

Genetic Testing for Neuropathy: Core Charcot-Marie-Tooth Panel Sequencing and Exon-Level Deletion/Duplication Testing of 6 Genes

Panel Gene List: *GDAP1, GJB1, MFN2, MPZ, PMP22, SH3TC2*

Clinical Features:

Collectively the Charcot-Marie-Tooth (CMT) neuropathies are the most common cause of hereditary neuropathy with a prevalence of approximately 1 in 2,500^{1,2}. Charcot-Marie-Tooth neuropathies are also known as hereditary motor and sensory neuropathies (HMSN) because they are characterized by predominately motor and sensory symptoms. The “classic” CMT presentation is characterized by progressive distal muscle weakness with the feet and legs being most severely affected, paresthesia and/or loss of sensation, a “drop foot” gait, depressed deep tendon reflexes, hammer toes, and pes cavus. A subset of patients present with a severe CMT phenotype in the first year of life, often called Dejerine-Sottas neuropathy (DSN) or congenital hypomyelinating neuropathy (CHN). These individuals with early-onset CMT commonly present with a severe demyelinating neuropathy and extremely slow nerve conduction velocities (NCV) (<10 m/s)^{1,7}. Hypotonia, developmental delay, pes cavus, scoliosis and sensory ataxia are also commonly reported¹⁻⁸. Many genes commonly associated with adult-onset CMT have been identified in individuals with DSN and CHN including: *PMP22* and *MPZ*. Pathogenic variants in four genes, *PMP22, GJB1, MPZ,* and *MFN2*, account for over 90% of molecular diagnoses in patients with CMT³. Biallelic pathogenic variants in the *GDAP1* and *SH3TC2* genes are common causes of autosomal recessive hereditary neuropathy. Biallelic variants in *GDAP1* account for approximately 1-5.4% of molecular diagnoses¹⁸. Biallelic variants in *SH3TC2* account for approximately 18% of CMT4 diagnoses²⁰. In a cohort of patients tested at GeneDx, pathogenic variants in *GDAP1* accounted for approximately 2% of positive diagnoses and biallelic pathogenic variants in *SH3TC2* accounted for approximately 4% of positive diagnoses made by the hereditary neuropathy panel¹⁹.

PMP22: Pathogenic variants in the *PMP22* gene, including whole gene aberrations, are the most common cause of CMT and have been associated with Charcot-Marie-Tooth type 1A (CMT1A), CMT1E, hereditary neuropathy with liability to pressure palsy (HNPP), and DSN. Individuals with CMT1A and CMT1E typically have a classic CMT phenotype and exhibit slow NCV of less than 38m/s in the arms, consistent with demyelinating neuropathy. Hearing loss and hip dysplasia may also be present.^{1,4} CMT1A is caused by a recurrent 1.5 Mb duplication including the *PMP22* gene, while CMT1E is caused by point mutations in the *PMP22* gene. Individuals with CMT1E tend to be more severely affected than individuals with CMT1A⁵.

HNPP is characterized by recurrent episodes of sensory and motor neuropathy in a single nerve. While any nerve in the peripheral nervous system can be affected, the ulnar, peroneal,

median, brachial plexus and radial nerves are most commonly affected.⁶ Almost all affected individuals show prolongation of distal nerve conduction latencies. Other features include: reduced or absent tendon reflexes, pes cavus, episodic foot drop, atrophy and weakness of the hands, carpal tunnel syndrome, and pain. Less common features include: motor brachial paralysis, proximal muscle atrophy, respiratory insufficiency, white matter lesions on brain MRI, hypoglossal nerve paralysis of the tongue, and scapulo-peroneal syndrome. An episode can last from minutes to months. Individuals typically present in the 2nd or 3rd decade, although the age of onset can range from neonatal period into the 7th decade⁶. Of individuals with HNPP who have a molecular diagnosis, approximately 80% have a recurrent 1.5 Mb deletion including the *PMP22* gene, while the remaining 20% of individuals have a pathogenic variant in *PMP22* detectable by sequence analysis.⁶

