



Clinical and Genetic Survey for Charcot-Marie-Tooth Neuropathy Based on the Findings in Turkey, a Country with a High Rate of Consanguineous Marriages

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Inherited peripheral neuropathies (IPNs) are a heterogeneous group of disorders of the peripheral nervous system. The most common type of IPN is Charcot-Marie-Tooth (CMT) disease, which constitutes an interesting research focus for neurologists and human geneticists alike. Most cases with CMT manifest with a slowly progressive symmetric distal weakness in the lower limbs that usually begin in the first to the third decade that causes atrophy and foot drop. Deep tendon reflexes are usually absent or reduced. A proven and efficient CMT therapy is yet available and may require different molecules and approaches due to its high clinical and genetic heterogeneity. Several ongoing clinical trials are promising and are mostly focused on the most frequent form, namely CMT Type 1A (CMT1A). Approximately, 60% of patients with CMT can be genetically diagnosed using the most advanced mutation screening techniques that cover approximately

100 IPN genes. Turkey has a 25% consanguineous marriage rate, and nearly 60% genetic diagnosis rate can still be reached when SH3 Domain and Tetratricopeptide Repeat Domain 2, Ganglioside-induced Differentiation-Associated Protein 1, and Histidine Triad Nucleotide Binding Protein 1 genes are also screened along with Myelin Protein Zero and Gap Junction Protein Beta-1 after exclusion of CMT1A duplication in families with probable recessive inheritance. The genetic diagnosis rates in different regions worldwide implicate that the most recent sequencing techniques should be more commonly used for both diagnosis and identification of further CMT genes. Herein, presented our 30 years of experience on genetic diagnosis and management strategies in CMT neuropathy in Turkey and review clinical and genetic features of this group of disorders.

Inherited peripheral neuropathies (IPNs) are a diverse group of disorders of the peripheral nervous system where neuropathy could be the only symptom or could be part of a multisystemic disorder. The most common type of IPN is Hereditary Motor and Sensory Neuropathy, a group of disorders that are more commonly called Charcot-Marie-Tooth (CMT) disease.¹ The disease is named after the clinicians who originally reported the clinical features simultaneously in 1886, namely, French neurologist Jean-Martin Charcot, his student Pierre Marie, and British neurologist Howard Henry Tooth.² CMT affects both motor and sensory axons, whereas motor axons are predominantly affected in hereditary motor neuropathy (HMN) and sensory and autonomic nerves in hereditary sensory/autonomic neuropathy.¹ These three disorders represent a phenotypic continuum and are collectively termed CMT and related neuropathies.³

Clinical Features

CMT is a clinically and genetically heterogeneous group of neurological disorders. Age of onset may be from birth to late adulthood and the symptoms may encompass a wide range of phenotypes.⁴ The hallmark of the disease is slowly progressive, symmetrical nerve degeneration, which results in chronic muscle weakness and wasting from the distal limbs^{4,5} as well as atrophy and foot drop. As the disease progresses, patients may develop distal upper limb weakness and skeletal deformities, such as pes cavus, hammertoes, and kyphoscoliosis, that are accompanying clinical features. Foot deformities may result in contractures, which cause further gait difficulties. Sensory symptoms are rare; however, they may be apparent in the neurological examination. Deep tendon reflexes are usually absent or reduced.^{2,4,5} Neuropathy is generally



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easily diagnosed in patients who present with distal muscle weakness and sensory loss starting at the feet and slowly ascending to the knees and hands. However, the findings should also be confirmed with nerve conduction studies.^{2,6} Developmental history evaluation of the individual, such as delayed motor milestones, poor performance in sports, lag behind peers, and difficulty of shoe-fitting during childhood, is essential in the clinical history.^{1,6}

Widespread population analyses on CMT epidemiology are still limited; therefore the true prevalence is currently speculative. Early estimates reported that 1 in 2500 individuals worldwide is affected by CMT⁷; however, most recent reports from different regions worldwide suggest variable prevalence rates. A systematic review on CMT epidemiology analyzed 802 studies and reported that CMT prevalence must be 9.7–82.3 in 100.000 individuals worldwide with no ethnic predisposition.⁸

Classification and Molecular Genetics

The molecular genetic era in the CMT field began in 1982 with the identification of the first disease-causing locus.⁹ In 1991, came the discovery of a 1.4-Mb tandem duplication of a region on chromosome 17 containing the Peripheral Myelin Protein 22 (*PMP22*) gene, which was the first report of an individual CMT causative gene and was classified as CMT Type 1A (CMT1A).¹⁰ The Human Genome Project data publication and subsequent next-generation sequencing (NGS) technology advances at the turn of

the century have led to a great acceleration in CMT discovery that causes genes and mutations. More than 90 causative genes have been reported at the time of writing. The genetic findings indicate that all modes of inheritance are possible for CMT, including autosomal dominant, autosomal recessive, X-linked, and maternal inheritance (Figure 1). The clinical and genetic heterogeneity of the disease is evident in observations and indicates different causative genes with similar clinical features or different mutations in the same gene with different disease subtypes, such as demyelinating and axonal pathologies.⁴ This second feature can be exemplified with Ganglioside-induced Differentiation-Associated Protein 1 (*GDAP1*) gene mutations that can be responsible for demyelinating form in one family but for axonal form in another. Even the same *GDAP1* mutation, p.D149Y, may present with demyelination or axonal loss.¹¹

