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REVIEW ARTICLE

Molecular Diagnostics of Charcot-Marie-Tooth Disease and Related Peripheral Neuropathies

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Abstract

DNA diagnostics plays an important role in the characterization and management of patients manifesting inherited peripheral neuropathies. We describe the clinical integration of molecular diagnostics with medical history, physical examination, and electrophysiological studies. Molecular testing can help establish a secure diagnosis, enable genetic counseling regarding recurrence risk, potentially provide prognostic information, and in the near future may be important for the choice of therapies.

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Index Entries: Molecular diagnostics; Charcot-Marie-Tooth disease; CMT; hereditary neuropathy with liability to pressure palsies; HNPP; Dejerine-Sottas neuropathy; DSN; congenital hypomyelinating neuropathy; CHN; CMT1A duplication; HNPP deletion.

Introduction

Molecular genetic diagnosis has become an integral part of the evaluation of patients with hereditary neuropathies. During the last decade, more than two dozen genes have been identified in which mutations cause Charcot-Marie-Tooth (CMT) disease and related neuropathies. When considering genetic testing, one needs to be familiar with the diagnostic tests available, choose the appropriate patients for testing, and utilize the diagnostic tools in a logical fashion to optimize the use of resources. This chapter summarizes the various methods used in clinical diagnosis and reviews an evidence-based testing scheme for molecular testing derived from population data.

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Epidemiology

CMT disease and related peripheral neuropathies, or hereditary motor and sensory neuropathies, represent a heterogeneous group of disorders affecting the peripheral nervous system with an estimated frequency of 1 in 2500 individuals (Skre, 1974). Epidemiological data from adult neuropathy clinics suggest that after the common causes of peripheral neuropathy associated with systemic illness, such as diabetes, uremia and nutritional deficiencies, hereditary polyneuropathy is more common than inflammatory or paraneoplastic polyneuropathy (Barohn, 1998; Dyck et al., 1981), and its prevalence might be as high as 29% (Barohn, 1998). Although it represents a relatively large group of patients manifesting neuropathy, inherited disease might not be readily apparent in recessive families or patients with *de novo* mutations. In fact, the high frequency of *de novo* duplication/deletion (37–90%) (Hoogendijk et al., 1992; Nelis et al., 1996) and point mutations (Boerkoel et al., 2002a) in sporadic neuropathy patients necessitates one having an index of suspicion for genetic disease even in the absence of a family history.

Diagnosis of CMT

The diagnostic process for evaluating a patient with suspected CMT starts with history and physical examination, which leads to the definition of the phenotype. Concomitantly, the inheritance pattern is determined from the pedigree. Further characterization of the neuropathy requires electrophysiology, which determines whether the neuropathy is primarily axonal, demyelinating, or the intermediate form. In clearly hereditary neuropathy with a known mutation in the family, the electrophysiology may be deemed unnecessary. Occasionally, sural nerve biopsy might be necessary, especially in cases in which there is no family history and clinical features raise the possibility of acquired neuropathy. Finally, other diagnostic tools are under evaluation, such as noninvasive magnetic resonance imaging of the peripheral nerve or skin biopsy for examining for small fiber neuropathy. Molecular testing is guided by the phenotype, inheritance pattern, electrophysiological data, and frequency data obtained in population-based studies (Szigeti et al., 2006).

Phenotype

The clinical picture is a combination of lower motor neuron-type motor deficits and sensory signs and symptoms. The lower motor neuron lesion manifests as the triad of flaccid paresis, atrophy, and reduced or absent reflexes. The severity of the disease ranges over a broad spectrum. CMT is included in the differential diagnosis from a floppy infant to an elderly patient with slowly progressive peripheral neuropathy. Depending on the age of onset, electrophysiological findings, and nerve pathology, several clinical phenotypes were classically defined (Lupski and Garcia, 2001). Features of specific clinical entities are reviewed later.

Adult-Onset Disease

Charcot-Marie-Tooth Disease Type 1

Most patients develop symptoms in the first or second decade. Patients have difficulties with motor function, including tripping on rough surfaces, inability to heel-walk, difficulty opening jars, difficulty buttoning. Secondary symptoms may develop, such as leg cramps and lumbar pain after long walks. Signs of chronic peripheral neuropathy such as tight heel cords, steppage gait, atrophic lower leg (peroneal atrophy), *pes cavus* (high arched feet), and hammertoes develop. The deep tendon reflexes are diminished or absent, weakness in distal muscle groups and mild sensory loss to pain develop. Neurophysiological testing shows uniform slowing of motor nerve conduction velocity (NCV) in all nerves examined. A conduction velocity less than 38 m/s is proposed as a cut-off value to distinguish between CMT types 1 (CMT1) and 2 (CMT2). A prospective study of patients with documented CMT1A duplication showed median NCV slowing to less than 43 m/s (Kaku et al., 1993). Sural nerve biopsy shows signs of demyelination and remyelination, characterized by onion bulb formation.