MPZ: Pathogenic variants in the *MPZ* gene cause a spectrum of CMT disorders including: DSN, congenital hypomyelinating neuropathy (CHN), CMT1B, CMT2I, CMT2J, and DI-CMTD^{4,9,10}. The early-onset phenotype, commonly called DSN or CHN, is characterized by infantile onset of hypotonia, motor delay, areflexia, and severely decreased NCV. Cranial nerve involvement, swallowing and respiratory difficulty, and elevated cerebrospinal fluid protein have been described in some individuals⁹. The adult-onset phenotype typically presents in the 4th decade (ranging from the 2nd to 7th decade) with typical features of progressive distal motor neuropathy and sensory neuropathy⁹. Adie's pupil and hearing loss are reported in some patients⁵. Individuals with the later onset phenotype can have NCV consistent with axonal or demyelinating neuropathy⁵.

MFN2: Pathogenic variants in *MFN2* cause CMT2A, a progressive neuropathy that typically affects the lower limbs more severely than the upper limbs¹¹. Motor NCV are typically normal or mildly decreased, consistent with axonal neuropathy¹¹. Approximately 80% of individuals with pathogenic *MFN2* variants have an early-onset (before age 10) severe phenotype; however, a smaller group of individuals with a mild phenotype and late-onset of symptoms has also been described^{1,12,13}. Scoliosis and knee joint contractures have been reported in individuals with early-onset of symptoms, while tremors of the arms or hands, pain, and sensorineural hearing loss were more commonly reported in some individuals with late-onset of symptoms¹². Pathogenic variants in the *MFN2* gene can also cause autosomal dominant CMT5 and autosomal dominant CMT6, also called hereditary motor and sensory neuropathy type VI (HMSN VI). CMT5 is characterized by classic features of axonal CMT neuropathy with pyramidal tract signs, such as extensor plantar response, hypertonia, and hyperreflexia¹¹. CMT6 is characterized by classic features of axonal CMT neuropathy and optic atrophy^{1,12}. Rarely, pathogenic *MFN2* variants have also been reported in families with autosomal dominant CMT¹²¹. In some families, up to 25% of individuals with *MFN2* variants may be asymptomatic²². Additionally, individuals who inherit two pathogenic *MFN2* variants have been reported to be more severely affected than either of their parents^{23,24}. In at least some of these

cases, the literature is supportive of autosomal recessive inheritance, while in other cases semi-dominant inheritance remains possible. Loss of function variants in particular are associated with autosomal recessive inheritance and commonly found with a second MFN2 variant on the opposite allele in CMT patients²⁵.

GJB1: Pathogenic variants in *GJB1* cause Charcot-Marie-Tooth neuropathy X, type 1 (CMTX1), the second most common cause of CMT. Male patients typically present between ages 5-25. In addition to the classic features of CMT, hearing loss and central nervous system symptoms, such as extensor plantar response, transient ataxia, dysarthria, hyperventilation, aphasia, and white matter abnormalities on brain MRI, have been reported in some individuals¹⁴. Female carriers of pathogenic *GJB1* variants can be asymptomatic; however, many have abnormal NCV and/or an abnormal neurological exam, and up to one-third of carrier females are as severely affected as males^{1,14}. Males typically have slow NCV, consistent with demyelinating neuropathy, while females usually have normal NCV; however, both males and females have been reported with NCV in the intermediate range¹⁵.