CMT is a term for a group of multiple related neuropathies, thus a classification was required from the discovery of this disease. Historically, CMT is classified into two broad groups based on nerve conduction studies of patients. Decreased nerve conduction velocities suggest myelin dysfunction and are generally interpreted as nerve hypomyelination or demyelination, whereas decreased compound muscle action potential amplitudes suggest axonal damage and/or loss of nerve fibers.¹² The clinical setting defined CMT1 as the demyelinating form of the disease, whereas CMT2 is the axonal form.⁴ The patients are diagnosed with demyelinating CMT (CMT1) when the upper limb motor nerve conduction

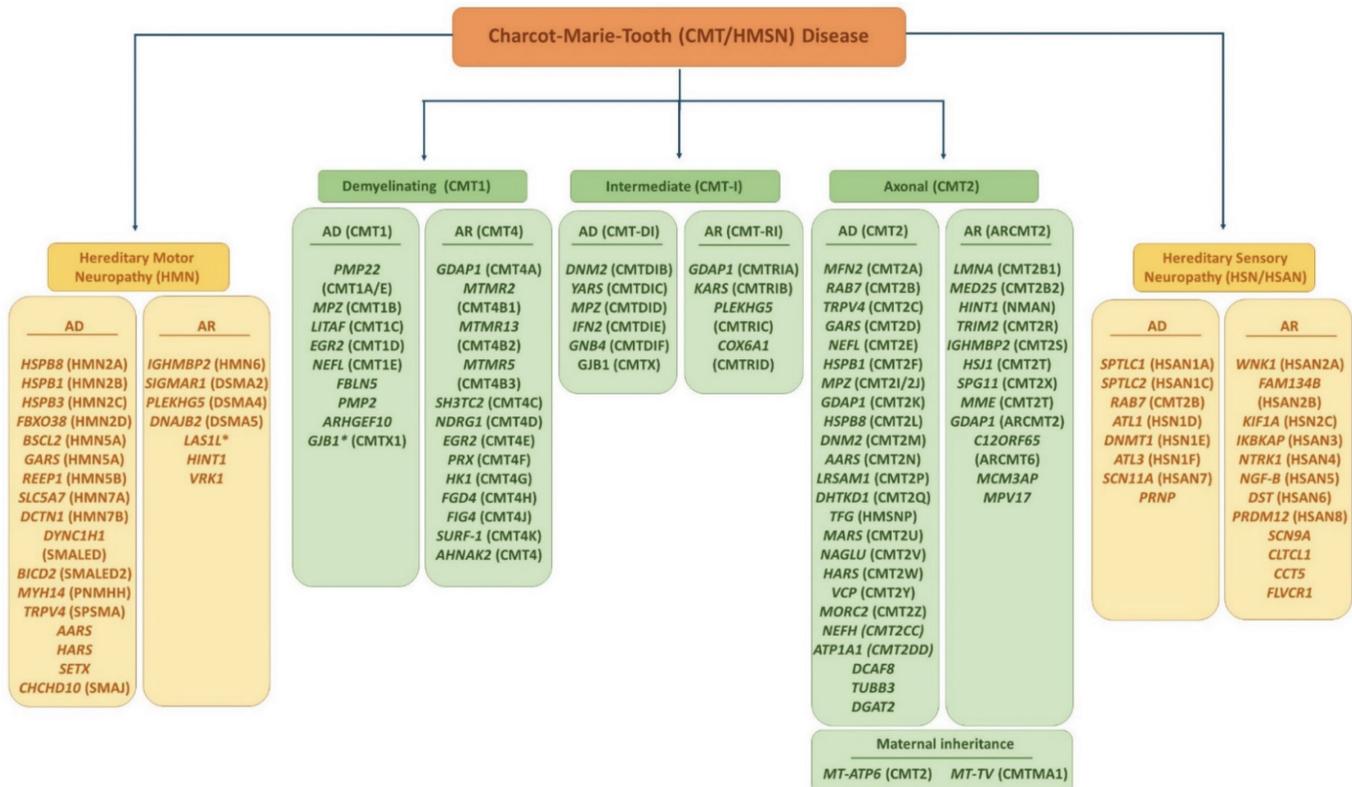


FIG. 1. An overview of subtype classification of Charcot-Marie-Tooth Disease and related neuropathies based on electrophysiological findings, inheritance pattern and causative gene.

