

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 heterogeneous 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, arthrogyposis, 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 contractures, 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 heterogeneous 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. Some of these disorders may be the result of a *de novo* variant.

Ninety-five percent of individuals with SMA have a defect in the *SMN1* gene.³ 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.⁵ 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. The *SMN2* gene sequence differs from the *SMN1* gene sequence by only 5 base pairs, with the most significant difference being a single C>T nucleotide change within the coding sequence of exon 8. This change in *SMN2* alters gene splicing, resulting in the skipping of exon 8 and the production of a truncated protein that is rapidly degraded. However, ~10% of *SMN2* transcripts undergo proper splicing, retaining exon 8, 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. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. 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:

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Spinal Muscular Atrophy Panel – 20 Genes

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
<i>ASAH1</i>	AR	Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy	Rare ¹
<i>ATP7A</i>	XL	X-linked Spinal Muscular Atrophy	Rare ²
<i>BICD2</i>	AD	Lower-Extremity Predominant Spinal Muscular Atrophy 2	Rare ³
<i>BSCL2</i>	AD	Distal motor neuropathy, type 5; Silver Syndrome	~7% of patients with distal hereditary neuropathy ⁴
<i>DNAJB2</i>	AR	Spinal Muscular Atrophy, Distal Autosomal Recessive, 5	Rare ⁵
<i>DYNC1H1</i>	AD	Spinal Muscular Atrophy, Lower Extremity Dominant	Rare ⁶
<i>GARS</i>	AD	Distal Hereditary Neuropathy 5	~3% of patients with CMT ⁷
<i>HSPB1</i>	AD	Distal Hereditary Neuropathy 2	8% of patients with distal hereditary motor neuropathy; 4% of patients with CMT ⁸
<i>HSPB8</i>	AD	Distal Hereditary Neuropathy 2	Unknown in CMT2 and dHMN II ⁸
<i>IGHMBP2</i>	AR	Spinal Muscular Atrophy and Respiratory Distress 1	~33% of patients with SMARD ¹⁹
<i>PLEKHG5</i>	AR	Distal Spinal Muscular Atrophy 4	Rare ¹⁰
<i>REEP1</i>	AD	Distal Hereditary Motor Neuropathy, 5	3-6.5% of patients with hereditary spastic paraplegia ¹¹
<i>SIGMAR1</i>	AR	Distal Spinal Muscular Atrophy 2	Rare ¹²
<i>SLC5A7</i>	AD/AR	Distal Hereditary Motor Neuropathy, 7A	Unknown ¹³

<i>SMN1</i> *	AR	Spinal Muscular Atrophy	95% of SMA Cases ¹⁴
<i>SMN2</i> *	N/A	Spinal Muscular Atrophy Modifier	N/A ¹⁴
<i>TRIP4</i>	AR	Spinal Muscular Atrophy with Congenital Bone Fractures	Unknown ¹⁵
<i>TRPV4</i>	AD	Congenital Distal Spinal Muscular Atrophy, Scapulo-peroneal Spinal Muscular Atrophy	Rare ¹⁶
<i>UBA1</i>	XL	X-linked Infantile Spinal Muscular Atrophy	Rare ¹⁷
<i>VRK1</i>	AR	Spinal Muscular Atrophy with Pontocerebellar Hypoplasia	Rare overall, founder mutation in the Ashkenazi Jewish population ¹⁸

*Dosage analysis only

1. Zhou et al., (2012) Spinal muscular atrophy associated with progressive myoclonic epilepsy is caused by mutations in *ASAH1*. American Journal of Genetics 91 (1): 5-14 (PMID: 22703880) 2. Kennerson et al., (2010) Missense mutations in the copper transporter gene *ATP7A* cause X-linked distal hereditary motor neuropathy. American Journal of Human Genetics (86 (3): 343-352 (PMID: 20170900) 3. Synofzik et al. (2014) Dominant spinal muscular atrophy due to *BICD2*: a novel mutation refines the phenotype, Journal of Neurology, Neurosurgery, and Psychiatry. 85 (5):590-2 (PMID: 24336790). 4. Dierick et al., (2008) Relative contribution of mutations in genes for autosomal dominant distal hereditary motor neuropathies: a genotype-phenotype correlation study. Brain 131: 1217-1227 (PMID: 18325928) 5. Gess et al., (2014) *HSJ1*-related hereditary neuropathies: novel mutations and extended clinical spectrum. Neurology 83 (19):1726-1732 (PMID: 25274842) 6. Tsurusaki et al. (2012). A *DYNC1H1* mutation causes a dominant spinal muscular atrophy with lower extremity predominance. Neurogenetics 13, 327-332 (PMID: 22847149) 7. Saporta et al. (2011) Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Annals of Neurology 69(1): 22-33 (PMID: 21280073). 8. Capponi et al. (2011) *HSPB1* and *HSPB8* in inherited neuropathies: study of an Italian cohort of *dHMN* and *CMT2* patients. Journal of the Peripheral Nervous System 16: 287-294 (PMID: 22176143) 9. Guenther et al. (2007). Clinical and mutational profile in spinal muscular atrophy with respiratory distress (*SMARD*): defining novel phenotypes through hierarchical cluster analysis. Human Mutation. 28, 808-815 (PMID: 17431882) 10. Rossor, A.M., et al. (2012). The distal hereditary motor neuropathies. The Journal of Neurology, Neurosurgery, and Psychiatry 83, 6-14 (PMID: 22028385) 11. Zuchner et al. (2006) Mutations in the novel mitochondrial protein *REEP1* cause hereditary spastic paraplegia type 31. American Journal of Human Genetics 79(2): 365-369. (PMID: 16826527) 12. Li et al., (2015) A *SIGMAR1* splice-site mutation causes distal hereditary motor neuropathy. Neurology 84 (24): 2430-2437 (PMID: 26078401) 13. Barwick et al., (2012) Defective presynaptic choline transport underlies hereditary motor neuropathy. American Journal of Human Genetics 91 (6):1103-1107 (PMID: 23141292) 14. Kolb SJ & Kissel JT. (2015) Spinal Muscular Atrophy. Neurologic clinics. 33 (4): 831-846. (PMID: 26515624) 15. Knierim et al., (2016) Mutations in Subunits of the Activating Signal Cointegrator 1 Complex Are Associated with Prenatal Spinal Muscular Atrophy and Congenital Bone Fractures. American Journal of Human Genetics 98 (3):473-489 (PMID: 26924529) 16. Cho, T., et al. (2012). *TRPV4*-pathy manifesting both skeletal dysplasia and peripheral neuropathy: a report of three patients. The American Journal of Medical Genetics Part A 158 A, 795-802. (PMID: 22419508) 17. Ramser, J., et al. (2008). Rare missense and synonymous variants in *UBE1* are associated with X-linked infantile spinal muscular atrophy. American Journal of Human Genetics 82, 188-193. (PMID: 18179898) 18. Renbaum, P., et al. (2009). Spinal muscular atrophy with pontocerebellar hypoplasia is caused by a mutation in the *VRK1* gene. American Journal of Human Genetics 85, 281-289. (PMID: 19646678)