NEUROGENETIC ANALYSIS OF HEREDITARY NEUROPATHIES IN THE ERA OF GENOMIC MEDICINE

Ph.D. thesis

György Máté Milley M.D.

Neuroscience Doctoral School
Semmelweis University

Supervisor: Anikó Gál Ph.D.

Official reviewers: Dóra Nagy Ph.D.
Eszter Jávorszky Ph.D.

Head of the Final Examination Committee: Miklós Szabó M.D. Ph.D.

Members of the Final Examination Committee: Bence Gunda MD. Ph.D.
György Báthori MD. Ph.D.

Budapest
2019

Table of Content

The list of Abbreviations

ACMG – American College od Medical Genetics and Genomics

ADOA – Autosomal dominant optic atrophy

ALS – Amyotrophic lateral sclerosis

ANA – Anti-nuclear antibody

ANCA – Anti-neutrophil cytoplasmic antibodies

ANS – Autonomic nervous system

ax – Axonal

CCFDN – Congenital cataracts, facial dysmorphism, and neuropathy

CDSMA – Dominant congenital spinal muscular atrophy

CHN – Congenital hereditary neuropathy

CI95% - 95% confidence interval

CIDP – Chronic inflammatory demyelinating polyradiculoneuropathy

CK – Creatinine kinase

CMAP – Compound muscle action potential

CMT – Charcot-Marie-Tooth disease

CMTES – Charcot-Marie-Tooth examination score

CMTNS – Charcot-Marie-Tooth neuropathy score

CNS – Central nervous system

CNV – Copy number variant

CSA – Cross-sectional area

CSF – Corticospinal fluid

CTDP1 – C-terminal domain of RNA polymerase II subunit A

Cx32 – Connexin 32

de – Demyelinating

dHSN – Distal hereditary motor neuropathy

DI-CMT – Dominant intermediate Charcot-Marie-Tooth disease

DNA – Deoxyribonucleic acid

DSS – Déjerine-Sottas syndrome

EGR2 – Early growth factor 2

EM – Electron microscopy

EMG – Electromyopgraphy

ENG – Electroneurography

ENMG – Electroneuromyography

FSGS – Focal segmental glomerulosclerosis

GDAP1 – Ganglioside-induced differentiation-associated protein 1

GJB1 – Gap junction beta 1

GTF – General transcription factor

HBV – Hepaitis B virus

HCV – Hepatitis C virus

HIV – Human immunodeficiency virus

HMSN – Hereditary sensory and motor neuropathy

HNPP – Hereditary neuropathy with pressure palsy

HR1/2 – Helical hapted repeat regions

HSAN – Hereditary sensory and autonomic neuropathy

HSN – Hereditary Sensory Neuropathy

HSP – Hereditary spastic paraplegia

in – Intermediate

INF2 – Inverted Formin 2

IVIg – Intravenous immunoglobulins

LDH – Lactate dehydrogenase

MADSAM – Multifocal acquired demyelinating sensory and motor neuropathy

MAG – Myelin-associated glycoprotein precursor

MFN2 – Mitofusin 2

MGUS – Monoclonal gammopathy with uncertain significance

MIRAS – Mitochondrial recessive ataxia syndrome

MLPA – Multiplex ligand probe assay

MME – Membrane metalloendopeptidase

MMN – Multifocal motor neuropathy

MNCV – Mean nerve conduction velocity

MRC – Medical Research Council

MRI – Magnetic resonance imaging

mtDNA – Mitochondrial deoxyribonucleic acid

NADH – Nicotinamid adenine dinucleotide

NCS – Nerve conduction study

NCV – Nerve conduction velocity

nDNA – Nuclear deoxyribonucleic acid

NDRG1 - N-myc downstream regulated 1

NEFL – Neurofilament L

NGS – New generation sequencing

NMD – Neuromuscular disorder

OR – Odds ratio

p.s. – Present study

P/F – Patient per family ratio

P0/MPZ – Myelin protein zero

PCR – Polymerase chain reaction

PEO – Progressive external ophtalmoplegia

PMP22 – Peripheral myelin protein 22

PNS – Peripheral nervous system

POLR2A – RNA polymerase 2 subunit A

qPCR – Quantitative polymerase chain reaction

RFLP – Restricted fragment length polymorphism

RLS – Roussy-Lévy syndrome

RNA – ribonucleic acid

SANDO – Sensory ataxia neuropathy dysarthria and opthalmoplegia

SAP – Sensory action potential

SLE – systematic lupus erythematosus

SMA – Spinal muscular atrophy

SMN1 – Survival of motor neuron 1

SNP – Singe nucleotide polymorphism

SNV – singe nucleotide variant

SPSMA – Scapuloperoneal spinal muscular atrophy

SPTLC1/2 – Serine palmitoyltransferase, long chain base subunit 1/2

TM1/2 – Transmembrane anchor domain ½

TRPV4 – Transient receptor potential cation channel subfamily V member 4

TSH – Thyroid stimulating hormone

UMN – Upper motor neuoron

1. Introduction

Peripheral neuropathy is a term for a group of conditions in which the peripheral nerves are damaged. Nerve damage can impair the muscle strength, the sensation, and different organ functions. In general, peripheral neuropathies can be classified according to the (i) number of affected nerves – mononeuropathy (one), multifocal neuropathy (multiple) or polyneuropathy (numerous nerves); (ii) pattern of impairment – symmetric or asymmetric (iii) type of involved nerves – motor, sensory or both types of nerves; (iv) type of nerve lesion – demyelinating, axonal or mixed, and (v) course of neuropathy – acute or chronic condition (1).

Numerous nerves are damaged in polyneuropathy at the same time resulting in broad spectrum of clinical symptoms. Length-dependent nerve degeneration causes the first symptoms in limbs, spreading proximal and worsening progressively. Common causes of polyneuropathy are (i) diabetes, (ii) alcohol abuse, (iii) infection or (iv) drug related nerve damage while other possible etiology with a lower incidence can be (v) tumors, (vi) metabolic and (vii) autoimmune diseases or (viii) hereditary disorders (1).

Hereditary neuropathies are chronic conditions affecting symmetric the motor and/or sensory nerves. It is one of the most common inherited neurodegenerative disorders, affecting approximately every one person from 2500 (2). The relative homogenous clinical appearance of the disease is associated with an especially wide genetic background. Charcot-Marie-Tooth disease is the eponym of hereditary motor and sensory neuropathy but related disorders – hereditary distal motor neuropathy (dHMN) and hereditary sensory neuropathy (HSN) – are also considered as subgroups of CMT (3, 4).

