

Congenital Hypotonia Evaluation

Panel Gene List: *SMN1*, *SMN2*, *DMPK*, 15q11.2-q13.1

Clinical Features:

Hypotonia is a general term that refers to reduced resistance to passive movement or low muscle tone, not to be confused with muscle weakness, which is defined as reduced muscle strength and power.¹ Hypotonia can be used to describe muscle groups throughout the body and is a feature of hundreds of neonatal disorders and diseases.^{2,3} Central hypotonia is due to defects in the upper motor neuron, whereas peripheral hypotonia is due to defects of the lower motor neuron, neuromuscular junction, or the skeletal muscle.² Central hypotonia is commonly associated with disorders of the CNS and presents with normal serum creatine kinase, hyperreflexia, cognitive delay, and/or seizures.² Infants with peripheral hypotonia often have elevated serum creatine kinase, significant muscle weakness, absent anti-gravity movements, head lag, and joint contractures.³ A large number of the disorders that present congenitally with hypotonia have a genetic etiology that can guide future testing, treatment, and medical management.¹⁻³ The disorders evaluated by this test have high incidences and contribute significantly to the number of infants with congenital hypotonia.^{1,2}

SMN1: Spinal muscular atrophies (SMA) result from the degeneration of the anterior horn cells in the spinal cord and brain stem nuclei, leading to progressive muscle weakness and wasting.⁴ Individuals typically present with symmetric proximal extremity weakness that may progress to distal, axial, intercostal, and bulbar muscles; however age-of-onset and disease severity vary.⁵ *SMN1*-related SMA accounts for 95% of cases of SMA.⁶ Individuals with *SMN1*-related SMA present with features that fall along a clinical spectrum and are categorized into 5 subtypes. Types 0, 1, and 2 are the most severe and are characterized by neonatal hypotonia, severe weakness, including facial weakness, joint contractures, swallow difficulties and often early respiratory failure.⁴ Subtype is influenced by *SMN2* copy number and individuals with a greater number of *SMN2* copies typically have milder disease.⁴

DMPK: Myotonic dystrophies are multisystem disorders that affect both smooth and skeletal muscle as well as the central nervous and endocrine systems. Myotonic dystrophy type 1 (DM1) presents with features that fall along a spectrum. The most severe form of the disease presents prenatally with polyhydramnios and reduced fetal movement or postnatally with severe muscle weakness, hypotonia, feeding difficulties, respiratory insufficiency, and intellectual disability.⁷

15q11.2-q13.1: Prader-Willi syndrome (PWS) is characterized by central hypotonia and feeding difficulties during infancy followed by excessive eating, rapid weight gain, and central

obesity in early childhood. Children with PWS exhibit characteristic facial features including bitemporal narrowing, almond-shaped palpebral fissures, and a down-turned mouth. Additional diagnostic characteristics of PWS include global developmental delay, mild to moderate intellectual disability, distinctive behaviors, short stature, hypopigmentation relative to other family members, and small hands and feet.^{8,9}

Inheritance Pattern/Genetics:

SMN1: Individuals with *SMN1*-related SMA most commonly harbor a homozygous loss of exon 8 (aka exon 7), although 5% of *SMN1*-related SMA is due to the presence of a pathogenic sequencing-based change on one allele and loss of exon 8 on the other allele.¹⁰ Loss of exon 8 occurs as a result of either a deletion including at least exon 8 of the *SMN1* gene or a conversion of the exon 8 *SMN1* sequence into the *SMN2* sequence.⁶ Subtype is influenced by *SMN2* copy number and individuals with a greater number of *SMN2* copies typically have milder disease.⁴

DMPK: DM1 is an autosomal dominant disorder caused by the expansion of a CTG trinucleotide repeat in the 3'UTR of the *DMPK* gene.¹¹ Normal alleles have 5-34 repeats, premutation (mutable normal) alleles have 35-49 repeats, and disease alleles have 50 or more repeats.¹² Linear correlation between repeat number and age-of-onset/disease severity has only been observed for expansions of <250 repeats and larger repeat expansions do not appear to have the same linear correlation.¹³⁻¹⁵ Although anticipation is well documented for DM1, several studies have identified individuals with >1000 repeats that do not have congenital DM1.¹⁶⁻¹⁸ Therefore, a repeat number of 50 or more is sufficient to make a diagnosis of DM1 and repeat number alone should not be used for prognosis.¹⁴ Clinical correlation is necessary to determine if the finding is consistent with a specific DM1 subtype.

15q11.2-q13.1: The most common cause of PWS (70%-75% of patients) is a recurrent deletion extending from 15q11.2 to 15q13.1 on the paternally inherited chromosome. Maternal uniparental disomy accounts for 25-30% of PWS cases, while approximately 1-3% of patients have an imprinting error that establishes a maternal chromosome-specific methylation pattern on both the maternally and paternally inherited chromosomes.^{8,9} Imprinting errors leading to abnormal methylation can be caused by a microdeletion within the imprinting center in 15q11.2 (8-15% of patients with an imprinting defect) or by an unknown mechanism that inappropriately silences genes regardless of the parental origin of the chromosome.¹⁹ The majority of cases are de novo with a recurrence risk of <1%; however, the recurrence is 50% for an inherited imprinting center deletion.