Roussy and Levy have described a combination of the demyelinating CMT phenotype with sensory ataxia and tremor, the Roussy–Levy syndrome (RLS). Molecular studies suggest that it might not be a distinct entity, rather the RLS phenotype is part of the CMT1 spectrum, as the same mutations may cause CMT1 or RLS in the same family (Harding and Thomas, 1980; Thomas et al., 1997; Auer-Grumbach et al., 1998; Garcia, 1999; Senderek et al., 1999).

The original family described by Roussy and Levy segregated an *MPZ* mutation (Planté-Bordeneuve et al., 1999).

Charcot-Marie-Tooth Disease Type 2

Clinical symptoms begin later than in CMT1, most commonly in the second decade of life, but often are delayed to middle age. The clinical features closely resemble those of CMT1, but foot deformities and upper limb involvement are less frequent. On clinical examination, it is often difficult to distinguish CMT1 from CMT2. Electrophysiology reveals normal or mildly reduced motor NCV with reduced sensory nerve action potentials. Nerve biopsy shows preferential loss of large myelinated fibers without signs of demyelination.

Hereditary Neuropathy With Liability to Pressure Palsies

Patients present with recurrent episodes of isolated mononeuropathies after relatively minor trauma, traction, or compression. The most frequently affected nerves in descending order are the common peroneal, ulnar, radial, and median nerves. Electrophysiological findings include nearly normal NCV with focal slowing in the ulnar nerve across the elbow and the peroneal nerve around the fibular head (Li et al., 2004). Concomitant with the palsies conduction blocks are found contributing to confusion with acquired neuropathy (Uncini et al., 1995).

Childhood-Onset Disease

Dejerine-Sottas Neuropathy

By definition, Dejerine-Sottas neuropathy (DSN) presents with motor developmental delay. Signs include hypotonia, weakness, and hyporeflexia or areflexia. In the demyelinating form, neurophysiological studies reveal severe slowing of NCV, usually less than 10 m/s. Neuropathology reveals more pronounced demyelination and a greater number of onion bulbs compared with CMT. Hypertrophic nerves can often be palpated in areas in which they come close to the skin surface. Cerebrospinal fluid proteins may be elevated.

Congenital Hypomyelinating Neuropathy

Congenital hypomyelinating neuropathy (CHN) presents at birth. However, it often gets recognized as motor developmental delay, and thus is difficult to differentiate from DSN on clinical grounds only. The distinction between DSN and CHN requires pathological evaluation, and is based on the presence or absence of onion bulbs associated with thin or absent myelin sheaths, respectively. CHN may present as arthrogryposis multiplex congenita (Boylan et al., 1992) suggesting prenatal onset.

Extended Phenotype

Classically, CMT and related neuropathies are disorders of the peripheral nervous system. If a patient exhibits symptoms and signs of central nervous system or systemic involvement, CMT is not likely. However, genotype–phenotype correlations have shown that other neurological and extraneurological signs can be part of the clinical phenotype with mutations in CMT genes. These include tremor, ataxia, glaucoma, cranial nerve involvement, scoliosis, and perhaps neutropenia. These findings do not preclude molecular testing for CMT. Furthermore, such features may point to the involvement of a specific gene and thus help direct molecular diagnostic testing.

Tremor is part of the RLS (Planté-Bordeneuve et al., 1999) and can be associated with mutations in *MPZ*, *GJB1*, and the CMT1A duplication (Harding and Thomas, 1980; Thomas et al., 1997; Auer-Grumbach et al., 1998; Garcia, 1999; Senderek et al., 1999). Ataxia, especially sensory ataxia, is part of the symptomatology of peripheral neuropathy, but true cerebellar ataxia is found in patients with *TDP1* mutations (Takashima et al., 2002). Special nerves might be affected in CMT, resulting in vocal cord paralysis (Klein et al., 2003; Sevilla et al., 2003) or respiratory insufficiency (phrenic nerve) (Hardie et al., 1990). Cranial nerve involvement may occur (Boerkoel et al., 2001; Szigeti et al., 2003). Hearing loss is associated with CMT caused by CMT1A duplication (Birouk et al., 1997), and with point mutations in *PMP22* (Kovach et al., 1999; Boerkoel et al., 2002b; Sambuughin et al., 2003), *MPZ* (Misu et al., 2000; Seeman et al., 2004), *GJB1* (Stojkovic et al., 1999), and *NDRG1* (Kalaydjieva et al., 1996; Kalaydjieva et al., 2000). Familial trigeminal neuralgia has been described in association with CMT (Coffey and Fromm, 1991). Scoliosis is a frequent finding in CMT as a group and can be associated virtually with any of the genes causing CMT. Subclinical white matter lesions are frequently observed in patients with *GJB1*

mutations (Nicholson and Corbett, 1996; Bähr et al., 1999), and expression of GJB1 in oligodendrocytes was shown (Kleopa et al., 2002). Spastic paraplegia has been observed in some patients with *MFN2* mutations (Zhu et al., 2005). *MFN2* mutations can cause optic atrophy as well (Züchner et al., 2004), and as its function is related to mitochondrial fusion, it is not an unexpected association. Early-onset glaucoma points to involvement of *SBF2* (Azzedine et al., 2003; Hirano et al., 2004). Neutropenia was observed in two families with *DNM2* mutations (Züchner et al., 2005).