The expanding knowledge about hereditary neuropathies has broken with the previous conventions and indicated more recent perspectives in the last two and half decades. Because of the extensive size of the topic and the scarce extent of the space, figures, flow charts, and tables are welcomed to use for detailed and easily understandable demonstration of topics.

1.1 Historical overview

The first descriptions of the inherited distal muscle weakness and wasting, calling it *peroneal muscular atrophy*, have been reported by Jean Martin Charcot with Pierre Marie and by Howard Henry Tooth in 1886, separately (5). Later, Johann Hoffman described a histological finding of thickened nerves in a case with peroneal muscular atrophy in 1912 (6). From this point, the disorder is also referred as Charcot-Marie-Tooth-Hoffmann disease.

In 1888, Herringham, ahead of his time, has observed a family in which only males were affected supposing a distinct genetic feature between males and females (7). In the following century, the existence of X-linked inherited neuropathy has been questioned by Harding (8); however, it has been later ascertained that the X-linked dominant form of CMT (CMTX1) is the second most common genetic cause of hereditary sensorimotor neuropathies. Eponyms, like Roussy-Lévy syndrome (RLS) and Déjerine-Sottas syndrome (DSS), has become obsolete in the last decades as well, after the genetic analyses have proved the same genetic background with CMT (9-13). In spite of that, Hereditary Neuropathy with Pressure Palsy (HNPP) has a distinct genetic cause and different clinical features than CMT so HNPP is still considered as an independent entity among hereditary motor and sensory neuropathy (HMSN) (14).

Nerve biopsy and pathological diagnosis meant to be the main diagnostic approach in the early decades of the 20th Century. Since the late 1970s, electroneuromyography (ENMG) has become a ubiquitous diagnostic tool providing new frontiers of non-invasive diagnosis, classification and efficient follow-up of hereditary and other neuropathies (15). Recently, nerve ultrasound and magnetic resonance imaging seem to be a new trend to assign the need for genetic testing, further improving the diagnostic algorithm of neuropathies and also proved their reliability in follow-up studies (5).

The genetic era has officially begun in 1991 with the identification of the first disease causing genetic variant in the *PMP22* gene (16). Since that time, the research has been accelerated and it is still at a bold pinnacle of discoveries of novel genes and genomic mechanisms [Fig. 1]. Until now, more than 90 genes have been related to hereditary neuropathies, however, there is a remarkable hiatus in the list of genes. The extended use

of next generation sequencing started in 2009 which provided a decent catalyst for novel findings (4).

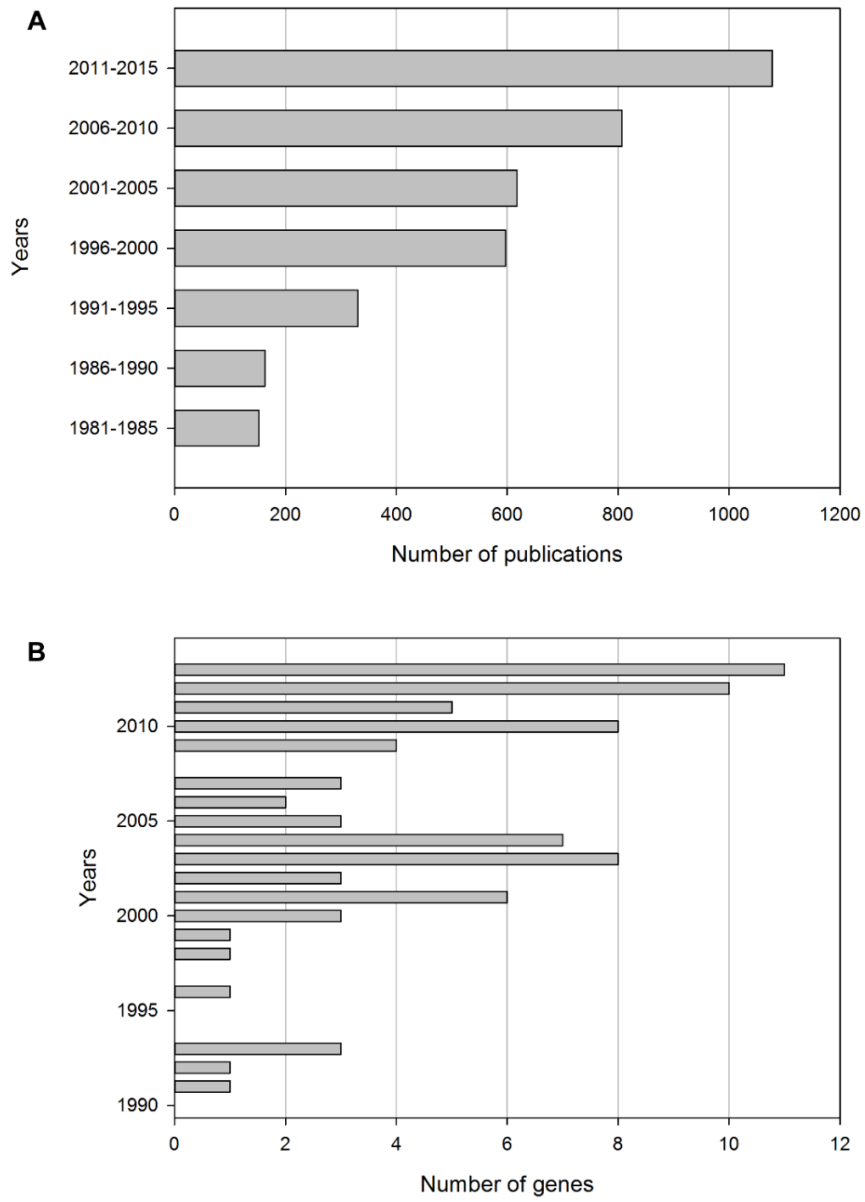


Fig. 1 A: The diagram indicates the extension of the literature related to Charcot-Marie-Tooth neuropathies in the previous decades. The number of publication (y axis) was determined by using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/). Data are visualized in five years divisions from 1981 to 2015 and they are arranged in chronological order (x axis). B: The diagram shows the number of discovered genes per year related to hereditary neuropathy (y axis) since 1991. The number of genes is showed per year arranged in decreasing order (x axis). Data are based on supplement 1.

1.2 Classification

The classification of hereditary neuropathies had undergone numerous changes depending on the prevailing views about the disease. The first subsets were based on the clinical description of the disease, usually used their eponyms for naming them. The expanding knowledge and the novel diagnostic possibilities have later required new approaches leading to further reconsideration of the actuel classificiations. Nowadays, the genetic classification is reckoned as the most sophisticated way of categorization but this detailed subdivision is quite a challenge in the daily practice (1) [Table 1, Suppl. 1].