CMT: Charcot-Marie-Tooth; AD: autosomal dominant; AR: autosomal recessive

velocity (mNCV) is <38 m/s, whereas the diagnosis is axonal CMT (CMT2) if mNCV is >38 m/s with reduced compound muscle action potential amplitudes (4). Later, a new subtype was defined as “intermediate CMT” (CMT-I) for patients with an upper limb mNCV of 25–45 m/s.¹³ Both axonal and demyelinating disease types will eventually result in an axonal loss in the later stages.¹⁴

Following the advancements in the field of human genetics, the identification of causative genes for CMT led to a further subclassification that combines electrophysiological findings, inheritance patterns, and causative genes.¹⁵ Accordingly, CMT1 and CMT4 groups consist of demyelinating types of the disease with dominant and recessive inheritance, respectively, whereas CMT2 and Autosomal Recessive Axonal Charcot-Marie-Tooth Disease (ARCM2) consist of axonal types of the disease with dominant and recessive inheritance, respectively. Moreover, causative genes are reflected in disease nomenclature with a certain letter for each gene. Figure 1 gives an overview of this common classification. Nevertheless, the advent of NGS technologies greatly accelerated the gene discovery rate, causing this classification to become less straightforward. The issue became even more complicated with discoveries that suggest that different mutations in the same gene may have different inheritance patterns or may cause different CMT phenotypes as in the case of *GDAP1* mutations.^{16,17} Recently, a new classification system was proposed to include all known information on a case, such as inheritance type, phenotypical disease form, and the causative gene using abbreviations for each.¹⁸

Nonetheless, this new classification system has not been fully implemented in the field yet.

This study aimed to review the clinical and genetic aspects of different CMT subtypes below and share our data accordingly for each of these forms. Our cohort consists of >1350 patients with IPN; however, clinical CMT diagnosis was confirmed in 645 cases. Of whom, 459 cases (71%) presented with demyelinating and 186 (29%) with the axonal form of the disease.

CMT1: CMT1 is defined as a dominantly inherited demyelinating subtype of the disease. In this disease form, the Schwann cell function and myelin sheath formation are affected.^{1,4} The most common type of CMT1 is CMT1A, which is approximately 70% of all demyelinating cases and 40% of all CMT cases.¹ CMT1A is caused by a 1.4 Mb tandem duplication of chromosome 17p11.2, which includes the *PMP22* gene.¹⁰ Deletion of the same region on chromosome 17p11.2 results in Hereditary Neuropathy with Liability to Pressure Palsies (HNPP).¹⁹ Point mutations in the *PMP22* gene are also disease-causing in 1–5% of demyelinating cases and this subtype is termed CMT1E.^{1,6} Other common disease-causing genes in CMT1 include *Gap Junction Protein Beta-1 (GJB1)* on the X-chromosome (Xq13.1) causing CMTX1 and *Myelin Protein Zero (MPZ)* on chromosome 1 causing CMT1B. CMTX1 has a prevalence of approximately 10% among demyelinating cases, with males being more severely affected than females possibly due to random X-inactivation.^{20,21} Contrarily, CMT1B cases are approximately 5% of all CMT cases.⁶ Other CMT1 causative genes include *LITAF* (CMT1C), *EGR2* (CMT1D), *NEFL* (CMT1F),

FBLN5, and *PMP2* (CMT1G) that are rarely mutated.³

In our population, genetic studies revealed the same frequencies of these gene mutations in which CMT1A duplication (151 cases) is followed by X-linked form (*GJB1* mutations in 47 cases), then by *MPZ* (6 cases), and finally by *PMP22* (3 cases). The rest of the CMT1 genes have either one or no patients. Another four patients with *PMP22* mutations presented with HNPP. The distribution of CMT gene mutations in our cohort of 645 cases is summarized in Figure 2.

CMT2: The dominantly inherited axonal disease form is called CMT2. The causative genes in this subtype have predominant impacts on neuronal function, metabolism, and maintenance and their mutations result in axonal degeneration.^{1,4} The axonal subtype of the disease is less common than demyelinating types.⁸ Both dominant and recessive axonal CMT is caused by many but rarely mutated genes that are reported in a small number of families.²² Pathogenic mutations in *Mitofusin 2 (MFN2)* gene on chromosome 1p36.22 makes up the most common cause of CMT2 with a prevalence of nearly 20% in all axonal cases.²³ Heterozygous mutations in the *MFN2* gene are classified as CMT2A2A²³, whereas a rare and more severe disease form called CMT2A2B is caused by homozygous or compound heterozygous mutations in the same gene.²⁴ Other CMT2 genes include *KIF1B* (CMT2A1), *RAB7* (CMT2B), *TRPV4* (CMT2C), *GARS* (CMT2D), *NEFL* (CMT2E), *HSPB1* (CMT2F), *GDAP1* (CMT2K), *HSPB8* (CMT2L), *DNM2* (CMT2M), and *AARS* (CMT2N).³