Inheritance Pattern

CMT and related neuropathies exhibit all forms of mendelian inheritance—autosomal-dominant (AD), autosomal-recessive (AR), and X-linked. AD-CMT1 is the most frequent pattern observed (Lupski and Garcia, 2001). HNPP and RLS show AD inheritance, whereas CHN is AR or sporadic. DSN shows both AD and AR forms. Sporadic disease is often a result of a new mutation and thus the absence of a family history does not preclude molecular genetic testing.

Electrophysiology

Electromyography (EMG)/NCV establish the diagnosis of demyelinating (CMT1) or axonal (CMT2) neuropathy by measuring NCV. In demyelination, there is slowing of the NCV (<38 m/s), whereas in axonal loss the NCV of the intact fibers remains normal, but the compound muscle action potentials decrease. In some patients, the electrophysiology is less straightforward and delineates an intermediate form with features of both demyelination and axonal loss (Villanova et al., 1998). The intermediate type is more frequently associated with mutations in certain genes such as *GJB1*, *DNM2* (Züchner et al., 2005), and some loci (Verhoeven et al., 2001; Jordanova et al., 2003). Neurophysiological studies found both demyelinating and axonal features in patients with *MPZ* and *PMP22* mutations (Hattori et al., 2003).

Methods for Molecular Testing

Hereditary polyneuropathy is common and powerful diagnostic tests are clinically available. More than 35 loci and more than 24 genes have been identified in CMT and related peripheral neuropathies (Lupski and Garcia, 2001; Saifi et al., 2003), out of which clinical molecular testing is available for mutations in 10 genes and alteration of copy number in one gene (*PMP22*) at the current time. Two major groups of methods are available for the detection of the two major mutation types, genomic rearrangements (CMT1A duplication and HNPP deletion), or point mutations/small deletions or insertions.

Methods used for the detection of genomic rearrangements in clinical diagnostic laboratories can be divided into qualitative (binary) and quantitative detection methods (Lupski, 1996). The binary methods include the detection of junction fragments by pulsed-field gel electrophoresis (PFGE) or polymerase chain reaction (PCR) and microsatellite analysis evaluating for three alleles. Fluorescence *in situ* hybridization (FISH) is a binary method by definition (detection of one, two, or three signals), but the interpretation is based on establishing the quantity of cells having the appropriate number of signals. Quantitative methods include dosage analysis by Southern blot, multiplex ligation probe amplification (MLPA), real-time PCR- dependent and, semiquantitative fluorescent PCR.

When utilizing any of these methods, their reproducibility, sensitivity, specificity, labor-requirement, and failure rate has to be considered. In 2001, a study was conducted in the United Kingdom to assess the sensitivity and specificity of the various methods used by clinical diagnostic laboratories at that time (Rowland et al., 2001). These included microsatellite analysis, detection of the junction fragment by Southern blotting or PCR (REP-PCR), and semiquantitative fluorescent PCR (sequence-tagged sites [STS] dosage) (Rowland et al., 2001). The other methods did not have formal assessment, however, comparisons between the REP-PCR and the RT-PCR (Choi et al., 2005), and between MLPA and FISH (Slater et al., 2004) are available. The calculated or estimated sensitivity and specificity of these methods are summarized in Table 1.

The reproducibility as determined by concordance between two independent laboratories, was the highest (100%) for junction fragment by PCR and STS dosage with capillary electrophoresis. Junction fragment detection by Southern blot and microsatellite analysis had a 2% discordance rate, whereas STS dosage with PAGE had a 5% discordance rate (Rowland et al., 2001). Data for FISH, MLPA, and

Table 1
Molecular Diagnostic Methods to Detect CMT1A Duplication/HNPP Deletion

	Sensitivity (%)	Specificity (%)	Obtained
Junction fragment by Southern blot	86	100	Calculated
Junction fragment by PCR (REP-PCR)	84	100	Calculated
Microsatellite	98	93	Calculated
STS by capillary electrophoresis	100	100	Calculated
STS by PAGE	100	100	Calculated
FISH	100	100	Estimated
RT-PCR	100	100	Estimated
MLPA	100	100	Estimated

STS, sequence-tagged sites; FISH, fluorescence *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification.

RT-PCR are not available. As each method has high sensitivity and specificity, cost-effectiveness, reproducibility, and the laboratory's resources and experience are the major determinants when a method is selected by a diagnostic laboratory.

Diagnostic laboratories in the United States and Europe use many of these methods, and as new, more sensitive, and/or cost-effective methods are developed, the laboratories change their methodology. A recent voluntary quality assurance survey conducted in Europe had 36 participants (Rautenstrauss et al., 2005). Out of these 36 labs, 20 use microsatellite analysis, 8 MLPA, 7 Southern dosage, 6 semiquantitative PCR, 5 junction fragment by PCR, and 2 PFGE. Several labs use more than one method. In the United States, one major commercial laboratory has used PFGE but has changed to MLPA, whereas another offers FISH for CMT1A duplication/HNPP deletion testing. Array comparative genome hybridization (aCGH) has more recently been utilized to detect the CMF1A duplication/HNPP deletion (Cheung et al., 2005).