Table 1. The different classifications of hereditary motor and sensory neuropathies since the first descriptions. **Notes:** *a:* it has been first introduced by Dyck and revised by Thomas and Harding in 1980 (17); *b:* The nerve conduction velocity (NCV) of the intermediate form is also referred to 30-40 m/s. Mixed pathological findings should be also a cirterion (18).

Original descriptions of inherited neuropathies	According to the clinical and pathological features along with the inheritance pattern (from 1968) [a]	According to the nerve conduction studies (from 1980)	Genetic classification (from 1997)
Charcot-Marie-Tooth-(Hoffmann) disease (peroneal muscular atrophy)	**HMSN type I** (autosomal-dominant form with low conduction velocities and segmental demyelination and remyelination)	**CMT1** (demyelinating form, NCV <38m/s)	**CMT1** autosomal dominant myelinopathy
Déjerine-Sottas disease (autosomal recessive, severe and early onset hypertrophic neuropathy with CSF protein elevation)	**HMSN type II** (autosomal dominant form with normal nerve conduction velocities and amplitude-reduction while nerve pathology shows axonal features)	**CMT2** (axonal form NCV >38m/s)	**CMT2** autosomal dominant and recessive axonopathy
Roussy-Lévy syndrome (inherited hypertrophic neuropathy and tremor)	**HMSN type III** (Déjèrine-Sottas disease)	**CMTI**[b] (intermediate form) NCV 25-45m/s	**DI-CMT** intermediate form
Congenital Hypomyelinating Neuropathy (neonatal hypotonia)	**HMSN type IV** (autosomal recessive form of hereditary motor and sensory neuropathies)	**HSAN and dHMN** (depending on the severity of motor or sensory deficiency)	**CMT4** either myelinopathy or axonopathy, recessive forms
Hereditary Neuropathy with Pressure Palsy (focal neuropathy in predilection spots)	**HMSN type V** (HMSN and spastic paraplegia are simultaneously present)		**CMTX** X-linked inheritance
	HMSN type VI and VII (HMSN with optic atrophy and/or pigmentary retinopathy)		**dHMN** overlapping forms with HMSN
	dHMN or HSAN[d] (depending on the severity of motor or sensory deficiency)		**HSAN** overlapping forms with HMSN

In addition, the genetic classification of CMT2 has reached and overcame the physical barriers of alphabets, therefore, its modification or expansion needs to be considered as well.

All the classification are presently used in different combinations to describe the disease accurately. Determining of genetic etiology is not always possible and genetic testing needs to be designed carefully. Prior the testing, multiple factors should be taken into account: (i) patient's phenotype (ii) country-specific genetic epidemiology data, (iii) availability of genetic screening, (iv) methodology, (v) aim of genetic testing (differential diagnostics, preconception, presymptomatic or prenatal counseling, therapy etc.) (1, 4).

1.3 Symptoms and signs

Charcot-Marie-Tooth neuropathy affects fundamentally both the motor and sensory nerves. It is characterized by length dependent nerve degeneration which slowly progresses with time leading to worsening of the condition and a gradually developing disability (19). It starts usually in the first three decades of life (20).

The symptoms of classical phenotype occur primarily in the areas of the nerve damage. Classical phenotype is characterized by distal muscle weakness (impaired tip toe and heel walking, paretic gait, difficulty using zippers and buttons as well as clumsiness in manipulating small objects), muscle atrophy (inverted champagne bottle appearance due to the loss of muscle bulk), hypotonia, reduction or abolition of tendon reflexes, distal sensory loss (e.g. paresthesia, hypoesthesia, anesthesia, sensory ataxia or pain) and often associated with fasciculation, cramps or orthopedic deformities, such as hollow feet, foot drop, scoliosis etc. [Fig. 2] (1, 19). The disease leads slowly and gradually to impaired mobility in most patients but they remain ambulatory supported by orthesis ofirst threer walking cane while wheelchair-dependency is infrequent and become required generally after the fourth decade of life (21). Pupils may have problems with handwriting in schools (22).

Associated features occur frequently due to the complex physiological roles of different CMT genes resulting in atypical signs and symptoms and leading to difficulties in distinguishing CMT from other disorders (see chapter 2.4.). If atypical clinical signs and

symptoms are present, it can inflict overlapping phenotypes with spinal muscular atrophy (SMA), hereditary spastic paraplegy (HSP), amyotrophic lateral sclerosis (ALS), autosomal dominant optic atrophy (ADOA), ataxias or mitochondrial disorders (4). Overlapping phenotypes lead to overlapping pharmacotherapies which further emphasize the importance of genetic profiling in the near and distant future.