Our cohort includes 15 patients with *MFN2* gene mutations, with only two patients having homozygous mutations in this gene. The rarity of homozygosity for *MFN2* mutations even in a population with a high rate of consanguinity, as in the case of our population, may indicate a considerably high rate for *de novo* mutations in this gene at the population level. Our studies mostly focused on CMT1 and in general on recessive cases, thus a high number of CMT2 cases were not analyzed. Still, two cases were identified with *NEFL*, two cases with *HSPB1*, and single cases with *KIF1B*, *DNM2*, and *AARS* mutations. Among all cases with *GDAP1* mutations, only one case had a heterozygous causative mutation, indicating the presence of dominant axonal CMT (CMT2).

CMT4: The demyelinating and recessively inherited CMT subtype is termed CMT4. This is a very rare and genetically highly diverse subtype.²⁵ These patients almost always have very severe clinical phenotypes with early onset of symptoms.² Homozygous mutations in *GDAP1* cause CMT4A, which is the most common subtype among CMT4. This is followed by mutations in *SH3 Domain and Tetratricopeptide Repeat Domain 2 (SH3TC2)* causing CMT4C.²⁶ Other genes causing recessive demyelinating CMT include *MTMR2* (CMT4B1), *MTMR13/SBF2* (CMT4B2), *MTMR5/SBF1* (CMT4B3), *NDRG1* (CMT4D), *EGR2* (CMT4E), *PRX* (CMT4F), *HK1* (CMT4G), *FGD4* (CMT4H), and *FIG4* (CMT4J).³

Our cohort with demyelinating phenotype has *SH3TC2* as the most commonly mutated gene with 17 cases. *GDAP1* mutations were associated with demyelinating disease form in nine patients. Additionally, four of these cases had c.786delG mutation, reflecting

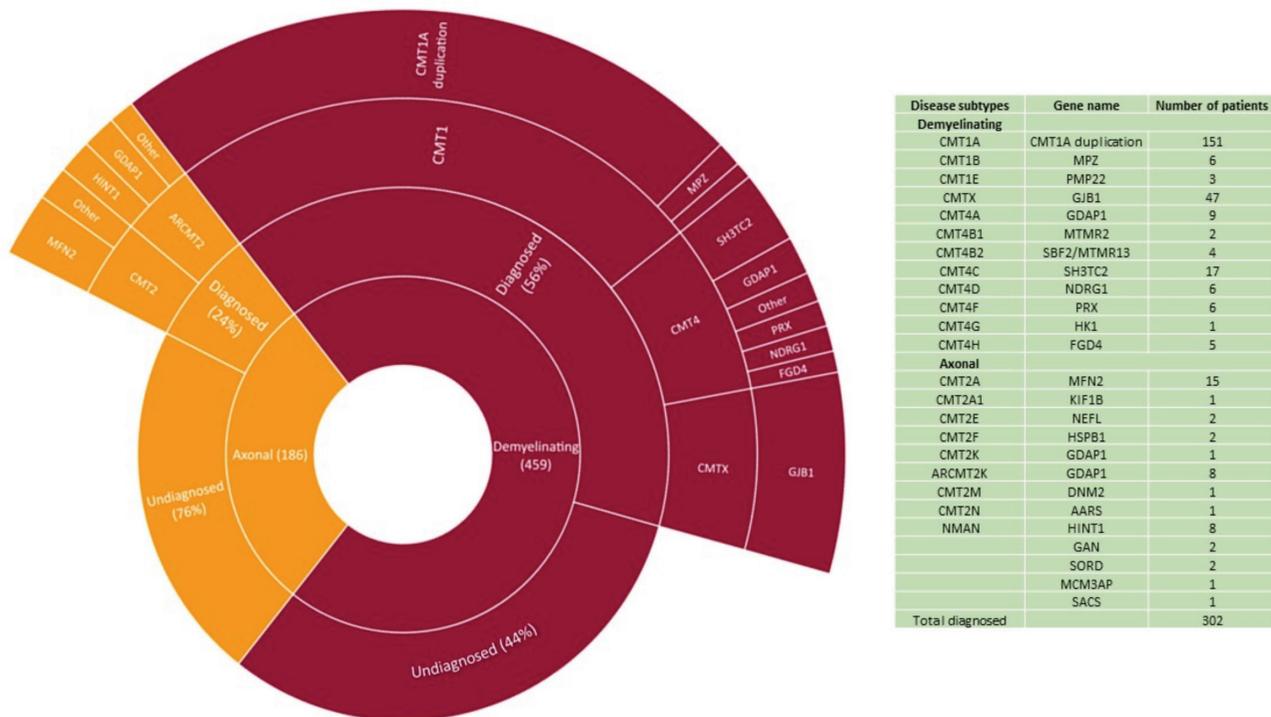


FIG. 2. Distribution of demyelinating and axonal phenotypes and mutations in the cohort of 649 Turkish patients with Charcot-Marie-Tooth disease. CMT: Charcot-Marie-Tooth

the possibility of a founder effect. The second most commonly mutated genes were *PRX* and *NDRG1* with six cases each, followed by *FGD4* (5 cases), *MTMR13/SBF2* (4 cases), *MTMR2* (2 cases), and a single case of *HK1*.

