Genetic Testing for Neuropathy: Charcot-Marie-Tooth (CMT) Panel Sequencing and Exon-Level Deletion/Duplication Testing of 43 Genes

Disorder also known as: Hereditary motor and sensory neuropathy (HMSN)

Panel Gene List: *AARS, AIFM1, BSCL2, DNAJB2, DNM2, DYNC1H1, EGR2, FGD4, FIG4, GAN, GARS, GDAP1, GJB1,GNB4, HARS, HINT1, HSPB1, HSPB8, IGHMBP2, INF2, KIF5A, LITAF, LMNA, LRSAM1, MFN2, MME, MORC2, MPZ, MTMR2, NDRG1, NEFL, PLEKHG5, PMP22, PRPS1, PRX, RAB7A, SBF1, SBF2, SH3TC2, SLC12A6, TRIM2, TRPV4, YARS*

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. Historically CMT neuropathies have been classified as demyelinating or axonal based on nerve conduction studies. Demyelinating forms of CMT primarily affect the myelin sheath of the peripheral nerve and are characterized by slow nerve conduction velocities (NCV) of less than 38 m/s in the arms, while axonal forms of CMT primarily affect the axons of the peripheral nerves and are characterized by normal or almost normal NCV of greater than 38 m/s in the arms[3, 4]. Axonal neuropathies are also typically associated with a decrease of compound muscle action potential (CMAP)[1]. Intermediate NCV of 25-45 m/s can be difficult to classify as axonal or demyelinating[1, 3, 4].

Inheritance Pattern/Genetics:

Neuropathy can be caused by a genetic disorder, metabolic disease, trauma, infection, or other inflammatory and immune related events, and in some cases the cause is not known. The inherited neuropathies show a great deal of genetic and phenotypic heterogeneity, and can be inherited in an autosomal dominant, autosomal recessive or X-linked manner. A genetic etiology can be identified in approximately 50-70% of individuals with CMT[4]. Specifically, a molecular diagnosis can be identified in approximately 80-95% of individuals with demyelinating neuropathy (CMT1) and a molecular diagnosis can be identified in approximately 25-35% of individuals with axonal neuropathy (CMT2)[5,6]. The CMT Panel at GeneDx includes sequencing and deletion/duplication analysis of genes associated with CMT. The complete list of genes and associated disorders is included in the table below.

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.

Sequencing and deletion/duplication analysis of the remaining genes on the Hereditary Neuropathy Panel is available as a reflex test if the 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 following table. Of note, four genes, *PMP22*, *GJB1*, *MPZ* and *MFN2*, are implicated in over 90% of cases of CMT.[2] 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.

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Disease Associations	Gene	Protein	Inh	Diagnostic yield in selected populations
CMT2N	*AARS*	alanyl-tRNA synthetase	AD	Unknown[1,2]
Cowchock syndrome/CMTX4; Combined oxidative phosphorylation deficiency 6; X-Linked deafness 5	*AIFM1*	apoptosis inducing factor, mitochondria-associated 1	XL	Rare[3]
dHMN VA; Silver syndrome	*BSCL2*	seipin	AD	~7% of dHMN; unknown in CMT2[4]
Distal SMA type 5	*DNAJB2*	DNAJ/HSP40 homolog subfamily B member 2	AR	Rare[5]

Disease Associations	Gene	Protein	Inh	Diagnostic yield in selected populations
CMT2M; CMTDIB	*DNM2*	dynamin 2	AD	~3% of CMT[6]
CMT2O; Spinal muscular atrophy with lower extremity predominance	*DYNC1H1*	cytoplasmic dynein 1 heavy chain 1	AD	Unknown in CMT; reported in 3 families with spinal muscular atrophy with lower extremity predominance and in 5.5% of patients with malformations of cortical development[7,8]
CMT1D; DSD; CHN	*EGR2*	early growth response 2	AD/AR	<2% of patients with CMT1[9]
CMT4H	*FGD4*	Fyve, RhoGEF and PH domain-containing protein 4	AR	~3% of autosomal recessive CMT[10]
CMT4J	*FIG4*	FIG4 homolog, SAC1 lipid phosphatase domain containing S. cerevisiae	AR	<1% of patients with CMT[10,11]
Giant axonal neuropathy 1	*GAN*	gigaxonin	AR	6% of patients with CMT2[12]
CMT2D; dHMN VA	*GARS*	glycyl-tRNA synthetase	AD	~3% of patients with CMT2[11]
CMT4A; CMT2K; CMTRIA; Axonal CMT with vocal cord paresis	*GDAP1*	ganglioside-induced differentiation-associated protein 1	AD/AR	~1-5% of autosomal recessive CMT[10]
CMTX1	*GJB1*	gap junction protein B1; connexin 32	XL	~90% of X-linked CMT[11;13]
CMT1DIF	*GNB4*	guanine nucleotide-binding protein, beta-4	AD	Rare[14]
CMT2W	*HARS*	histidyl-tRNA synthetase	AD	Unknown[15]
Neuromyotonia and axonal neuropathy	*HINT1*	histidine triad nucleotide binding protein 1	AR	Up to 11% of autosomal recessive neuropathies in patients from Czech Republic,