For point mutations, short sequence alterations (small insertions or deletions), the diagnostic method of choice is direct DNA sequencing. Denaturing high-performance liquid chromatography (DHPLC) is used in research laboratories only, and results always have to be confirmed by direct sequencing.

Evidence-Based Stepwise Aproach to Molecular Testing

Molecular diagnostics have increased the possibility of establishing a secure and specific diagnosis, provide for accurate recurrence rate estimates, and enable prenatal diagnosis, however, the expense associated with evaluating multiple genes has also escalated. When deciding on genetic testing one should consider multiple factors, including (1) availability of clinical testing, (2) the yield of a specific molecular test, (3) the specificity and sensitivity of the method used, (4) the aim of establishing a molecular diagnosis, and (5) in sporadic cases, the frequency of *de novo* mutations.

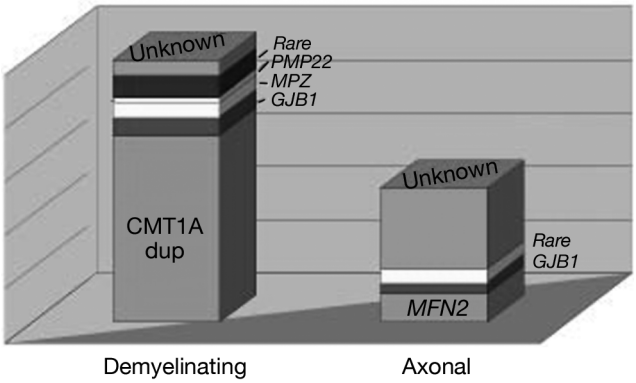

Fig. 1. The approximate expected yield of genetic testing in demyelinating and axonal CMT by using the depicted molecular testing.

Once the clinical phenotype is defined, including age of onset, demyelinating vs axonal vs intermediate form established, and inheritance pattern is determined, molecular testing should proceed. An evidence-based mutation distribution study (Szigeti et al., 2006) analyzing data from 11 population-based studies (Wise et al., 1993; Nelis et al., 1996; Bort et al., 1997; Janssen et al., 1997; Leonardis et al., 1998; Silander et al., 1998; Nicholson, 1999; Mersiyanova et al., 2000; Mostacciuolo et al., 2001;

Table 2
Molecular Testing Scheme in Familial CMT and Related Peripheral Neuropathies.

	Electrophysiology		
Inheritance	Demyelinating	Intermediate	Axonal
Autosomal dominant	***PMP22 dup*** 70% ***MPZ mut*** 5% ***PMP22 mut*** 2.5% *Others to consider:LITAF, NEFL, EGR2*	*DNM* *MPZ*	*MFN2* 20% ***MPZ*** *Others to consider:RAB7, GARS, NEFL, HSP27, HSP22*
Autosomal recessive	*Rare: PRX, GDAP1, EGR2, MTMR2, SBF2, NDRG1, SH3TC2* *Prioritize by extended phenotype*		*Rare: GDAP1, LMNA, TDP1* *Prioritize by extended phenotype*
X-linked	***GJB1*** 12%	***GJB1*** 12%	***GJB1*** 12%

Bold indicates frequencies for which population-based studies are available.

Boerkoel et al., 2002a; Choi et al., 2004; Marques et al., 2005) is depicted in Fig. 1.

In a demyelinating case, CMT1A duplication and *GJB1* mutation account for a significant fraction of patients and should be the tests for initial consideration. Duplication of a chromosomal segment harboring *PMP22* (i.e., the CMT1A duplication) (Lupski et al., 1991; Raeymaekers et al., 1991) represents 43% of the total CMT cases, whereas the yield of duplication detection rises to 70% in CMT1. The frequency of CMT1A duplication is fairly consistent across populations. Because of the predominance of this molecular defect drug toxicity studies (Nakamura et al., 2001; Naumann et al., 2001) addresses this population so far and the potential novel molecular therapeutic interventions (Sereda et al., 2003; Passage et al., 2004) are aiming at this group of patients as well. As clinical trials of these novel treatments are underway, the aim is to identify all subjects with the CMT1A duplication, therefore the test should be utilized as a screening test.

GJB1 mutation testing is appropriate in an even larger population of patients, as the neurophysiological phenotype can be intermediate, having features of both demyelination and axonopathy. As it has an X-linked dominant inheritance pattern, families in which male-to-male transmission is observed should not be tested for *GJB1* mutations. Genetic testing in families with *GJB1* mutations enables both genetic counseling and accurate estimation of recurrence risk. The population-based studies suggest that in patients with the demyelinating phenotype, *MPZ* and *PMP22* mutations are the next most common.

HNPP is a characteristic phenotype, although at the time of presentation, when the first entrapment syndrome occurs it might be difficult to recognize because entrapment neuropathies are common. Once the clinical diagnosis is established, molecular testing is straightforward, as the vast majority of cases have the HNPP deletion. Because the phenotype is characteristic, HNPP deletion testing is a confirmatory test. Occasionally, it can mimic multifocal neuropathy (Tyson et al., 1996; Tabaraud et al., 1999), a frequently inflammatory disorder that requires immunosuppressant therapy. The individuals with HNPP among this group of patients need to be identified in order to do no harm. Rare cases of HNPP without deletion can be found to have *PMP22* loss-of-function mutations.