ARCMT2: The recessively inherited axonal CMT disease is a very rare subtype termed ARCM2.¹ Mutations in *Histidine Triad Nucleotide Binding Protein 1 (HINT1)* are disease-causing in approximately 10% of all recessive CMT cases. Interestingly, this gene is responsible for nearly 80% of axonal neuropathy cases that present with neuromyotonia.^{27,28} Other genes that cause ARCM2 include *LMNA* (CMT2B1), *MED25* (CMT2B2), *TRIM2* (CMT2R), *IGHMBP2* (CMT2S), *HSJ1* (CMT2T), *SPG11* (CMT2X), *MME*, *GDAP1*, and *C12ORF65*.³

HINT1 gene mutations were identified in eight families among the patient with ARCM2 in our cohort based on presentation with neuromyotonia. The most commonly mutated gene in our ARCM2 cohort was *GDAP1* with nine families presenting axonal neuropathy. Two families had *GAN* and another two had *SORD* gene mutations. Single cases with mutations in *SPG11*, *MME*, and *C12ORF65*, *MCM3AP*, and *SACS* were also identified.

CMT-I: CMT cases with mNCV values of 25–45 m/s are classified as CMT-I.¹³ Median NCV values may be different in different nerves of the same patients or among other affected family members.¹ Disease-causing genes in CMT-I may be dominantly (CMT-DI) or recessively (CMT-RI) inherited, which include *MPZ*, *GJB1*, *DNM2* (CMTDIB), *YARS* (CMTDIC), *IFN2* (CMTDIE), *GNB4* (CMTDIF), *GDAP1* (CMTRIA), *KARS* (CMTRIB), *PLEKHG5*

(CMTRIC), and *COX6A1* (CMTRID).³ None of our cases with *MPZ* mutations, but almost all cases with *GJB1* mutations had mNCV values of 25–45 m/s. Almost all axonal cases with *GDAP1* mutations had mNCV values in this range. Thus, they can be accepted as CMT-I, as well as CMT1 or CMT2.

Molecular Mechanisms Leading to Charcot-Marie-Tooth Disease

Demyelinating and axonal forms of the disease may be clinically distinguished using nerve conduction studies, but pathologies are also well-established to eventually converge into a final pathway, which results in axonal degeneration and muscle denervation.¹⁴ The pathology may either be due to mutations that alter nerve function or their proper myelination. The influence of these genes in disease progression is another focus in CMT research. The genes implicated in CMT pathogenesis belong to a vast range of functional classes, including structural components of myelin, signaling proteins, proteins in mitochondrial dynamics, cytoskeletal proteins, and proteins in axonal transport. Understanding the molecular mechanisms is important since they may provide molecular targets for therapeutic approaches.

CMT1A, as the most common subtype, has historically been the leading subject in experimental research. The discovery that the duplication of the 17p11.2 locus causes CMT1A, and its deletion causes HNPP, made it clear that a gene-dosage mechanism was responsible for the pathology. The *PMP22* gene located in this locus produces a small integral membrane protein in Schwann

cells, which is a major myelin component.²⁹ Similarly, MPZ, the causative gene for CMT1B, encodes for a transmembrane protein that makes up >50% of all proteins purified from the myelin sheaths.³⁰ The myelin may be destabilized when the ratio between *PMP22* and *MPZ* gene products is altered.³¹ The *Pmp22-null* mice have myelination to some extent; however, frequent tomacula formation was observed in nerve biopsies.³² Contrarily, observations in *Mpz-null* mice suggest that *MPZ* is essential for myelination and membrane compaction.³³ An interesting finding implicated that overexpressed *PMP22* causes formation of ubiquitinated aggregates in late endosomes both in vitro and in vivo. Cellular response against misfolded proteins showed to be the cause of perturbed Schwann cell function due to overloaded protein degradation machinery.³⁴ Another breakthrough discovery made through CMT1A, CMT1B, and CMTX mouse models revealed that immune cells, particularly T cells and macrophages, were involved in demyelination during disease progression, perhaps while attempting to repair myelin defects due to pathogenic mutations.^{35,36}