Disease Associations	Gene	Protein	Inh	Diagnostic yield in selected populations
				Austria, Serbia, Bulgaria and Turkey[16]
CMT2F; dHMN IIB	*HSPB1*	heat shock 27 kDa protein 1	AD	~8% of patients with distal hereditary motor neuropathy (dHMN); ~4% of patients with CMT2[17]
CMT2L; dHMN IIA	*HSPB8*	heat shock 22 kDa protein 8	AD	Unknown in CMT2 and dHMNII[17,18]
CMT2S; SMARD/ HMNVI	*IGHMBP2*	immunoglobulin u binding	AR	~33% of patients with SMARD[19]; ~2% of CMT2[20]
CMTDIE; focal segmental glomerulosclerosis (FSGS)	*INF2*	inverted formin 2	AD	~75% of patients with CMT-FSGS[21]
Spastic paraplegia 10	*KIF5A*	kinesin heavy chain, neuron-specific	AD	~3% of familial HSP[22]; ~10% of complicated HSP in French European population[23]
CMT1C	*LITAF*	lipopolysaccharide-induced TNF factor gene	AD	1-2% CMT1[9]
CMT2B1	*LMNA*	lamin A/C nuclear-envelope proteins	AR	Rare in CMT2[24]
CMT2P	*LRSAM1*	leucine-rich repeat and sterile alpha motif-containing 1	AD/AR	Rare in CMT2[25,26]
CMT2A2A (AD); CMT2A2B (AR); HMSN VIA (AD)	*MFN2*	mitofusin 2	AD/AR	10-30% of CMT2[13;24]
CMT2T	*MME*	membrane metalloendopeptidase	AD/AR	~13% of patients with a clinical diagnosis of autosomal recessive CMT undergoing exome

Disease Associations	Gene	Protein	Inh	Diagnostic yield in selected populations
				sequencing[27]
CMT2Z	*MORC2*	MORC family CW-type zinc finger protein 2	AD	Rare in CMT2[28,29]
CMT1B; CMT2I; CMT2J; CMTDID; DSD; CHN	*MPZ*	myelin protein zero	AD	6-10% of patients with CMT1[11;30]; Rare in CMT2[24]
CMT4B1	*MTMR2*	mytotubularin-related protein 2	AR	Rare in autosomal recessive CMT[10]
CMT4D	*NDRG1*	N-myc downstream-regulated gene 1	AR	Rare in autosomal recessive CMT[10]
CMT1F; CMT2E	*NEFL*	neurofilament light	AD	~1% of CMT with onset during the first year of life[31]; 2%-5% of CMT2[11]
CMTRIC; dSMA 4	*PLEKHG5*	Pleckstrin homology domain-containing family C member 5	AR	Unknown[32,33]
CMT1A; CMT1E; HNPP; DSD	*PMP22*	peripheral myelin protein 22	AD	Duplication: ~70% of CMT113;34 Deletion: ~80% of HNPP35 Point mutations: <5% of CMT19 and 20% of HNPP35
CMTX5	*PRPS1*	phosphoribosylpyrophosphate synthetase 1	XL	Unknown36
CMT4F; DSD	*PRX*	periaxin	AR	~5% of autosomal recessive CMT10
CMT2B	*RAB7A*	RAS-associated protein 7	AD	Up to 7% HSAN patients37; rare in CMT224
CMT4B3	*SBF1*	SET binding factor 1; mytotubularin-related protein 5	AR	Rare in autosomal recessive CMT38
CMT4B2	*SBF2*	SET binding factor 2; mytotubularin-related protein 13	AR	~4% of autosomal recessive CMT10

Disease Associations	Gene	Protein	Inh	Diagnostic yield in selected populations
CMT4C	*SH3TC2*	SH3 domain and tetratricopeptides repeats 2	AR	~18% of CMT439
Agenesis of the corpus callosum with peripheral neuropathy	*SLC12A6*	solute carrier family 12 member 6	AR	Carrier frequency of 1/23 in the Charlevoix and Saguenay-Lac-St-Jean regions of Quebec40
CMT2R	*TRIM2*	tripartite motif-containing protein 2	AR	Rare in axonal neuropathy41
CMT2C; congenital distal SMA	*TRPV4*	transient receptor vallanoid 4	AD	Rare in CMT224
CMTDIC	*YARS*	tyrosyl-tRNA synthetase	AD	Unknown42

Abbreviations: AD – autosomal dominant; AR – autosomal recessive; CMT – Charcot-Marie-Tooth neuropathy; dHMN – distal hereditary motor neuropathy; FSGS – focal segmental glomerulosclerosis; HNPP – hereditary neuropathy with liability to pressure palsy; HSAN – hereditary sensory and autonomic neuropathy; HSP – hereditary spastic paraplegia; SMARD – spinal muscular atrophy with respiratory distress; XL – X-linked

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