In clearly axonal CMT, *MFN2* mutations are the most common, approx 20% of CMT2 cases (Züchner et al., 2004; Lawson et al., 2005; Reilly, 2005), followed by *GJB1* mutations, whereas *MPZ* is involved less frequently. In the third electrophysiological class, the intermediate form, there are features consistent with demyelination and axonal loss. This finding suggests the involvement of *GJB1*, *MPZ* (Hattori et al., 2003), or *DNM2* (Züchner et al., 2005). Mutations in other genes are responsible for the CMT phenotype in only a small minority of patients and population-based

Table 3
Molecular Testing Scheme in Sporadic CMT and Related Peripheral Neuropathies

Electrophysiology		
Demyelinating	Intermediate	Axonal
PMP22 dup *MPZ* *PMP22* *GJB1*	*MPZ* *GJB1*	*MFN2* *MPZ* *GJB1*
Rare: LITAF, EGR2 PRX, GDAP1, EGR2, MTMR2, SBF2, NDRG1, SH3TC2, NEFL *Prioritize by extended phenotype*	*Rare: DNM2* *Prioritize by extended phenotype*	*Rare: RAB7, GARS, NEFL, HSP27, HSP22, TDP1 GDAP1, LMNA* *Prioritize by extended phenotype*

data are not available. An evidence-based testing scheme guided by electrophysiology and inheritance pattern is depicted in Table 2.

The high frequency of *de novo* duplication/deletion (37–90%) (Hoogendijk et al., 1992; Nelis et al., 1996) and point mutations (Boerkoel et al., 2002a) necessitates having an index of suspicion for genetic disease even in the absence of a family history. A rational diagnostic approach in sporadic cases is presented in Table 3. Finally, when performing genetic testing, one must consider the specific question posed and the likelihood of whether the result alters medical management (Szigeti et al., 2006). In adults with the CMT phenotype, *PMP22* duplication and *GJB1* mutation analysis establishes the molecular diagnosis in 65% of patients. It identifies the candidates for the clinical trials and patients potentially at risk for idiosyncratic drug reactions. It also determines inheritance pattern establishing grounds for accurate genetic counseling and prenatal diagnosis. If patients with the demyelinating form are tested as a group, the diagnostic yield increases to more than 80% by performing *PMP22* duplication and *GJB1* mutation testing (Szigeti et al., 2006).

In the pediatric population, the major questions are prognosis and recurrence risk, thus an accurate molecular diagnosis is very important. All genes implicated in the given phenotype should be considered, but the testing in these cases should also be prioritized and performed in a serial manner to maximize the utilization of medical resources. The initial tests one has to consider are *PMP22* duplication and *GJB1* mutation testing, followed by panel testing according to the phenotype.

Limitations

Molecular testing occasionally identifies new sequence variations of unknown pathogenic significance (Szigeti et al., 2006). In these cases, further studies (segregation analysis, functional assay) are required to establish pathogenicity, which are performed in research laboratories. However, the ambiguous results affect only a small number of patients, not more frequently than borderline autoimmune test results. As natural history studies are lacking, and variable expressivity and age-dependent penetrance are key features of the inherited peripheral neuropathies, it is often difficult to answer questions regarding disability and impact on quality of life.

General Information and Educational Resources

Inherited Peripheral Neuropathies Mutation Database:
http://www.molgen.ua.ac.be/CMTMutations/

GeneClinics:
http://www.geneclinics.org

CMT Association:
2700 Chestnut Street
Chester, PA 19013-4867
610-499-9264 or 610-499-9265
1-800-606-CMTA (2682)
Fax 610-499-9247
http://www.charcot-marie-tooth.org

Muscular Dystrophy Association (MDA):
Muscular Dystrophy Association, USA
National Headquarters
3300 E. Sunrise Drive
Tucson, AZ 85718
(800) 572-1717
mda@mdausa.org

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Disclosure

JRL is a coinventor of patented molecular diagnostic test for CMT (US Patents 5,306,616; 5,599,920; 5,780,223) and a consultant for Athena Diagnostics.

References

Auer-Grumbach M., Strasser-Fuchs S., Wagner K., Korner E., and Fazekas F. (1998) Roussy-Levy syndrome is a phenotypic variant of Charcot-Marie-Tooth syndrome IA associated with a duplication on chromosome 17p11.2. *J. Neurol. Sci.* **154,** 72–75.

Azzedine H., Bolino A., Taieb T., et al. (2003) Mutations in MTMR13, a new pseudophosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma. *Am. J. Hum. Genet.* **72,** 1141–1153.

Bähr M., Andres F., Timmerman V., Nelis E., Van Broeckhoven C., and Dichans J. (1999) Central visual, acoustic and motor pathway involvement in a Charcot-Marie-Tooth family with an Asn205Ser mutation in the connexin 32 gene. *J. Neurol. Neurosurg. Psychiatry* **66,** 202–206.

Barohn R. J. (1998) Approach to peripheral neuropathy and neuronopathy. *Semin. Neurol.* **18,** 7–18.