Test Methods:

Using genomic DNA from the submitted specimen, multiplex ligation-dependent probe amplification (MLPA) is completed to determine the copy number of the *SMN1* and *SMN2*

genes in the provided specimen, compared to control specimens. Methylation sensitive MLPA (MS-MLPA), is used to identify deletions and establish the methylation status of the Prader-Willi critical region on chromosome 15. Although this analysis will identify abnormal methylation patterns it cannot distinguish between maternal UPD and imprinting center defects and imprinting center sequence analysis is not completed as part of this test.

Standard PCR fragment analysis is performed to identify alleles with 100 or fewer repeats and the Asuragen AmplideX PCR/CE *DMPK* kit is used to identify alleles with >100 repeats, as well as determine the number of repeats in alleles with fewer than 200 repeats. Nucleotide repeat numbers up to 50 are reported with an accuracy of +/- 2 repeats and repeat numbers from 50-200 are reported with an accuracy of +/- 5 repeats. Internal standards were analyzed along with clinical samples to evaluate assay performance. The exact number of repeats cannot be determined for alleles with greater than 200 repeats. Southern blot analysis is required to determine the number of repeats in alleles larger than this and is not completed as part of this test. If desired, Southern blot can be ordered from GeneDx, however it is not available for samples from New York State.

Test Sensitivity:

The clinical sensitivity of the genes included in this panel depends in part on the patient's clinical phenotype. Published cohort studies have shown that *SMN1*-related SMA, Congenital DM1, and Prader-Willi syndrome contribute 2%, 4% and 5% of hypotonic infants, respectively.¹ The technical sensitivity of MLPA analysis for the dosage of *SMN1* and *SMN2* is estimated to be >99%. MLPA analysis is unable to determine whether two copies of *SMN1* are present on opposite chromosomes (92-97% of cases) or on the same chromosome (3-8% of cases) in an individual. MLPA analysis is also unable to detect sequence changes in the *SMN1* gene which occur in 3-5% of *SMN1*-related SMA cases. MS-MLPA can detect imprinting abnormalities in greater than 99% of individuals with PWS. All individuals with DM1 have an expansion of the repeat in the 3' UTR the *DMPK* gene, which is detectable by this targeted analysis. However, the exact number of repeats will not be determined for those alleles with more than 200 repeats. The technical sensitivity of fragment analysis is estimated to be greater than 95%.

References:

1. Peredo DE and Hannibal MC. The floppy infant: evaluation of hypotonia. *Pediatrics In Review*. 2009 Sep 30(9):e66-76.19726697
2. Lisi EC and Cohn RD. Genetic evaluation of the pediatric patient with hypotonia: perspective from a hypotonia specialty clinic and review of the literature. *Developmental Medicine and Child Neurology*. 2011 Jul 53(7):586-99.21418198
3. Harris SR. Congenital hypotonia: clinical and developmental assessment. *Developmental Medicine and Child Neurology*. 2008 Dec 50(12):889-92.19046184
4. Prior TW & Finanger E. (Updated 2016). Spinal Muscular Atrophy. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1352/>
5. Kolb SJ and Kissel JT. Spinal Muscular Atrophy. *Neurologic Clinics*. 2015 Nov 33(4):831-46.26515624
6. Arnold WD et al. Spinal muscular atrophy: diagnosis and management in a new therapeutic era. *Muscle & Nerve*. 2015 Feb 51(2):157-67.25346245

7. Bird T (Updated 2018). Myotonic Dystrophy Type 1. In: GeneReviews at GeneTests Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1993-. Available at <http://www.genetests.org>;
8. Cassidy, S. and Schwartz, S. (Updated September 3, 2009). Prader-Willi Syndrome. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1993-. Available at <http://www.genetests.org>.
9. Jin and DK. Systematic review of the clinical and genetic aspects of Prader-Willi syndrome. *Korean Journal of Pediatrics*. 2011 54(2):55-63.21503198
10. Labrum R et al. The molecular basis of spinal muscular atrophy (SMA) in South African black patients. *Neuromuscular Disorders: NMD*. 2007 Oct 17(9-10):684-92.17627822
11. Arsenault ME et al. Clinical characteristics of myotonic dystrophy type 1 patients with small CTG expansions. *Neurology*. 2006 Apr 25 66(8):1248-50.16636244
12. Martorell et al., (2001) Frequency and stability of the myotonic dystrophy type 1 premutation. *Neurology* 56 (3): 328-335 (PMID: 11171897)
13. Savić D et al. 250 CTG repeats in DMPK is a threshold for correlation of expansion size and age at onset of juvenile-adult DM1. *Human Mutation*. 2002 19(2):131-9.11793472
14. Hamshere MG et al. Myotonic dystrophy: the correlation of (CTG) repeat length in leucocytes with age at onset is significant only for patients with small expansions. *Journal of Medical Genetics*. 1999 Jan 36(1):59-61.9950368
15. Thornton CA. Myotonic dystrophy. *Neurologic Clinics*. 2014 Aug 32(3):705-19, viii.25037086
16. Tsilfidis C et al. Correlation between CTG trinucleotide repeat length and frequency of severe congenital myotonic dystrophy. *Nature Genetics*. 1992 Jun 1(3):192-5.1303233
17. Meola G and Cardani R. Myotonic dystrophies: An update on clinical aspects, genetic, pathology, and molecular pathomechanisms. *Biochimica Et Biophysica Acta*. 2015 Apr 1852(4):594-606.24882752
18. Lavedan C et al. Myotonic dystrophy: size- and sex-dependent dynamics of CTG meiotic instability, and somatic mosaicism. *American Journal of Human Genetics*. 1993 May 52(5):875-83.8098180
19. Buiting K et al. Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *American Journal of Human Genetics*. 2003 72(3):571-7.12545427