is also available.

In this AMBRY SEQUENCE, full gene sequence analysis of *MECP2* is performed by PCR-based double-stranded automated sequencing in the sense and antisense directions for exons 1-4 of the *MECP2* gene, plus at least 20 bases into the 5' and 3' ends of all the introns. Gross deletion/duplication analysis of *MECP2* is performed by MLPA®. Gene sequence analysis of *CDKL5* is performed by PCR-based double-stranded automated sequencing in the sense and antisense directions for exons 2-21, plus at least 20 bases in at least one direction into the 5' and 3' ends of all the introns.

### Mutation Detection Rate

As described above, the combination of *MECP2* gene sequence analysis and deletion/duplication testing identifies mutations in approximately 95% of classic Rett cases<sup>14</sup> and 58-75% of atypical cases<sup>9,10</sup>. Another 12.9% of patients with atypical Rett and negative *MECP2* analysis are positive for *CDKL5* sequence mutations (clinical sensitivities).<sup>16</sup> The Ambry SEQUENCE: Rett Syndrome identifies approximately 99% of described *MECP2* and *CDKL5* mutations (analytic sensitivity).

### Turn-Around-Time

Ambry SEQUENCE: Rett Syndrome .....	14 – 49 days
MECP2 AMPLIFIED .....	21 - 35 days
MECP2 Gene Sequence Analysis .....	14 - 21 days
MECP2 Del/Dup .....	7 - 14 days
CKDL5 Gene Sequence Analysis .....	14 – 28 days
Specific mutation analysis .....	10 – 14 days

### Specimen Requirements

**Blood:** Collect 3-5 cc from adult or 2 cc minimum from child into EDTA purple-top tube (first choice) or ACD yellow-top tube (second choice). Store at room temperature or refrigerate. Ship at room temperature.

**Blood Spot:** Call for availability.

**Saliva:** Collect 2 ml into Oragene™ DNA Self-Collection container. Store and ship at room temperature.

**DNA:** Send 20 µg in TE at 50-100 ng/µl. Store frozen and ship on ice or dry ice.

**Prenatal:** Prenatal testing is available. Please call an Ambry Genetic Counselor to discuss your case.

### CPT Codes

SEQUENCE: Rett Syndrome or MECP2 AMPLIFIED .....	83891, 83894, 83898, 83900, 83901, 83904, 83909, 83912
Gene sequence or specific mutation analysis .....	83891, 83894, 83898, 83904, 83909, 83912
Deletion/duplication analysis .....	83891, 83894, 83900, 83901, 83909, 83912

### References

- Moretti P& Zoghbi H. *Curr Opin Genet Dev.* 2006;16:276-281.
- Kerr AM, Nomura Y, Armstrong D, et al. *Brain Dev* 2001;23:208-11.
- Laurvick CL, de Klerk N, Bower C, et al. *J Pediatr.* 2006; 148: 347-52.
- Villard L. *J Med Genet.* 2007;44(7):417-423.
- Van Esch H et al. *Am J Hum Genet.* 2005;77:442-453
- Setoguchi H, Namihira M, Kohyama J, et al. *J Neurosci Res.* 2006;84(5):969-979.
- Galvao TC & Thomas JO. *Nucleic Acids Res.* 2005;33(20):6603-6609.
- Mari F, Caselli R, Russo S, et al. *Clin Genet.* 2005;67(3):258-60.
- Shahbazian MD, Zoghbi HY. *Curr Opin Neurol.* 2001;14:171-176.
- Percy AK, Lane JB, Childers J, et al. *J Child Neurol.* 2007;22(12):1338-41.
- Archer HL, Whatley SD, Evans JC, et al. *J Med Genet.* 2006;43(5):451-6.
- Weaving LS, Ellaway CJ, Gecz J, Christodoulou J. *J Med Genet.* 2005;42:1-7.
- Percy AK. *J Child Neurol.* 2008;23:543-549.
- Schaefer GB et al. *Genet Med.* 2008;10(4):301-305.
- Schollen E, Smeets E, Deflem E, et al. *Hum Mut.* 2003;22:116-20.
- Bahi-Buisson N, Nectoux, J, Rosas-Vargas, H, et al. *Brain.* 2008; 131, 2647-2661.