Peripheral neurones have exceptionally long axons that require high energy and regular transport of specific cargo; therefore, healthy mitochondria and proper axonal transport are essential for these cells. Mitochondrial dynamics, a collective term for mitochondrial fusion and fission, describes the regulation of shape, size, number, and transport of mitochondria, which is fundamental for the proper distribution of these organelles along the axons.³⁷ Numerous CMT causative genes are implicated in this regulation, among which *MFN2* and *GDAP1* are the most well-established genes. *MFN2*, together with *MFN1*, regulates mitochondrial fusion, whereas *GDAP1* regulates mitochondrial fission. Pathogenic mutations in these genes lead to improper distribution of these organelles and/or abnormally shaped mitochondria, which are likely incapable of performing fundamental tasks. Therefore, cytosolic calcium imbalance and increased oxidative stress occur, leading to axonal degeneration and neuronal death.^{37,38}

Axonal transport of vesicles and organelles along microtubules is also essential for proper neurone function. As expected, axonal neuropathy is observed due to mutations in important members of the neurofilament family (*NEFL* and *NEFH* genes).^{39,40} Additionally, *RAB7*, which encodes for a protein that acts as a regulator of vesicular transport, also causes axonal CMT.⁴¹ Similarly, the *DNM2* gene, underlying intermediate CMT, encodes for a protein involved in receptor-mediated endocytosis, actin assembly, and membrane trafficking.⁴²⁻⁴⁴ Mutations in the heavy chain of dynein motor protein (*DYNC1H1*) and in a part of a multi-subunit complex protein, which binds dynein (*DCTN1*), are associated with axonal CMT and distal HMN, respectively.^{45,46} Both *DYNC1H1* and *DCTN1* were vital for microtubule-mediated axonal transport.⁴⁷ Moreover, *HSPB1*, *HSPB3*, and *HSPB8* coding for small heat shock proteins were also associated with axonal CMT and distal HMN. They act as molecular chaperones and may be involved in actin and intermediate filament assembly.⁴⁸⁻⁵⁰ The identification of numerous CMT genes that are involved in cytoskeletal function and assembly further implies axonal transport dysregulation as a common disease mechanism.

Genetic Diagnosis Strategies in Charcot-Marie-Tooth Disease

Correct molecular diagnosis of inherited neuropathies can only be achieved by active communication between clinicians and molecular geneticists. Once a neuropathy diagnosis is established by clinical examination and electrophysiological findings, looking for evidence of a genetic origin is the next step. Clues for genetic origin are relatively easy to find in large families with multiple cases but could be challenging in isolated cases or adopted individuals.⁶ Therefore, family history should be thoroughly investigated with specific questions on developmental milestones of the affected individual and their physical performance in childhood. Acquired neuropathy could be distinguished from genetic neuropathy using clinical markers, such as asymmetrical weakness, specific electrophysiological clues, magnetic resonance imaging, and cerebrospinal fluid protein level.⁶ The patient should be referred to molecular diagnosis only with suspicious inherited neuropathy. Genetic testing should not be offered to eliminate the possibility of an inherited neuropathy since the causative gene and mutation cannot be identified even in >40% of inherited cases due to its heterogenic nature. Additionally, it causes unnecessary labor and financial loss.

Multiple tools are available for genetic testing in CMT disease. One should address several issues to correctly choose a strategy. Initially, the likely mode of inheritance must be determined. Autosomal recessive inheritance is likely with multiple affected individuals in the same generation and/or parental consanguinity in the pedigree. X-linked inheritance is excluded in male-to-male transmission. In more challenging pedigrees, such as small families and sporadic cases, autosomal dominant or *de novo* dominant inheritance are considered in Northern Europe and North America, whereas in regions with high consanguinity rates, like Turkey, autosomal recessive inheritance can still be possible.⁶ Axonal neuropathy with strict maternal inheritance indicates mitochondrial DNA mutations.^{51,52} Meanwhile, informing the molecular geneticist on the axonal or demyelinating pathology or predominantly motor, sensory, or both pathology is crucial since these different phenotypes, alongside other indicators, may direct the geneticist to look for certain genes.⁶

Sequential screening of known genes: Genetic testing strategies based on mutational frequency have been suggested by multiple studies, which use sequential screening of most causative genes based on clinical features of patients.^{15,20,53,54} This is generally a high-yield-low-cost strategy since several genes are responsible for a great number of cases.¹⁵ For instance, *PMP22* duplication is shown to be responsible for nearly 40% of all CMT cases, thus it is the initial target for molecular testing.^{20,54} When the patient has demyelinating neuropathy and is negative for *PMP22* duplication, testing for *GJB1* mutations is reasonable without the male-to-male transmission in the pedigree and followed by testing for *MPZ* mutations. For axonal neuropathy cases, mutations in the *MFN2* gene could be initially screened.²⁰ Studies that retrospectively analyze large patient cohorts revealed that mutations in four genes (*PMP22*, *MPZ*, *GJB1*, and *MFN2*) were the underlying cause in >90% of all diagnosed CMT cases.⁵⁵⁻⁵⁷ Therefore, initial screening

of these four genes before moving to more advanced tools is highly advised.⁵⁷ Additionally, certain genes could be considered for initial screening when the patient has some distinct clinical features. For instance, neuromyotonia is established as a very frequent symptom in patients with recessive axonal neuropathy with causative mutations in the *HINT1* gene.²⁸ Likewise, patients with demyelinating neuropathy with *SH3TC2* mutations frequently present with scoliosis or kyphoscoliosis.⁵⁸ Similarly, diaphragmatic dysfunction and vocal cord paresis are frequently observed in patients with *GDAP1* mutations.⁵⁹ Therefore, initial screening of these genes may be pivotal in patients with relevant modes of inheritance and clinical features.