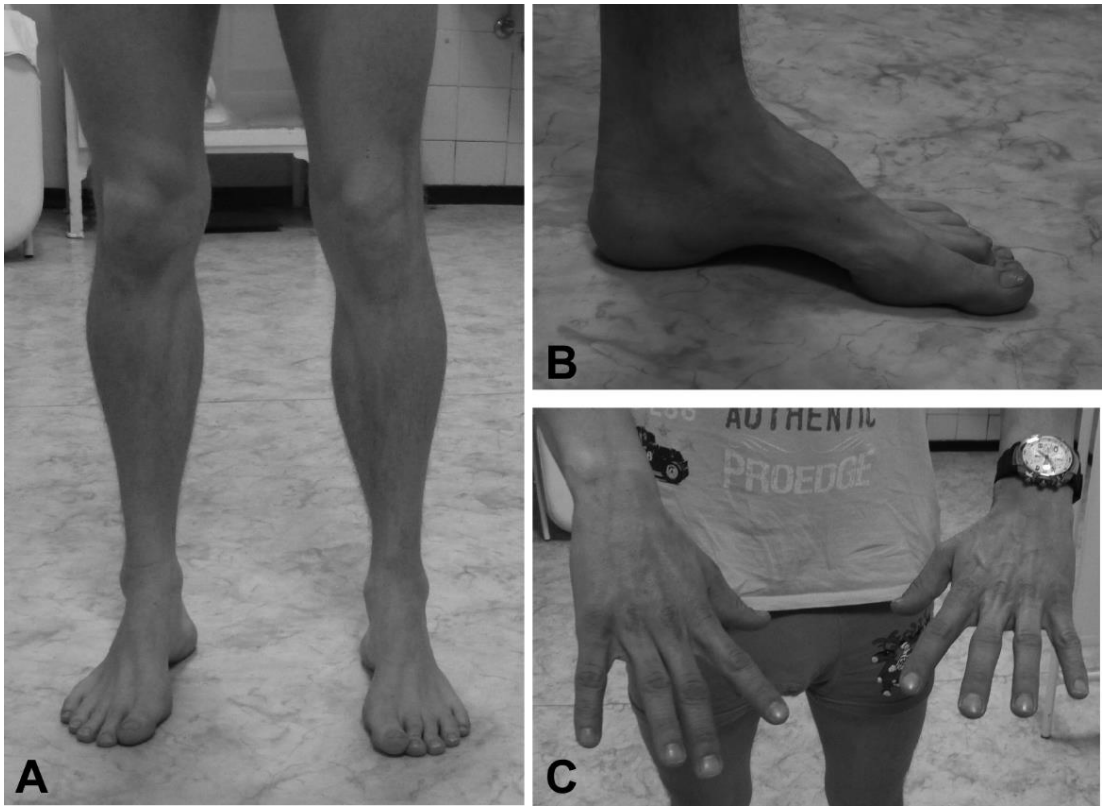


Fig. 2 27 -year-old male patient harboring the entire deletion of the coding region of *GJB1* gene. The patient above has moderate sensomotoric neuropathy with a CMTNS of 11/36. The photos were taken by the authors. Patient has consented to take the photographs and to press them.

A: inverted champagne bottle appearance due to the loss of muscle bulk while proximal musculature is spared; **B:** pes cavus and atrophy of the small foot muscles; **C:** atrophy of the small hand muscles and deformation of the fingers.

1.4 Genetic background

Until now, more than 90 genes are known to be related to Charcot-Marie-Tooth disease [Suppl. 1]. The proteins, encoded by CMT genes, are involved in many different

physiological functions of neuronal cells, i.e. (i) myelin proteins; (ii) cytoskeleton, nucleoskeleton and nuclear envelope; (iii) transcriptional regulation (iv) protein biosynthesis; (v) protein modification, folding and degradation (vi) intracellular transport; (vii) ER membrane shaping; (viii) mitochondrial dynamics; (ix) mitochondrial energy production; (x) sphingolipid biosynthesis; (xi) phosphoinositide metabolism; (xii) Rho GTPase signaling; (xiii) interaction with the extracellular environment (xiv) ion channels and (xv) others [Fig. 3] (23).

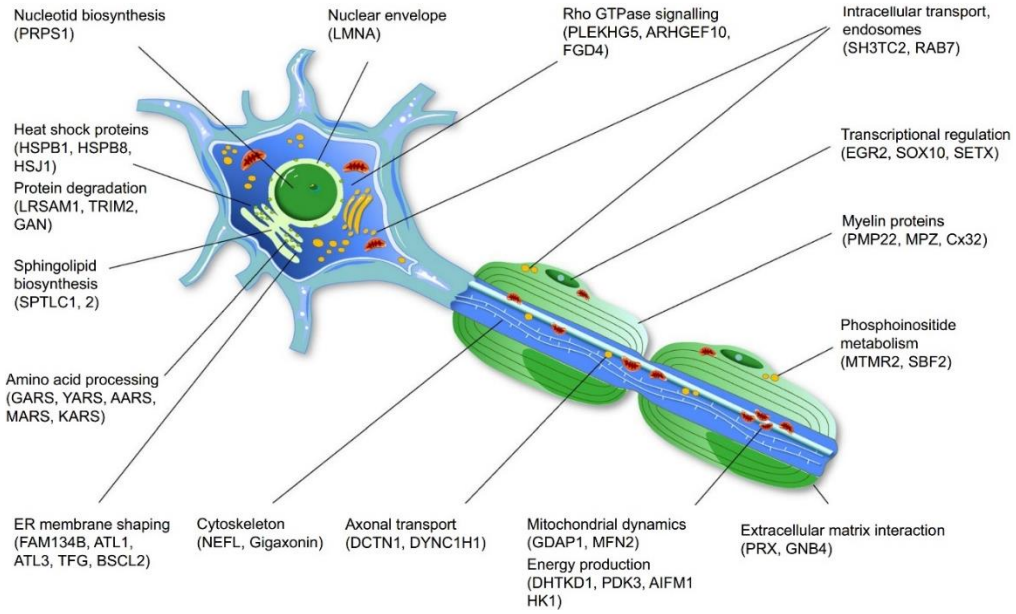


Fig. 3 Functions of proteins encoded by different CMT associated genes (23).

Depending on the causative CMT gene, inheritance can be autosomal dominant or recessive, or X-linked dominant or recessive. Interestingly, few genes show recessive and dominant inheritance, as well (19). Different genes cause a different type of neuropathies while the clinical presentation can further vary the overall picture (see par. *2.2 and 2.3*) [Fig. 4].

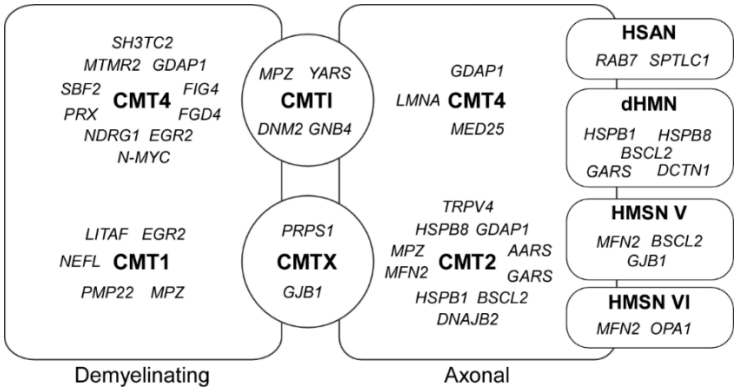


Fig. 4 The figure demonstrates the overlap of clinical and electrophysiological features along with the genetic classification of HMSN without a claim to completeness. It is worth to note that some of the genes (*MFN2*, *MPZ*, *HSPB1*, *EGR2* etc.) can be categorized differently which further complicate the classification (5).

The four most frequent causative genes are *PMP22*, *GJB1*, *MPZ* and *MFN2* in order and these are responsible for 70-90 percent of all CMT cases (24). The frequency of major and minor genes varies widely in different cohorts and because of the founder effect, some of the genetic variants are mainly specific for a certain ethnicity or geographical area. Because of the high number of CMT genes, this paper is not suitable for a detailed enumeration of genes, therefore, we discuss here the actually studied part of CMT genetics (2.4.1. and 2.4.2). Further genes are collected in the supplement (Suppl. 1).

