Genetic Testing for Neuropathy: Core Charcot-Marie-Tooth Panel Sequence Analysis and Exon-level Deletion/Duplication

Panel Gene List: PMP22, MPZ, MFN2, and GJB1

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) [7]. Hypotonia, developmental delay, pes cavus, scoliosis and sensory ataxia are also commonly reported[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.

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 1.5Mb 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 scapuloperoneal 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 decade[6]. 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 and CMT2J[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 individuals[9]. 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 neuropathy[9]. Adie's pupil and hearing loss are reported in some patients[5]. Individuals with the later onset phenotype can have NCV consistent with axonal or demyelinating neuropathy[5].

MFN2: Pathogenic variants in MFN2 cause CMT2A, a progressive neuropathy that typically affects the lower limbs more severely than the upper limbs[11]. Motor NCV are typically normal or mildly decreased, consistent with axonal neuropathy[11]. 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 symptoms[12]. 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 hyperreflexia[11]. CMT6 is characterized by classic features of axonal CMT neuropathy and optic atrophy[1,12].

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 individuals[14]. 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 range[15].

Genetics:

The Core CMT Panel at GeneDx includes sequencing and deletion/duplication analysis of 4 genes causing Charcot-Marie-Tooth neuropathy. Pathogenic variants in the PMP22, MPZ, and MFN2 are inherited in an autosomal dominant manner while pathogenic variants in the GJB1 gene are inherited in an X-linked manner. 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 CMT1X are inherited, as only approximately 5% of pathogenic GJB1 variants are de novo[14]. The percentage of de novo variants in the MFN2 gene is currently unknown; however, most individuals have an affected parent[11].

Test Methods:

Using genomic DNA, coding exons and flanking splice junctions of the genes on this panel are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. Concurrently, targeted array CGH analysis with exon-level resolution is performed to evaluate for a deletion or duplication of one or more exons for the genes included on the panel. The presence of any potentially disease-associated sequence variant(s) or copy number alteration(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other appropriate methods. Sequencing and deletion/duplication analysis of the remaining genes on the Hereditary Neuropathy Panel is available as a separate 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 the sequencing test is estimated to be 98%. Deletions involving more than 20 bp and insertions involving more than 10 bp are not reliably detected by the sequencing methodology, and deletions or duplications of less than 500 bp are not reliably detected by array CGH. Note that small sections of a few genes have inherent sequence properties that result in suboptimal data and variants in those regions may not be reliably identified.

Gene	Protein	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
GJB1	gap junction protein beta 1; connexin 32	XL	CMTX1	~90% of X-linked CMT1[1,16]
MPZ	myelin protein zero	AD	CMT1B; CMT2I; CMT2J; CMTDID; DSD; CHN	6-10% of patients with CMT1[15,16]; Rare in CMT2[17]
MFN2	mitofusin 2	AD	CMT2A; HSAN VI	10-30% of CMT2[1,4]
PMP22	peripheral myelin protein 22	AD	CMT1A; CMT1E; HNPP; DSD	Duplication: ~70% of CMT1[1,2] Deletion: ~80% of HNPP[8] Point mutations: <5% of CMT1[5]; 20% of HNPP[6]

References:

1. Siskind et al. (2013) Journal of genetic counseling 22 (4):422-36 (PMID: 23604902).
2. Vallat et al. (2013) Current opinion in neurology 26 (5):473-80 (PMID: 23945280).
3. Murphy et al. (2012) Journal of neurology, neurosurgery, and psychiatry 83 (7):706-10 (PMID: 22577229).
4. Bird (Updated May 2015). Charcot-Marie-Tooth Hereditary Neuropathy Overview. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010. Available at http://www.genetests.org.
5. Bird (Updated March 2015). Charcot-Marie-Tooth Neuropathy Type 1. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010. Available at http://www.genetests.org.
6. Bird (Updated September 2014). Hereditary Neuropathy with Liability to Pressure Palsies. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010. Available at http://www.genetests.org.
7. Al-Thihli et al. (2008) American journal of medical genetics. Part A 146A (18):2412-6 (PMID: 18698610).
8. Baets et al. (2011) Brain : a journal of neurology 134 (Pt 9):2664-76 (PMID: 21840889).
9. Shy, et al. (2006) Journal of the neurological sciences 242 (1-2):55-66 (PMID: 16414078).
10. Mazzeo et al. (2008) Acta neurologica Scandinavica 118 (5):328-32 (PMID: 18422810).
11. Zuchner (Updated August 2013). Charcot-Marie-Tooth Neuropathy type 2A. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010. Available at http://www.genetests.org.
12. Chung et al. (2006) Brain 129: 2103-2118.
13. Verhoeven et al. (2006) Brain 129: 2093-2102.
14. Bird (Updated March 2015). Charcot-Marie-Tooth Neuropathy X Type 1. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010. Available at http://www.genetests.org.
15. Reilly et al. (2011) Journal of the peripheral nervous system: JPNS 16 (1):1-14 (PMID: 21504497).
16. Saporta et al. (2011) Annals of neurology 69 (1):22-33 (PMID: 21280)
17. Bird (Updated April 2015). Charcot-Marie-Tooth Neuropathy Type 2. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2010.
18. Nelis et al. (2006) Eur J Hum Genet 4(1): 25-33.