Spinal Muscular Atrophy and Related Disorders Panel

Dosage Analysis of SMN1 and SMN2 & Sequence Analysis and Exon-Level Deletion/Duplication Testing of 18 Genes

Panel Gene List: ASAH1, ATP7A, BICD2, BSCL2, DNAJB2, DYNC1H1, GARS, HSPB1, HSPB8, IGHMBP2, PLEKHG5, REEP1, SIGMAR1, SMN1*, SMN2*, SLC5A7, TRIP4, TRPV4, UBA1, VRK1

*Dosage analysis only

Clinical Features:

The genes evaluated on this panel cause Spinal muscular atrophies (SMA) and related disorders. SMAs are a clinically and genetically heterogenous group of disorders characterized by the progressive degeneration of the anterior horn cells in the spinal cord and brain stem nuclei, leading to progressive muscle weakness and wasting.[1] Individuals typically present with symmetric proximal extremity weakness that may progress to distal, axial, intercostal, and bulbar muscles; however age-of-onset, disease severity, and additional clinical findings can be variable.[2] Some forms of SMA may present with additional features including seizures, respiratory failure, arthrogryposis, or congenital bone fractures.[1]

Autosomal recessive proximal SMA, caused by pathogenic variants in the *SMN1* gene, accounts for 95% of individuals with SMA.[3] *SMN1*-related SMA was originally classified into five clinical subtypes (SMA 0-4) prior to the utilization of genetic testing in the diagnosis of SMA. These subtypes include **SMA 0**, characterized by prenatal onset, neonatal hypotonia, severe weakness, early respiratory failure, and death within 6 months of life. **SMA I** has an onset prior to 6 months, mild joint conctractures, minimal facial weakness, swallow difficulties, and an average lifespan of less than 2 years of age. **SMA II** has an onset between 6-18 months, independent sitting when placed, and a lifespan typically into adulthood. **SMA III** has an onset after 18 months, independent ambulation, and normal lifespan. **SMA IV**, the mildest form, is associated with muscle weakness in 2nd-3rd decade of life and a normal lifespan.[1] These subtypes are influenced by *SMN2* copy number and individuals with a greater number of *SMN2* copies typically have milder disease.[1]

This panel also includes genes that share clinical overlap with SMA and are associated with distal hereditary motor neuropathy (dHMN). dHMN is a clinically and genetically heterogenous disorder characterized by slowly progressive symmetrical distal lower motor neuron weakness.[4] This disorder can present with varying age of onset, although the genes included on this panel have typically been associated with an earlier age of onset, similar to many forms of SMA.

Genetics:
The disorders included on this panel are inherited in either an autosomal recessive, autosomal dominant, or X-linked manner. Many of these have disorders may be the result of a *de novo* variant.

Ninety-five percent of individuals with SMA have a defect in the *SMN1* gene. *SMN1*-related SMA is most commonly due to the homozygous loss of exon 7, whereas 5% of *SMN1*-related SMA isdue to the presence of a pathogenic sequencing-based change on one allele and loss of exon 7 on the other allele.[5] Loss of exon 7 occurrs as a result of either the deletion of at least exon 7 of the *SMN1* gene or the conversion of the exon 7 *SMN1* sequence into the *SMN2* sequence. The *SMN2* gene sequence differs from the *SMN1* gene sequence by only 5 base pairs. The most significant difference being a single C>T nucleotide change within the coding sequence of exon 7. This change in *SMN2* alters gene splicing, resulting in the skipping of exon 7, resulting in a truncated protein that is rapidly degraded. However, ~10% of *SMN2* transcripts undergo proper exon splicing, thus retaining exon 7 and producing full length protein.[3] The greater the number of *SMN2* copies present, the greater the amount of functional SMN protein is produced. Therefore the greater the number of *SMN2* copies an individual has, the less severe the disease will be.

Test Methods:
Using genomic DNA from the submitted specimen, 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 NCBI RefSeq transcripts and 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.

For the *SMN1* and *SMN2* genes, multiplex ligation-dependent probe amplification (MLPA) is completed to determine copy number of the genes in the provided specimen, compared to control specimens.

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. 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 unable to detect sequence changes in the *SMN1* gene which occur in 3-5% of *SMN1*-related SMA cases.

References:

1. Prior TW & Finanger E. Spinal Muscular Atrophy. 2000 Feb 24 [Updated 2016 Dec 22]. 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/
2. Kolb SJ & Kissel JT. (2015) Spinal Muscular Atrophy. Neurologic clinics. 33 (4): 831-846. (PMID: 26515624)
3. Arnold, Kassar, & Kissel (2015) Spinal muscular atrophy: diagnosis and management in a new therapeutic era, Muscle and Nerve 51 (2): 157-167 (PMID: 25346245)
4. Rossor et al., (2012) The distal hereditary neuropathies, Journal of Neurology, Neurosurgery, and Psychiatry 83 (1): 6-14 (PMID: 22028385)
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Spinal Muscular Atrophy Panel – 20 Genes

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
ASAH1	AR	Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy	Rare[1]
ATP7A	XL	X-linked Spinal Musuclar Atrophy	Rare[2]
BICD2	AD	Lower-Extremity Predominant Spinal Musuclar Atrophy 2	Rare[3]
BSCL2	AD	Distal motor neuropathy, type 5; Silver Syndrome	~7% of patients with distal hereditary neuropathy[4]
DNAJB2	AR	Spinal Muscular Atrophy, Distal Autosomal Recessive, 5	Rare[5]
DYNC1H1	AD	Spinal Muscular Atrophy, Lower Extremity Dominant	Rare[6]
GARS	AD	Distal Hereditary Neuropathy 5	~3% of patients with CMT[7]
HSPB1	AD	Distal Hereditary Neuropathy 2	8% of patients with distal hereditary motor neuropathy; 4% of patients with CMT2[8]
HSPB8	AD	Distal Hereditary Neuropathy 2	Unknown in CMT2 and dHMN II[8]
IGHMBP2	AR	Spinal Muscular Atrophy and Respiratory Distress 1	~33% of patients with SMARD1[9]
PLEKHG5	AR	Distal Spinal Muscular Atrophy 4	Rare[10]
REEP1	AD	Distal Hereditary Motor Neuropathy, 5	3-6.5% of patients with hereditary spastic paraplegia[11]
SIGMAR1	AR	Distal Spinal Musuclar Atrophy 2	Rare[12]

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
SLC5A7	AD/AR	Distal Hereditary Motor Neuropathy, 7A	Unknown[13]
*SMN1**	AR	Spinal Muscular Atrophy	95% of SMA Cases[14]
*SMN2**	N/A	Spinal Muscular Atrophy Modifier	N/A[14]
TRIP4	AR	Spinal Muscular Atrophy with Congenital Bone Fractures	Unknown[15]
TRPV4	AD	Congenital Distal Spinal Muscular Atrophy, Scapuloperoneal Spinal Muscular Atrophy	Rare[16]
UBA1	XL	X-linked Infantile Spinal Muscular Atrophy	Rare[17]
VRK1	AR	Spinal Muscular Atrophy with Pontocerebellar Hypoplasia	Rare overall, founder mutation in the Ashkenazi Jewish population[18]

*Dosage analysis only

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