The expanding number of clinical data have proved the heterogeneity of the appearance of CMT. Natural histories of patients varies greatly even in families with same mutation. This is especially true in *GJB1* families where females tend to have milder phenotypes than males [24]. High number of potential modifying factors, such as gene-gene interactions, mutation load, epigenetic modification effects, co-morbidities or disease management, likely have an impact on its characteristics and leads to a less predictable disease progression (25-29). Recently, extensive genotype-phenotype meta-analyses studied the possible correlation between various symptoms, disease severity and genes (30, 31). It is well known that progression of neuropathy is linked to age but its dynamics and age of onset vary widely. It worth to note that around seventy percent of CMT starts before the third decade of life (32).

Depending on the causative gene, additional features can occur in CMT as well. Signs of chronic pyramid tract involvement, spasticity, nystagmus or ataxia are sensitive markers for *MFN2* and *GJB1* mutations (33, 34). Hearing impairment is a common feature in *PMP22, GJB1, MPZ, NDRG1* neuropathies (35-38). Genes, which are involved in mitochondrial dynamics (*MFN2, GDAP1, DNM2*), frequently cause mtDNA deletion or depletion, optic atrophy or cerebellar ataxia (39-42) while myopathy might be present with *DNM2* or *LMNA* mutation (40). The connection between *PMP22* duplication and immunological abnormalities has been also reported multiple occasions (43, 44). Proteinuria, FSGS, and deafness occur together in *INF2* mutations (45, 46). Furthermore, respiratory difficulties, phonation disturbances, and age at onset can be related to certain genotypes as well. For a detailed collection of reported associated features see Suppl. 1.

1.4.1 Frequent CMT genes

1.4.1.1 Peripheral Myelin Protein 22 (PMP22) MIM #601097

Cytogenetic location of *PMP22* is on 17p12 chromosome region (MIM #601097). *PMP22* encodes a myelin structure protein which comprises 2 to 5% of myelin sheet in the PNS (47). Although mainly expressed in PNS, *PMP22* mRNA expression has been also found in CNS. Studies also suggest a potential role in nerve regeneration, Schwann cell differentiation, and growth (48).

In 1991, *PMP22* duplication was the first mutation identified in CMT and later its deletion was linked to HNPP in 1993 (14). *PMP22* gene is functionally not disrupted in eithers so gene-dosage effect seems to be the potential explanation for the disorder. Reiter et al identified the molecular etiology where they observed the homologous recombination event that was responsible for the unequal crossing over causing a deletion and a duplication at the same time in two sister chromatids (49).

PMP22 duplication and point mutations have been linked to CMT1A and CMT1E, respectively. CMT1A is the most common cause of hereditary neuropathies (35-45%) whereas CMT1E incidence seems to be less prominent (<1%) (50, 30, 51). Symptoms, severity, and age of onset in CMT1A vary widely. Molecular genetic analysis has linked the gene to Dejerine-Sottas syndrome and Roussy-Levy syndrome as well which should be considered as part of the CMT1A spectrum (MIM #601097). Frequently, the disease

starts in a couple of years after birth but some cases can be completely symptomless during entire life. CMT1A is characterized by classic symptoms of demyelinating neuropathy but may occur along with tremor, cranial nerve involvement – facial weakness, hearing impairment, and vocal cord palsy – or autonomic dysfunction, too. CNS may also be affected by nystagmus, pyramidal signs, white and grey matter volume reduction or white matter lesions (31). An elevated level of PMP22 protein may trigger the immune dysfunction since CMT1A occasionally associate with chronic inflammatory demyelinating polyneuropathy (CIDP) and other autoimmune diseases (52).

1.4.1.2 Gap Junction Protein Beta 1 (GJB1)

Gap junction beta 1 (*GJB1*) gene is located in Xq13.1 chromosome region and encodes Connexin 32 (Cx32) proteins. *GJB1* is a member of connexin protein family (53) which form gap junction channels and are involved in the transport of small molecular weight substances (<1kDa). A connexin protein contains four transmembrane (M1-M4), two extracellular (E1-2), and one cytoplasmic loop domain (IC) [Fig. 5/A]. Six of same connexins form a connexon also called hemichannel [Fig. 5/C] which pairs with the connexons of adjacent membrane creating gap junction channels [Fig. 5/B]. Due to the highly homologous connexin proteins, different connexons can form heterodimer pores as well (54) (55).

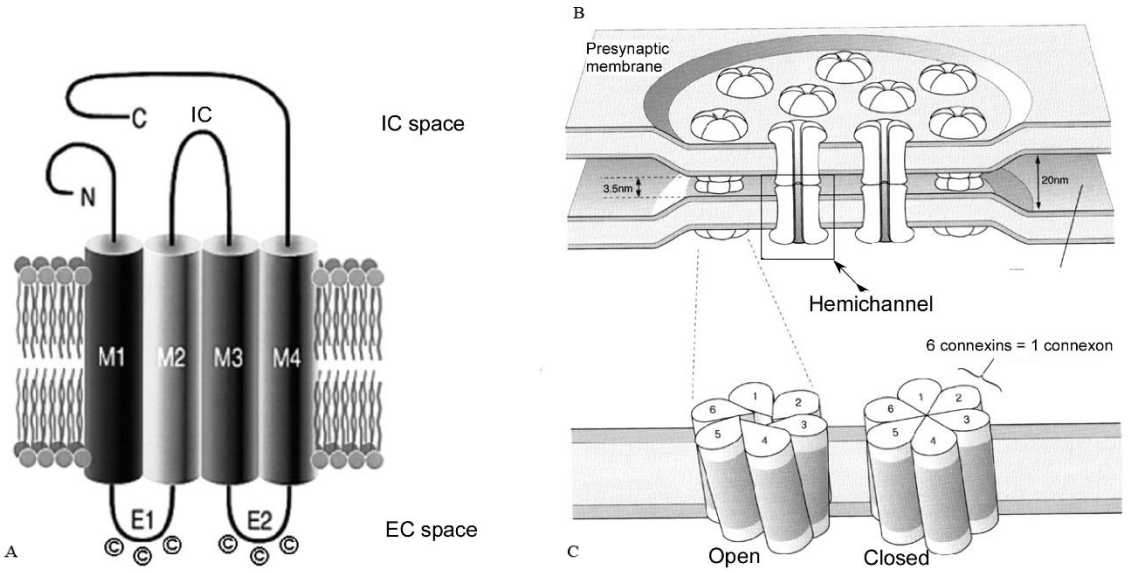


Fig. 5 (A) Structure of connexin in the membrane. © refers to cysteine rich regions of extracellular domains. **(B)** Hemichannels of adjacent membranes create the gap junction channel. **(C)** The structure of connexon is formed by six homolog connexin molecules where the pore in the middle is gathered around by them. Pores can be both opened or closed state influenced by current voltage (54).

