

Myotonia Panel

DISORDER ALSO KNOWN AS

Dystrophic myotonia, Non-dystrophic myotonia, Muscle Channelopathies

PANEL GENE LIST

DMPK, CNBP, ATP2A1, CACNA1S, CAV3, CLCN1, GLRA1, HINT1, PTRF, SCN4A

CLINICAL FEATURES

Myotonia, defined as the failure of muscle relaxation after activation or contraction, is observed in a small number of genetically heterogeneous disorders.¹ It can present as painless muscle stiffness, triggered by cold, stress, or exercise; or as spontaneous discharges on EMG without clinical manifestations.^{1,2} The dystrophic myotonias, are associated with progressive muscle damage, while non-dystrophic myotonias are not typically associated with muscle damage.^{1,3} Myotonic dystrophy type 1 (DM1, *DMPK*) and type 2 (DM2, *CNBP*), are multisystem disorders that affect both smooth and skeletal muscle, as well as the central nervous and endocrine systems.⁴ Whereas DM2 typically presents in adulthood, age of onset and disease severity for DM1 fall along a broad spectrum that is broken into three subtypes: mild (onset 20–70 years), classic (onset 10–30 years), and congenital (onset birth–10 years).⁴ Clinical features associated with both adult-onset DM1 and DM2 include myotonia, muscle weakness, cardiac conduction defects, insulin insensitivity, and cataracts.^{4,5} Although much less common, non-dystrophic myotonias generally result from defects in chloride and sodium ion channel genes and present with muscle stiffness in the absence of defined weakness or atrophy.^{1,2} Over time, individuals may experience pain, fatigue, and muscle weakness.^{2,3} Additionally, disorders such as periodic paralysis and rippling muscle disease can present similarly and occur in the differential of myotonia.^{1,2}

INHERITANCE PATTERN/GENETICS

Myotonia can be inherited in an autosomal dominant or an autosomal recessive manner. Reduced penetrance, intra and inter-familial variability, and variable age-of-onset may be observed in disorders with myotonia. The genes investigated on this panel include voltage gated ion channels, ion pumps, and transporters.^{1,3}

DM1 is caused by the expansion of a CTG trinucleotide repeat in the 3'UTR of the *DMPK* gene.⁴ Normal alleles have 5–34 repeats, premutation (mutable normal) alleles have 35–49 repeats, and disease alleles have greater than 50 repeats.^{6,7} Repeat expansions of <250 generally result in the mild form of DM1; however, larger repeat expansions do not appear to have the same linear correlation with age-of-onset and disease severity.^{8–10} Although anticipation is well documented for DM1, multiple studies have shown considerable phenotypic variation among individuals with similar repeat numbers, including classically affected individuals with >1000 repeats.^{11–13} Therefore, a repeat number of 50 or more is sufficient to make a diagnosis of DM1 and use of repeat number to predict disease subtype or prognosis is not recommended by multiple guidelines.^{7,9} Clinical correlation is necessary to determine the specific DM1 subtype.

DM2 is caused by an expansion of the CCTG tetranucleotide repeat within the complex repeat motif [TG(n)TCTG(n)CCTG(n)] in intron 1 of the *CNBP* gene.⁵ The three repeating units (TG, TCTG, CCTG) within this

motif are all highly variable in both individuals with DM2 and the general population.¹⁴ The CCTG repeat is the only repeat that expands to pathogenic lengths, although the highly polymorphic TG and TCTG repeats contribute to the overall length of the expansion. Consequently, the exact number of CCTG repeats cannot be determined as the TG and TCTG repeats make up a significant and unknown proportion of the overall length of the expansion.⁷ Normal alleles have 11–26 CCTG repeats and disease alleles have greater than 75 repeats. Alleles with 27–74 repeats are not well characterized in the literature and their clinical significance and disease association is uncertain.¹⁵

TEST METHODS

Using genomic DNA from the submitted specimen, standard PCR fragment analysis is performed to identify normal and minimally expanded alleles in the *DMPK* and *CNBP* genes. For *DMPK*, 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 <200 repeats. For *CNBP*, repeat-primed PCR is used to identify alleles with >75 repeats, as well as determine the number of repeats in alleles with <75 repeats. Internal standards are analyzed along with clinical samples to evaluate assay performance. Nucleotide repeat numbers are reported with an accuracy of ± 2 repeats up to 50 repeats, but larger expansions are reported with an accuracy of ± 5 repeats, if a specific number is reported. The exact number of repeats will not be determined for *DMPK* alleles with >200 repeats or *CNBP* alleles with >75 repeats. Southern blot analysis is required to determine the number of repeats in large expansions and is not completed as part of this test. The technical sensitivity of fragment analysis is estimated to be greater than 95%.

Simultaneously, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size.

TEST SENSITIVITY

The clinical sensitivity of the genes included in this panel depends in part on the patient's clinical phenotype. All individuals with DM1 and DM2 have expansions of the polynucleotide repeats targeted by this

analysis.^{4,5} Pathogenic variants in *CLCN1* and *SCN4A* account for the majority of molecular findings in non-dystrophic myotonias.^{1,3}

MYOTONIA PANEL

Sequencing and Exon-level Deletion/Duplication Analysis of 10 genes,
Plus Repeat Analysis of *DMPK* and *CNBP*

Gene	Disease Associations	Inheritance	Diagnostic Yield in Selected Population(s)
<i>CNBP</i>	Myotonic Dystrophy Type 2	AD	100% of DM2 ⁵
<i>DMPK</i>	Myotonic Dystrophy Type 1	AD	100% of DM1 ⁴
<i>ATPIA2</i>	Brody Disease/Syndrome; HypoPP	AR	31% of Brody Disease ¹⁶ Rare contribution to myotonia ²
<i>CACNA1S</i>	HypoPP; MH; Thyrotoxic PP; Congenital myopathy	AD	40–60% of hypoPP ^{17,18} Rare contribution to myotonia ¹⁹
<i>CAV3</i>	Rippling muscle disease; LGMD1C; Distal Myopathy; Isolated hyperCKemia	AD	2.7% of Rippling muscle disease/ exercise intolerance in the UK ²⁰
<i>CLCN1</i>	Myotonia congenita; HypoPP	AD/AR	95% of myotonia congenita ^{21,22} 42–44% of non-dystrophic myotonia in the Netherlands & US ^{23, 24}
<i>GLRA1</i>	Hyperekplexia 1	AD/AR	63–94% of hyperekplexia ^{25,26} Rare contribution to myotonia ²⁶
<i>HINT1</i>	Neuromyotonia and axonal neuropathy	AR	Up to 11% of autosomal recessive neuropathies in patients from Czech Republic, Austria, Serbia, Bulgaria and Turkey ^{27,28}
<i>PTRF</i>	Lipodystrophy, congenital generalized, type 4	AR	Rare overall ²⁹
<i>SCN4A</i>	Paramyotonia congenita; Atypical myotonia	AD/AR	56–59% of non-dystrophic myotonia in the Netherlands ^{23,24}

	congenita; Hyper/hypoPP; Congenital Myasthenia syndrome;		80% of hyperPP ¹⁸ 7–14% of hypoPP ¹⁹
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ABBREVIATIONS

HyperPP – Hyperkalemic periodic paralysis
 LGMD – Limb girdle muscular dystrophy
 MH – Malignant hyperthermia
 HypoPP – Hypokalemic periodic paralysis
 PP – Periodic paralysis

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