NGS: Sequential screening strategy may be time- and cost-effective in demyelinating cases; however, the presence of many individually rare disease-causing genes in axonal neuropathies may cause the procedure to become very expensive and cumbersome.⁶ The development and commercialization of NGS technologies caused a shift in the trend for genetic diagnosis strategies. High-throughput DNA sequencing, more commonly referred to as NGS, describes the massively parallel sequencing of DNA fragments.⁶⁰ This technology is an upgrade to the first generation of DNA sequencing (Sanger sequencing) and generates sequence data using “sequencing by synthesis.” This method utilizes initial random shearing of genomic DNA and capture of sheared fragments in separate chambers or through adaptors, followed by the amplification of fragments using modified nucleotides. Finally, short-read sequence data are generated by detecting incorporated nucleotides on each synthesis round.⁶⁰

NGS technologies may be used for whole-genome sequencing (WGS), for targeted sequencing of certain genes/loci in the genome (gene panels), or sequencing of only the protein-coding regions of the genome termed, whole-exome sequencing (WES).⁶ NGS technology choice will depend on the purpose of the analysis. When using gene panels, high-quality sequence data will be obtained by screening a small set of genes, which allows easy genetic diagnosis if the patient has a pathogenic mutation in one of the genes present in the gene panel. Using WES, sequencing data with relatively less read-depth will be obtained from a large set of genes, and this unbiased sequencing technique identifies novel causative genes in individuals without a recurrent gene mutation.⁶

Currently, the use of gene panels is the common practice in large diagnostic centers for the sequencing of recurrent genes in the molecular diagnosis of CMT disease.⁶¹⁻⁶⁵ CMT panels covering genes associated with its specific subtypes are increasingly discouraged due to the presence of phenotypically overlapping features between different CMT subtypes and neurological disorders, such as inherited ataxias, hereditary spastic paraplegias, and distal myopathies.⁶³ Nevertheless, the development of large gene panels, including all known CMT-causing genes, will drastically reduce costs for sequencing and improve the characterization of genotype-phenotype correlation in CMT. Moreover, gene panels will likely unveil less common inheritance patterns, such as digenic inheritance.⁴ The gene hunt for CMT disease is not over, thus the gene panels currently do not have full coverage of all

relevant genes and loci and are not widely commercially available. However, this technology is shortly expected to entirely replace sequential Sanger sequencing.

According to our experience, the most effective approach in CMT molecular diagnosis is to use sequential sequencing after excluding CMT1A duplication and then WES or WGS as the last step. Sequential screening can be substituted with a panel to screen for most commonly mutated CMT genes; however, it would be more expensive compared to sequential screening. In Turkey, just considering recessive cases, both demyelinating and axonal forms reached a 60% success rate in molecular diagnosis with initial screening for *GDAP1* mutations followed by WES. Initial filtering of the WES data with relaxed filtering criteria (MAF < 0.05) for known neurology disease genes has a major contribution to the success rate since it identifies the mutations in the genes that overlap with other neurological diseases like hereditary spastic paraplegia and amyotrophic lateral sclerosis.⁶⁶

WES has been an exceedingly popular tool in the past decade for novel disease-causing gene identification. The technology allows unbiased sequencing of nearly all protein-coding regions with high read-depth; however, it generally results in nearly 20,000 single nucleotide variants and small indels for each individual for which the analysis and interpretation require great expertise that limit its widespread use in the clinical setting.⁶³ Still, WES is a great tool for an unbiased novel gene hunt since 85% of all mutations were suggested to cause Mendelian disorders in the protein-coding of 1%–2% of the human genome.⁶⁷ Meanwhile, some research centers perform WGS for families that remain undiagnosed after WES analysis, though WGS is more costly compared to WES for novel gene identification.⁶⁸

Third-generation sequencing, also called large fragment single-molecule sequencing, is currently the most advanced sequencing technology.⁶⁰ This technology does not require fragment amplification and allows the long molecule sequencing (up to 30–50 kb) in a single run instead of clusters of amplified short DNA sequences. This helps avoid the problems that arise from GC-rich region amplification and results in a more evenly distributed coverage along the genome.⁶⁹ This technology is not yet widely common due to high cost and novelty; however, it is likely to identify novel causative genes/mutations and non-conventional disease mechanisms in CMT disease, especially in regions that are poorly covered by short-read sequencing technologies, including non-coding DNA sequences, large deletions, insertions, repeat sequences, inversions, and translocations.⁷⁰