Cx32 is localized in many different cell types including central and peripheral nerves, hepatocytes, pancreatic and embryonal cells (56). In peripheral nerves, Cx32 is located in the paranodal region and the Schmidt-Lantermann incisures of Schwann cells. It has a crucial role in maintaining normal myelination in the peripheral nervous system (57, 58).

Pathogenic mutations of *GJB1* cause X-linked dominant form (CMTX1). To date, more than 400 *GJB1* pathogenic variants have been identified as the cause of CMTX1 including CNVs. CMT1X is responsible for 8.3 to 12.8 percent of all CMT cases, and after *PMP22* gene duplication, it is the second most common cause of CMT (59). CMTX1 shows a wide spectrum of sensory and motor symptoms, and the nerve conduction studies reveal all forms of sensorimotor neuropathy (60). Though CMTX1 is considered to show X-dominant inheritance, female carriers usually show milder clinical symptoms than males with the same genotype (61, 62). Certain *GJB1* pathogenic alterations were in association with central nervous system involvement and sensorineural hearing loss as well (63-65). Most of the *GJB1* mutations cause CMT through loss of normal connexin function but it

is also suspected that the mutant protein has a dominant negative effect and suppresses the function of other gap junction proteins which would be able to replace them (66, 67). Presumably, both pathogenic changes are possible but depending on certain variants (68).

1.4.1.3 Myelin Protein Zero (MPZ, P0)

Myelin protein zero (*MPZ*) gene is located on 1q23.3 chromosome and encodes the major structural protein of peripheral myelin sheet and accounts for more than 50% of myelin proteins. It expresses only in PNS (69). *MPZ* mutations may refer to the third most frequent genetic cause of CMT, it accounts for 3-7% of all cases (50, 30, 51).

In 1993, Hayasaka and al identified *MPZ* as the cause of CMT1B (70). *MPZ* pathogenic alterations can cause a wide spectrum of hereditary neuropathy – it was found in association with Dejerinne-Sottas syndrome, Roussy-Lévy syndrome, congenital hypomyelinating neuropathy (71). Electrophysiological patterns vary as well, demyelinating, axonal or intermediate types can be also recorded with ENG. Cranial nerve involvement, pupil abnormalities, skeletal deformities, deafness, cognitive impairment, CNS involvement may be associated features, too (31).

1.4.1.4 Mitofusin2 (MFN2)

MFN2 is located on 1p36.2 chromosome region which encodes a dynamin homolog protein involved in mitochondrial dynamics (72) (MIM #608507). The protein structure is similar to GTPase family members, it consists of two transmembrane anchor domain (TM1 and TM2), two helical hapted repeat regions (HR1 and HR2), a GTPase domain and a very short region in intermembrane space. Both N and C terminal ends are located in the intracellular space [Fig. 6]. Major domains are taxonomically highly conserved (73).

MFN2 has a crucial role in mitochondrial dynamics, especially in mediating fusion, but it is involved in calcium homeostasis, ER-mitochondrion tethering, axonal transport and intrinsic apoptosis as well. These processes are operating from the energy of GTP hydrolysis resulting in the altered structure of protein (74).

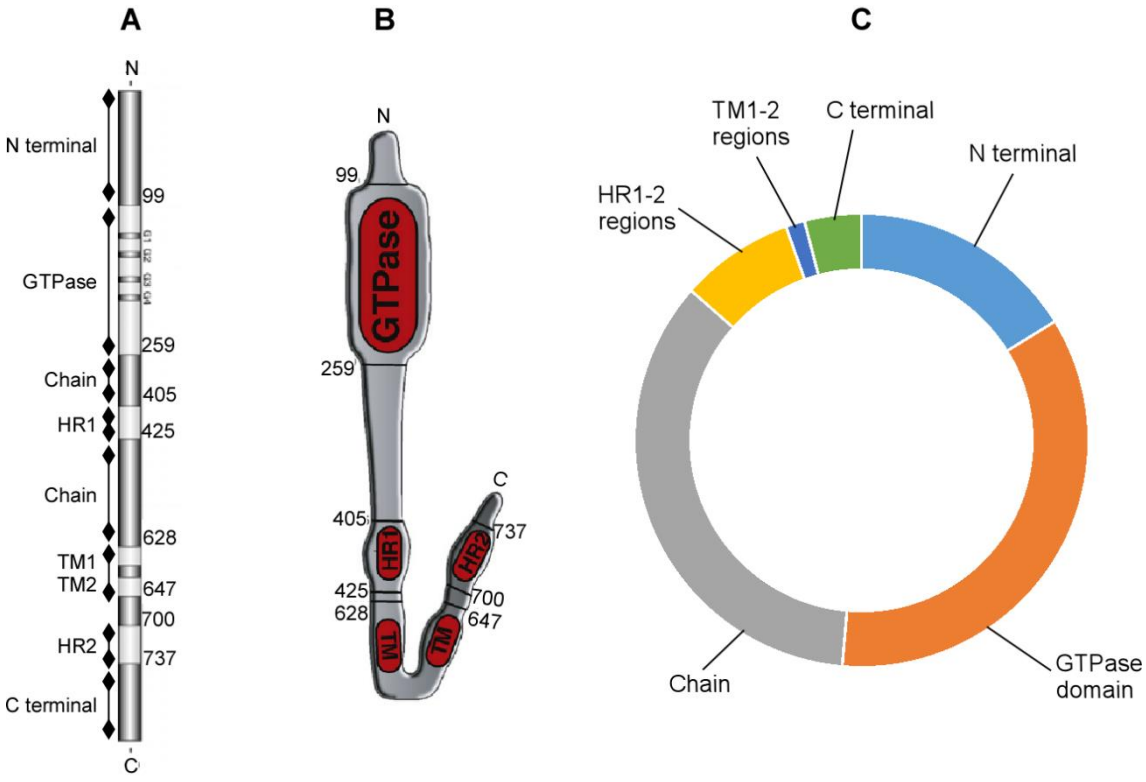


Fig. 6 (A) Gene and (B) protein structure of *MFN2 (75, 76)*. (C) Colors indicate different *MFN2* regions where slices represent the number of known mutations in percentages. Abbreviations: (A) G1-G4 indicate GTP binding domains.