Birouk N., Gouider R., LeGuern E., et al. (1997) Charcot-Marie-Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. *Brain* **120,** 813–823.

Boerkoel C. F., Takashima H., and Lupski J. R. (2002b) The genetic convergence of Charcot-Marie-Tooth disease types 1 and 2 and the role of genetics in sporadic neuropathy. *Curr. Neurol. Neurosci. Rep.* **2,** 70–77.

Boerkoel C. F., Takashima H., Bacino C. A., Daentl D., and Lupski J. R. (2001) EGR2 mutation R359W causes a spectrum of Dejerine-Sottas neuropathy. *Neurogenetics* **3,** 153–157.

Boerkoel C. F., Takashima H., Garcia C. A., et al. (2002a) Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation. *Ann. Neurol.* **51,** 190–201.

Bort S., Nelis E., Timmerman V., et al. (1997) Mutation analysis of the MPZ, PMP22 and Cx32 genes in Charcot-Marie-Tooth disease and hereditary neuropathy with liability to pressure palsies patients with Spanish ancestry. *Hum. Genet.* **99,** 746–754.

Boylan K. B., Ferriero D. M., Greco C. M., Sheldon R. A., and Dew M. (1992) Congenital hypomyelination neuropathy with arthrogryposis multiplex congenita. *Ann. Neurol.* **31,** 337–340.

Cheung S. W., Shaw C. A., Yu W., et al. (2005) Development and validation of a CGH microarray for clinical cytogenetic diagnosis. *Genet. Med.* **7,** 422–432.

Choi J. R., Lee W. H., Sunwoo I. N., Lee E. K., Lee C. H., and Lim J. B. (2005) Effectiveness of real-time quantitative PCR compare to repeat PCR for the diagnosis of Charcot-Marie-Tooth Type 1A and hereditary neuropathy with liability to pressure palsies. *Yonsei Med. J.* **46,** 347–352.

Choi B. O., Lee M. S., Shin S. H., et al. (2004) Mutational analysis of PMP22, MPZ, GJB1, EGR2 and NEFL in Korean Charcot-Marie-Tooth neuropathy patients. *Hum. Mutat.* **24,** 185, 186.

Coffey R. J. and Fromm G. H. (1991) Familial trigeminal neuralgia and Charcot-Marie-Tooth neuropathy. Report of two families and review. *Surg. Neurol.* **35,** 49–53.

Dyck P. J., Oviatt K. F., and Lambert E. H. (1981) Intensive evaluation of reffered unclassified neuropathies yealds improved diagnosis. *Ann. Neurol.* **10,** 222–226.

Garcia C. A. (1999) A clinical review of Charcot-Marie-Tooth. *Ann. NY Acad. Sci.* **883,** 69–76.

Hardie R., Harding A. E., Hirsch N., Gelder C., Macrae A. D., and Thomas P. K. (1990) Diaphragmatic weakness in hereditary motor and sensory neuropathy. *J. Neurol. Neurosurg. Psychiatry* **53,** 348–350.

Harding A. E. and Thomas P. K. (1980) Autosomal recessive forms of hereditary motor and sensory neuropathy. *J. Neurol. Neurosurg. Psychiatry* **43,** 669–678.

Hattori N., Yamamoto M., Yoshihara T., et al. (2003) Demyelinating and axonal features of Charcot-Marie-Tooth disease with mutations of myelin-related proteins (PMP22, MPZ and Cx32): a clinicopathological study of 205 Japanese patients. *Brain* **126,** 134–151.

Hirano R., Takashima H., Umehara F., et al. (2004) SET binding factor 2 (SBF2) mutation causes CMT4B with juvenile onset glaucoma. *Neurology* **63,** 577–580.

Hoogendijk J. E., Hensels G. W., Gabreëls-Festen A. A. W. M., et al. (1992) De-novo mutation in hereditary motor and sensory neuropathy type 1. *Lancet* **339,** 1081, 1082.

Janssen E. A. M., Kemp S., Hensels G. W., et al. (1997) Connexin32 gene mutations in X-linked dominant Charcot-Marie-Tooth disease (CMTX1). *Hum. Genet.* **99,** 501–505.

Jordanova A., Thomas F. P., Guergueltcheva V., et al. (2003) Dominant intermediate Charcot-Marie-Tooth type C maps to chromosome 1p34-p35. *Am. J. Hum. Genet.* **73,** 1423–1430.

Kaku D. A., Parry G. J., Malamut R., Lupski J. R., and Garcia C. A. (1993) Nerve conduction studies in Charcot-Marie-Tooth polyneuropathy associated with a segmental duplication of chromosome 17. *Neurology* **43,** 1806–1808.

Kalaydjieva L., Gresham D., Gooding R., et al. (2000) N-myc downstream-regulated gene 1 is mutated in hereditary motor and sensory neuropathy—Lom. *Am. J. Hum. Genet.* **67,** 47–58.

Kalaydjieva L., Hallmayer J., Chandler D., et al. (1996) Gene mapping in Gypsies identifies a novel demyelinating neuropathy on chromosome 8q24. *Nature Genet.* **14,** 214–217.