GDAP1: Pathogenic variants in *GDAP1* are associated with several types of Charcot-Marie-Tooth (CMT) disease including: type 4A, type 2K/2H, and CMTR1A. Additionally, *GDAP1* variants are identified in patients with axonal, demyelinating, and intermediate forms of CMT^{26,27}. *GDAP1* variants are most often associated with an autosomal recessive CMT disorder, which typically presents during the first two years of life and is characterized by severe and progressive muscle weakness, initially of the lower limbs, sensory impairment, vocal cord paresis, absent reflexes, pes cavus, and contractures; most individuals require wheelchair use by the end of the second decade²⁷. Although rare, autosomal dominant *GDAP1*-related disorders have also been reported and typically have late-onset, slowly progressive peripheral neuropathy, characterized by distal muscle weakness and atrophy, although most patients remain ambulatory; incomplete penetrance has been reported in some families^{28,29}. Additionally, the age of onset and severity of symptoms associated with *GDAP1* variants has been noted to vary considerably, even within a family^{29,30}.

SH3TC2: Homozygous and compound heterozygous variants in *SH3TC2*, also known as *KIAA1985*, have been reported in association with Charcot-Marie-Tooth type 4C (CMT4C), an autosomal recessive disorder characterized by early onset scoliosis or kyphoscoliosis, progressive sensorimotor neuropathy typically presenting in childhood or adolescence, foot deformities, hypoventilation and/or respiratory insufficiency, and motor nerve conduction velocities in the demyelinating range²⁰. Hypoacusis, deafness, nystagmus, facial nerve paralysis, abnormal pupillary light reflexes, lingual fasciculation, head tremor, sensory ataxia, and diabetes mellitus have also been reported in a small percentage of patients with CMT4C²⁰. In addition, heterozygous variants in the *SH3TC2* gene have been reported in association with mononeuropathy of the median nerve (MNMN), an autosomal dominant disorder with

phenotypic variability ranging from carpal tunnel syndrome to more widespread axonal neuropathy resembling hereditary neuropathy with liability to pressure palsies³¹.

Genetics:

Pathogenic variants in the *PMP22* and *MPZ* genes are inherited in an autosomal dominant manner, pathogenic variants in the *SH3TC2* gene are inherited in an autosomal recessive manner, pathogenic variants in the *GJB1* gene are inherited in an X-linked manner, and pathogenic variants in the *MFN2* and *GDAP1* genes can be inherited in an autosomal dominant or autosomal recessive manner. In *GDAP1*, autosomal recessive inheritance is more common¹⁸. Approximately 20-33% of *PMP22* duplications are de novo, while approximately 20% of *MPZ* variants and *PMP22* deletions are de novo^{15, 16, 6, 5}. Most cases of CMTX1 are inherited, as only approximately 5% of pathogenic *GJB1* variants are de novo¹⁴. The percentage of de novo variants in the *MFN2* gene is currently unknown; however, most individuals have an affected parent¹¹.

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.

Sequencing and deletion/duplication analysis of the remaining genes on the Hereditary Neuropathy Panel is available as a reflex test if the Core CMT Panel is negative.

Test Sensitivity:

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the table below. 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.

Disease Associations	Gene	Protein	Inh	Diagnostic Yield in Selected Populations
CMT4A, CMT2H, CMTRIA, CMT2K	<i>GDAP1</i>	ganglioside-induced differentiation-associated protein 1	AD/AR	1-5% of autosomal recessive CMT ¹⁸
CMTX1	<i>GJB1</i>	gap junction protein beta 1; connexin 32	XL	~90% of X-linked CMT ^{1,16}
CMT2A; HMSN VI	<i>MFN2</i>	mitofusin 2	AD/AR	10-30% of CMT ^{2,17}
CMT1B; CMT2I; CMT2J; CMTDID; DSD; CHN	<i>MPZ</i>	myelin protein zero	AD	6-10% of patients with CMT ^{15,16} ; Rare in CMT ² ¹⁷
CMT1A; CMT1E; HNPP; DSD	<i>PMP22</i>	peripheral myelin protein 22	AD	Duplication: ~70% of CMT ^{1,2} Deletion: ~80% of HNPP ⁶ Point mutations: <5% of CMT ¹ ⁵ ; 20% of HNPP ⁶
CMT4C	<i>SH3TC2</i>	SH3 domain and tetratricopeptides repeats 2	AR	~18% of CMT ⁴ ²⁰

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