MFN2 is expressed by most cells where mitochondria are present although its dysfunction leads to disease only in the high-energy demanding tissues such as nerve cells. *MFN2* is linked foremost to the axonal form of hereditary sensorimotor neuropathies (CMT2A2) but optic atrophy (HMSN-VI) and CNS involvement (HMSN-V) can associate as well (23). *MFN2* mostly inherits autosomal dominantly but recessive pattern has been also described (MIM #608507, CMT2A2B). There are more than 110 known pathogenic alterations (77) where phenotypic spectrum, expressivity varies widely (31).

The precise mechanisms are not yet entirely unfolded but more crucial pathways have been identified. Overexpression of *MFN2* causes a profound formation of mitochondrial network whereas gene silencing results in extensive fragmentation of mitochondria (78). In lack of fusion, cell cultures have shown decreased mitochondrial respiration and apoptosis while embryonal lethality and neurodegeneration were observed in animal models (79). *MFN2* is also contributing to mitophagy due to *PARK2* ubiquitination, *PARK2* recruitment and *PINK1* phosphorylation which impairments can lead to

unnecessary apoptosis or necrosis of nerve cells (80). In *MFN2* carrier patients' fibroblast, bioenergetic studies showed mitochondrial coupling defect and an increase of the respiration rate linked to complex II (81).

1.4.2 Less frequent CMT genes

1.4.2.1 EGR2 (Early Growth Response 2) (MIM 129010)

EGR2 gene is localized on 10q21.3 chromosomal region encoding a transcription regulator protein with 3 tandem zinc finger. *EGR2* induces the expression of many essential myelin proteins including Cx32, MPZ and MAG where the mutated *EGR2* protein has a dominant negative effect on the proteins above, by lowering their overall expression and resulting in neuropathy (82).

Warner et al identified the first heterozygous mutation of *EGR2* in CMT. Later, pathogenic alterations were linked to autosomal dominant and recessive forms of DSS and CHN, as well (13, 83). The electrophysiological characteristics are primary demyelination whereas sural nerve biopsy may show a severe loss of myelinated and unmyelinated fibers, onion bulb formation, and focally folded myelin sheaths (13). There are less clinical informations about *EGR2* pathogenic alterations, since it proved to be less frequent in CMT population; however, it is commonly associated with severe phenotype indicating its screening especially in cases where early onset (<12 months), rapid progression and premature death (<6 years) is present (84). *EGR2* screening should be considered in these cases (13).

1.4.2.2 CTDP1 (C-Terminal Domain of RNA Polymerase II Subunit A, Phosphatase of Subunit 1) (MIM 604927)

The cytogenetic location of *CTDP1* is found on 18q23 chromosome region. *CTDP1* encodes a general transcription factor which is essential in RNA synthesis. The protein regulates the activity of RNA polymerase II subunit A (*POLR2A*) with a posttranslational modification. CTDP1 makes possible the initiation of gene expression through *POLR2A* dephosphorylation (85).

First pathogenic alteration has been described in Rudari Vlax Roma patients with a well-defined syndrome consisted of congenital cataract, facial dysmorphism and neuropathy (CCFDN) (86). CCFDN inherits autosomal recessive and is considered as a founder

mutation since high carrier rates are present in Roma individuals (~6.9% in Rudari Gypsy, 0.6% in other Roma tribes and 0.0% in non-Roma people) (87, 86). Independently of signs and symptoms, abnormal cell function was observed in each tissue studied (86). In addition to demyelinating neuropathy, skeletal deformities, short stature, congenital nystagmus, cataract, facial dysmorphism, and impaired visus may be present as well.

1.4.2.3 NDRG1 (N-Myc Downstream-Regulated Gene 1)

NDRG1 is localized on 8q.24.22 chromosome region. NDRG1 protein plays a role in cell growth and differentiation as a signaling protein shuttling between the cytoplasm and nucleus and its expression is especially high in peripheral nerve cells, mainly in Schwann cells (88). All the findings indicate the necessity of *NDRG1* in nerve cell survival (88).

NRDG1 mutations cause autosomal recessive hereditary sensorimotor neuropathy named as CMT4D or Lom neuropathy after a Bulgarian town. To date, there are only six described mutations where the most frequent p.R148X is considered as a founder mutation in Wallachian Roma patients (89, 88, 90, 91). The disease is characterized by early onset neuropathy (<10 ys), distal paresis and atrophy, sensory impairment including hearing loss, and skeletal deformities (92). Nerve biopsy reveals hypertrophic onion bulbs with partial ensheathment of axons, and ENG shows demyelinating type of neuropathy (93).

1.5 Evaluation of the hereditary neuropathies

Finding the cause of neuropathy is not always easy. A considerable amount of cases stays unsolved after excluding all the treatable disorders. Basically, there are two types of diagnostic approach – pragmatist and completist. Doctors, who are pragmatists, are aiming at minimal possible diagnostic tests to reveal the cause. Other doctors, the completists, try to investigate each possibility, even if it may not have a therapeutic application. These doctors are usually working in tertiary centers as experts of the field (94).

1.5.1 Clinical assessment

The medical history should be always carefully recorded. Clinicians should ask about age of onset, first symptoms, course of progression, family history, possible exposition of

neurotoxic substances (alcohol, chemotherapeutics, medications, toxins etc.), various infectious diseases (Lyme disease or tick bite, HIV, HCV, HBV) and other medical conditions (malignant neoplasm, lymphoma, multiple myeloma, amyloidosis, autoimmune disease, thyroid dysfunction, trauma, uremia etc.) (1) [Fig. 7]

The routine neurological examination should be performed thorough. To follow the progression, the weakness of muscles (e.g. using MRC scale) and the type and area of sensory involvement should be given exactly by the physician (94). CMT Neuropathy and Examination Scores (CMTNS and CMTES) are powerful and quantitative methods to determine the disease severity and to follow-up the progression. CMTNS consist nine different scores in three groups: symptoms (3), signs (4) and neurophysiology (2). Each score is rated from 0 to 4 regarding the severity (0 – normal, 4 – severe) which means a maximum possible score of 36 points per individual. Severity is ranked as follows: ≤10 mild, 11-20 moderate, >20 severe. CMT examination score can be used as well which exclude the scores of nerve conduction study thus the maximum possible score is 28 points in this case (95). CMT Pediatric Score is designed for children contenting 11 different items with a total score of 44 (96). These measurements are based on Rasch methodology which provides a linear evaluation of progression and follow-up (97). The 6-min walking test is helpful in measuring walking capability and stamina, and prolonged ambulation test can be performed with StepWatch™ medical device (98).

