Congenital Hypotonia Panel: Spinal Muscular Atrophy Deletions, Prader-Willi/Angelman Syndrome Methylation, Myotonic Dystrophy, and Uniparental Disomy 14

Condition Description

The Congenital Hypotonia Panel consists of tests for five genetic conditions most often associated with isolated congenital hypotonia in newborns: spinal muscular atrophy, myotonic dystrophy (type 1), Prader-Willi syndrome, Angelman syndrome, and maternal UPD 14.

Spinal muscular atrophy (SMA) is the second most common lethal, autosomal recessive disorder in Caucasians. SMA is characterized by anterior horn cell degeneration which causes a symmetrical muscle weakness and wasting. Approximately 95%-98% of patients will have loss of both copies of the SMN1 gene. Approximately 2%-5% of patients are compound heterozygotes for an SMN1 deletion and an SMN1 intragenic mutation.

Myotonic dystrophy, type I (DM), is the most common adult form of muscular dystrophy, ranging from congenital to adult onset. The congenital form typically presents with hypotonia, muscle weakness, or respiratory distress, and may progress to include mental retardation or early death. Myotonic dystrophy type I is an autosomal dominant disorder, associated with an expansion of an unstable triplet (CTG) repeat in the DMPK gene. The size of the repeat expansion correlates with the severity of the phenotype.

Prader-Willi syndrome (PWS) is characterized by neonatal onset of severe hypotonia, feeding difficulty and failure to thrive, which may be preceded by decreased fetal movement in utero. An insatiable appetite may develop in later infancy or early childhood and can lead to obesity, if not controlled. Many children with PWS have short stature, hypogonadism, small hands and feet, and mild to moderate mental retardation. Many individuals with PWS may have recognizable patterns of behavior marked by stubbornness and temper tantrums. However, in the neonatal period, PWS may manifest only as isolated hypotonia, usually accompanied by feeding problems, with no obvious dysmorphic features.

Prader-Willi syndrome is caused by absence of the paternally derived 15q11.2-q13 region by one of several genetic mechanisms. Approximately 70% of individuals have a deletion in the paternal 15q11.2-q13 region. Approximately 25%-35% of individuals have maternal uniparental disomy, receiving two copies of chromosome 15 from the mother and no chromosome 15 from the father. Approximately 2% of individuals have other imprinting mutations which repress expression of the genes critical to the Prader-Willi region.

Angelman syndrome (AS) is caused by loss of expression of the maternally inherited UBE3A gene and is characterized by severe developmental delay, severe speech impairment, gait ataxia and/or tremulousness of the limbs, and a unique behavior with an inappropriate happy demeanor that includes frequent laughing, smiling, and excitability. During infancy individuals with AS typically have a normal phenotype. However, some affected individuals may have feeding problems and hypotonia.

Maternal UPD 14 is characterized by neonatal hypotonia, prenatal and postnatal growth retardation, small hands and feet, feeding difficulties and precocious puberty. Many of these characteristics are similar to those found in Prader-Willi syndrome and studies have indicated that a significant number of patients with maternal UPD 14 are suspected to have Prader-Willi syndrome in infancy. Distinctive features of maternal UPD 14 include constant prenatal growth failure, less-specific facial characteristics, and better intellectual development. In addition to patients reported with complete maternal UPD 14, patients with epimutations and patients with a microdeletion of the 14q32.2 imprinted region have been reported with a upd(14)mat-constant phenotype. Hosoki et al. enrolled 78 patients with infant hypotonia who tested negative for Prader-Willi syndrome into their study. Abnormal hypomethylation of the MEG3 promoter was identified in 5 of the 78 patients. Three of these patients had complete maternal UPD 14, one had an epimutation, and one was mosaic for maternal UPD 14. The patients reported with epimutations have clinical features that are indistinguishable from patients with complete maternal UPD 14. Their patient with mosaic maternal UPD 14 had clinical features typical of complete maternal UPD 14 as well.

Genes

DMPK, MEG3, SMN1, SNRPN

Indications

This test is indicated for newborns with isolated, congenital hypotonia.

Methodology

This hypotonia panel includes four assays:

- Testing for spinal muscular atrophy consists of SMN1 gene deletion analysis by multiplex ligation polymerase chain reaction amplification (MLPA) of exons 7 and 8.
- Testing for myotonic dystrophy consists of PCR amplification and fragment size analysis, as well as triplet-primed PCR analysis. Large size expansions may require Southern analysis for accurate size estimation.
- Testing for PWS and AS consists of methylation sensitive PCR analysis of the SNRPN gene.
- Testing for maternal UPD 14 consists of methylation sensitive PCR analysis of the MEG3 gene.

Detection

- Spinal muscular atrophy: This assay tests for the common SMN1 deletion that is found in approximately 95% of individuals with SMA; other pathogenic variants will not be detected.
- Myotonic dystrophy: Nearly all CTG expansions in the DMPK gene will be detected by this assay.
- Prader-Willi syndrome: Approximately 99% of individuals with Prader-Willi syndrome will be detected by this assay. In addition, approximately 70% of individuals with Angelman syndrome will be identified by this assay. (Hosoki et al. (2009). J Pediat; 155: 900-903.)

**Reference Range**

- Spinal muscular atrophy is a qualitative assay.
- For myotonic dystrophy testing, normal individuals carry less than 40 repeats, while affected individuals carry 50 to over a few thousand repeats.
- For Prader-Willi testing, an unmethylated and a methylated allele are detected in normal samples. Only the methylated maternal allele is detected in individuals with PWS. Only the unmethylated paternal allele is detected in individuals with AS.
- For maternal UPD 14 testing, an unmethylated and a methylated allele are detected in normal samples. Only the unmethylated allele is detected in individuals with maternal UPD 14.

**Specimen Requirements**

*Submit only 1 of the following specimen types*

**Type: DNA, Isolated**

**Specimen Requirements:**
- Microtainer
- 15µg
- Isolation using the Perkin Elmer™Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

**Specimen Collection and Shipping:**
- Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

**Type: Whole Blood (EDTA)**

**Specimen Requirements:**
- EDTA (Purple Top)
- Infants and Young Children (2 years of age to 10 years old): 3-5 ml
- Older Children & Adults: 5-10 ml
- Autopsy: 2-3 ml unclotted cord or cardiac blood

**Specimen Collection and Shipping:**
- Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

**Related Tests**

- Newborns or older children with neurologic distress or encephalopathy may require additional studies for inborn errors of metabolism, including: plasma acylcarnitine, plasma amino acids, and urine organic acids. Consult with neurology or genetics is suggested.
- Each of the five tests in the panel is also available separately.