Klein C. J., Cunningham J. M., Atkinson E. J., et al. (2003) The gene for HMSN2C maps to 12q23-24: a region of neuromuscular disorders. *Neurology* **60,** 1151–1156.

Kleopa K. A., Yum S. W., and Scherer S. S. (2002) Cellular mechanisms of connexin32 mutations associated with CNS manifestations. *J. Neurosci. Res.* **68,** 522–534.

Kovach M. J., Lin J. -P., Boyadjiev S., et al. (1999) A unique point mutation in the PMP22 gene is associated with Charcot-Marie-Tooth disease and deafness. *Am. J. Hum. Genet.* **64,** 1580–1593.

Lawson V. H., Graham B. V., and Flanigan K. M. (2005) Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene. *Neurology* **65,** 197–204.

Leonardis L., Zidar J., Ekici A., Peterlin B., and Rautenstrauss B. (1998) Autosomal dominant Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies: detection of the recombination in Slovene patients and exclusion of the potentially recessive Thr118Met PMP22 point mutation. *Int. J. Mol. Med.* **1,** 495–501.

Li J., Krajewski K., Lewis R. A., and Shy M. E. (2004) Loss-of-function phenotype of hereditary neuropathy with liability to pressure palsies. *Muscle Nerve* **29,** 205–210.

Lupski J. R. (1996) DNA diagnostics for Charcot-Marie-Tooth disease and related inherited neuropathies. *Clin. Chem.* **42,** 995–998.

Lupski J. R. and Garcia C. A. (2001) Charcot-Marie-Tooth peripheral neuropathies and related disorders., in *The metabolic and molecular bases of inherited disease*, Scriver C. R., Beaudet A. L., Sly W. S., and Valle D., eds., McGraw-Hill, New York.

Lupski J. R., Montes de Oca-Luna R., Slaugenhaupt S., et al. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* **66,** 219–239.

Marques W., Jr., Freitas M. R., Nascimento O. J., et al. (2005) 17p duplicated Charcot-Marie-Tooth 1A: characteristics of a new population. *J. Neurol.* **252,** 972–979.

Mersiyanova I. V., Ismailov S. M., Polyakov A. V., et al. (2000) Screening for mutations in the peripheral myelin genes PMP22, MPZ and Cx32 (GJB1) in Russian Charcot-Marie-Tooth neuropathy patients. *Hum. Mutat.* **15,** 340–347.

Misu K., Yoshihara T., Shikama Y., et al. (2000) An axonal form of Charcot-Marie-Tooth disease showing distinctive features in association with mutations in the peripheral myelin protein zero gene (Thr124Met or Asp75Val). *J. Neurol. Neurosurg. Psychiatry* **69,** 806–811.

Mostacciuolo M. L., Righetti E., Zortea M., et al. (2001) Charcot-Marie-Tooth disease type I and related demyelinating neuropathies: Mutation analysis in a large cohort of Italian families. *Hum. Mutat.* **18,** 32–41.

Nakamura Y., Shimizu H., Nishijima C., Ueno M., and Arakawa Y. (2001) Delayed functional recovery by vincristine after sciatic nerve crush injury: a mouse model of vincristine neurotoxicity. *Neurosci. Lett.* **304,** 5–8.

Naumann R., Mohm J., Reuner U., Kroschinsky F., Rautenstrauss B., and Ehninger G. (2001) Early recognition of hereditary motor and sensory neuropathy type 1 can avoid life-threatening vincristine neurotoxicity. *Br. J. Haematol.* **115,** 323–325.

Nelis E., Van Broeckhoven C., De Jonghe P., et al. (1996) Estimation of the mutation frequencies in Charcot-Marie-Tooth disease type 1 and hereditary