Genetic diagnosis success in CMT disease has rapidly increased as the gene discovery rate accelerated following the development of high-throughput sequencing technologies.⁴ Genetic diagnosis rate ranges from 18% to 31% in studies that used gene panels^{61,64,65,71}, whereas 45% to 60% in studies that utilize WES.^{16,20,54,72} One striking observation is the great diagnostic gap between demyelinating and axonal CMT. For instance, a study published by Inherited Neuropathy Consortium (INC) reviewed 1652 patients from 13 INC centers and revealed a total genetic diagnosis rate of 60.4%; however, the diagnosis rate was 91.4%

in CMT1 cases, whereas 42% in CMT2 cases.⁷³ Likewise, another study on 1206 patients from Germany reported genetic diagnosis success in 56% of CMT1 cases and 17% in CMT2 cases.⁵³ The total genetic diagnosis rate was 47% (302/645) in our cohort; however, not all patients in the cohort could be analyzed by WES. Sequential sequencing was used for selected cases based on clinical data and pedigree analysis. Still, the *GJB1* mutations constitute 10% of cases (47/459), reflecting the use of a correct strategy in testing for the relevant genes. The genetic diagnosis was possible in approximately 56% (257/459) in demyelinating (CMT1 and CMT4) and 24% (45/186) in axonal forms (CMT2 and ARCT2), in line with the report from Germany. The strategy also helped to unravel novel genes in our cohort, including *MFN2* and *SH3TC2*, *HINT1*, *FGD4*, *MCM3AP*, and *AHNAK2*, and a novel phenotype segregating with a homozygous missense mutation in the *FXN* gene.^{23,27,74-78}

The missing heritability indicates the disease-causing genes yet to be identified, especially in CMT2, and non-Mendelian aspects of CMT disease, such as modifier genes and multigenic inheritance, that may be uncovered in the future.

Treatment

No proven efficient therapy for CMT is available. Clinical trials are compelling to design due to the rather slow progression and the heterogeneity of the disease. Nonetheless, several still ongoing trials are promising, of which most are focused on CMT1A as the most frequent form. These studies aimed to reduce the *PMP22* expression to overcome the effects of CMT1A duplication.

Progesterone is known to increase *PMP22* expression and ascorbic acid enhances myelination.^{79,80} Thus, progesterone antagonists and ascorbic acid are known to be the first candidates for CMT1A treatment. Several transgenic mouse models that overexpress *PMP22* have been developed and testing these molecules gave promising results; however, human trials have failed to reproduce these results.^{81,82} Antisense oligonucleotide suppression of *PMP22* mRNA levels in CMT1A and gene replacement therapy in loss-of-function mutations are recent emerging therapies.⁸³ Another therapeutic approach to lower the toxic *PMP22* gene overexpression PXT3033 molecule is used in combination with gamma aminobutyric acid-B receptor agonist baclofen, opioid receptor antagonist naltrexone, and intracellular metabolite D-Sorbitol.⁸⁴ Neurotrophin-3 (NT-3) has been shown to enhance axonal regeneration along with associated myelination in animal models.⁸⁵ AAV1.NT-3 surrogate gene therapy phase 1/2a clinical trial is underway to improve nerve regeneration in CMT1A.⁸⁶

Several other mouse models are produced to develop novel treatment options. Niacin-mediated Tace activation in a mouse model was shown to ameliorate CMT neuropathies with focal hypermyelination such as CMT4B1 and HNPP.⁸⁷ Another study of a mouse model with CMT1A revealed Curcumin to restore myelinated axons.⁸⁸ A small molecule acting as *MFN2* agonist in CMT2A and intrathecal gene therapy for different *GJB1* mutations were also designed and are currently tested.^{89,90}

In conclusion, gene therapy approaches to deliver different therapeutic molecules to patients in different ways or silencing the genes with allele-specific oligonucleotides are all promising therapies that are expected to be successful shortly. Having some of such therapeutic products in the market for neurological disorders, such as spinal muscular atrophy, increases the expectations for other similar disorders and CMT. Unraveling the mutated gene in each CMT patient is necessary to choose one of these personalized treatment options as a preliminary step. Genetic diagnosis should start with CMT1A duplication, followed sequentially with *MPZ* and *GJB1* in demyelinating CMT in Europe and the United States. However, populations with a high rate of consanguinity also need to consider *SH3TC2*, *GDAP1*, and *HINT1* genes after excluding CMT1A duplication in families with probable recessive inheritance. Today, approximately 60% of patients with CMT can get a genetic diagnosis, even though approximately 100 IPN genes have been identified. This finding implicates the need to use more advanced techniques in genetic diagnosis and identification of further CMT genes, like third-generation sequencing approaches.

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