neuropathy with liability to pressure palsies: a European collaborative study. *Eur. J. Hum. Genet.* **4,** 25–33.
Nicholson G. A. (1999) Mutation testing in Charcot-Marie-Tooth neuropathy. *Ann. NY Acad. Sci.* **883,** 383–388.
Nicholson G. and Corbett A. (1996) Slowing of central conduction in X-linked Charcot-Marie-Tooth neuropathy shown by brain stem auditory evoked responses. *J. Neurol. Neurosurg. Psychiatry* **61,** 43–46.
Passage E., Norreel J. C., Noack-Fraissignes P., et al. (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. *Nature Medicine* **10,** 396–401.
Planté-Bordeneuve V., Guiochon-Mantel A., Lacroix C., Lapresle J., and Said G. (1999) The Roussy-Levy family: from the original description to the gene. *Ann. Neurol.* **46,** 770–773.
Raeymaekers P., Timmerman V., Nelis E., et al. (1991) Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). *Neuromusc. Disord.* **1,** 93–97.
Rautenstrauss B., Nelis E., and O'Brian O. (2005) Final report of EMQN External Quality Assessment scheme for Charcot-Marie-Tooth disease.
Reilly M. M. (2005) Axonal Charcot-Marie-Tooth disease: the fog is slowly lifting! *Neurology* **65,** 186, 187.
Rowland J. S., Barton D. E., and Taylor G. R. (2001) A comparison of methods for gene dosage analysis in HMSN type 1. *J. Med. Genet.* **38,** 90–95.
Saifi G. M., Szigeti K., Snipes G. J., Garcia C. A., and Lupski J. R. (2003) Molecular mechanisms, diagnosis, and rational approaches to management of and therapy for Charcot-Marie-Tooth disease and related peripheral neuropathies. *J. Investig. Med.* **51,** 261–283.
Sambuughin N., de Bantel A., McWilliams S., and Sivakumar K. (2003) Deafness and CMT disease associated with a novel four amino acid deletion in the PMP22 gene. *Neurology* **60,** 506–508.
Seeman P., Mazanec R., Huehne K., Suslikova P., Keller O., and Rautenstrauss B. (2004) Hearing loss as the first feature of late-onset axonal CMT disease due to a novel P0 mutation. *Neurology* **63,** 733–735.
Senderek J., Hermanns B., Bergmann C., et al. (1999) X-linked dominant Charcot-Marie-Tooth neuropathy: clinical, electrophysiological, and morphological phenotype in four families with different connexin32 mutations(1). *J. Neurol. Sci.* **167,** 90–101.
Sereda M. W., zu Horste G. M., Suter U., Uzma N., and Nave K. -A. (2003) Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). *Nature Med.* **9,** 1533–1537.
Sevilla T., Cuesta A., Chumillas M. J., et al. (2003) Clinical, electrophysiological and morphological findings of Charcot-Marie-Tooth neuropathy with vocal cord palsy and mutations in the GDAP1 gene. *Brain* **126,** 2023–2033.
Silander K., Meretoja P., Juvonen V., et al. (1998) Spectrum of mutations in Finnish patients with Charcot-Marie-Tooth disease and related neuropathies. *Hum. Mutat.* **12,** 59–68.
Skre H. (1974) Genetic and clinical aspects of Charcot-Marie-Tooth's disease. *Clin. Genet.* **6,** 98–118.
Slater H., Bruno D., Ren H., et al. (2004) Improved testing for CMT1A and HNPP using multiplex ligation-dependent probe amplification (MLPA) with rapid DNA preparations: comparison with the interphase FISH method. *Hum. Mutat.* **24,** 164–171.
Stojkovic T., Latour P., Vandenberghe A., Hurtevent J. F., and Vermersch P. (1999) Sensorineural deafness in X-linked Charcot-Marie-Tooth disease with connexin 32 mutation (R142Q). *Neurology* **52,** 1010–1014.
Szigeti K., Garcia C., and Lupski J. R. (2006) Charcot-Marie-Tooth disease and related hereditary polyneuropathies: molecular diagnostics determine aspects of medical management. *Genet. Med.* **8,** 86–92.
Szigeti K., Saifi G. M., Armstrong D., Belmont J. W., Miller G., and Lupski J. R. (2003) Disturbance of muscle fiber differentiation in congenital hypomyelinating neuropathy caused by a novel myelin protein zero mutation. *Ann. Neurol.* **54,** 398–402.
Tabaraud F., Lagrange E., Sindou P., Vandenberghe A., Levy N., and Vallat J. M. (1999) Demyelinating X-linked Charcot-Marie-Tooth disease: unusual electrophysiological findings. *Muscle Nerve* **22,** 1442–1447.
Takashima H., Boerkoel C. F., John J., et al. (2002) Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat. Genet.* **32,** 267–272.
Thomas P. K., Marques W. Jr., Davis M. B., et al. (1997) The phenotypic manifestations of chromosome 17p11.2 duplication. *Brain* **120,** 465–478.
Tyson J., Malcolm S., Thomas P. K., and Harding A. E. (1996) Deletion of chromosome 17p11.2 in multifocal neuropathies. *Ann. Neurol.* **39,** 180–186.
Uncini A., Di Guglielmo G., Di Muzio A., et al. (1995) Differential electrophysiological features of neu-

ropathies associated with 17p11.2 deletion and duplication. *Muscle Nerve* **18,** 628–635.

Verhoeven K., Villanova M., Rossi A., Malandrini A., De Jonghe P., and Timmerman V. (2001) Localization of the gene for the intermediate form of Charcot-Marie-Tooth to chromosome 10q24.1-q25.1. *Am. J. Hum. Genet.* **69,** 889–894.

Villanova M., Timmerman V., De Jonghe P., et al. (1998) Charcot-Marie-Tooth disease: an intermediate form. *Neuromusc. Disord.* **8,** 392, 393.

Wise C. A., Garcia C. A., Davis S., N. et al. (1993) Molecular analyses of unrelated Charcot-Marie-Tooth (CMT) disease patients suggest a high frequency of the CMT1A duplication. *Am. J. Hum. Genet.* **53,** 853–863.

Zhu D., Kennerson M. L., Walizada G., Zucher S., Vance J. M., and Nicholson G. A. (2005) Charcot-Marie-Tooth with pyramidal signs is genetically heterogeneous: Families with and without MFN2 mutations. *Neurology* **65,** 496, 497.

Züchner S., Mersiyanova I. V., Muglia M., et al. (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* **36,** 449–451.

Züchner S., Noureddine M., Kennerson M., et al. (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat. Genet.* **37,** 289–294.