Laboratory investigation helps to rule out the acquired causes of polyneuropathy and the following lab parameters are advised to check routinely: serum glucose, calcium (Ca^{2+}), creatinine kinase (CK), lactate dehydrogenase (LDH), thyroid stimulating hormone (TSH), immunoglobulins and B12 vitamin levels, sediment rate; and if it is suspected: anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), ganglioside profile, immunoglobulin electrophoresis, postinfectious panel (Borellia burgerdorfii, human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV and HCV), Clostridium jejuni etc.) or paraneoplastic markers (94).

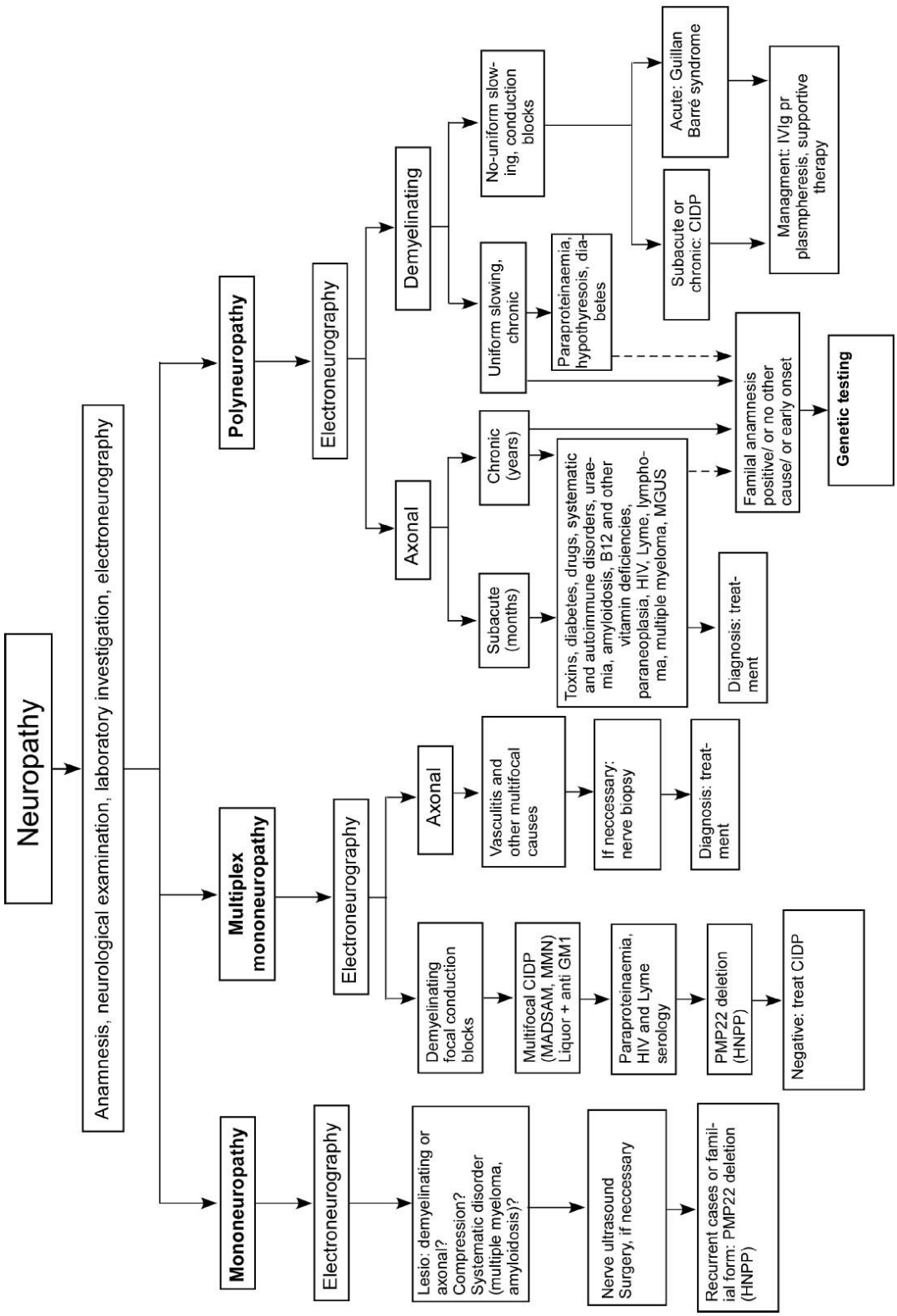


Fig 7. Evaluation and differential diagnostic considerations of neuropathies [8].

1.5.2 Nerve conduction study

Nerve conduction study (NCS) is a rapid, non-invasive and cost-effective method to diagnose and follow-up the polyneuropathy. The three different form of neuropathy in CMT are demyelinating, axonal and intermediate types.

Demyelinating neuropathy is characterized by the progressive loss of myelin sheet. According Kelly electrodiagnostic criteria for predominant demyelination, findings consists at least 3 of the following features (1) prolonged distal latency in ≥2 nerves, (2) <60% reduction of motor conduction velocity in ≥2 nerves, (3) prolonged latency of F waves as follows: >3ms in arms or >5ms in legs (≥1 nerve), or absence of F-waves in ≥1 nerve (4) partial motor conduction block of ≥1 nerve. The criterion of CIDP also comprises >130% increased temporal dispersion in ≥2 nerves (99, 100).

Axonal polyneuropathy is considered if no definitive signs of demyelination are present. Kelly electrodiagnostic criteria specify it as follows: (1) >90% of normal NCV if CMAP amplitude is >30% of normal, and >60% of normal NCV if CMAP is <30% of normal, (2) normal or prolonged distal latency in proportion to conduction velocities, (3) normal F waves, (4) no conduction block and (5) fibrillation potentials and neurogenic motor unit changes. Axonal loss has two basic patterns of motor conduction alteration: (1) sparing of few of the fast fibers with severe amplitude reduction and spared MNCV (>50-60 m/s), and (2) sparing only few of slowly conducting fibers which cause a moderate mean nerve conduction velocity (MNCV) (>35 m/s) and prolonged distal motor latency beside the compound muscle action potential (CMAP) amplitude reduction (101).

The definition and criterion of intermediate neuropathy are frequently controversial in the lack of specific electrophysiological protocol. Most recently, Saporta et al considered intermediate neuropathy if MNCV is 38-45 m/s in ulnar nerves and CMAP amplitude reduction is >0.5 mV. However, making a difference between axonal and intermediate types is sometimes hard due to the sparing only of slowly conduction fibers in axonal neuropathy (18).

Certain genes are specific for distinct types of neuropathy but many cannot be unambiguously categorized (e.g. *GDAP1*, *GJB1, MPZ* etc.). In special cases, uncommon

signs can be also present such as severe temporal dispersion (CMTX1) or conduction blocks (HNPP) which may implicate difficulties in differential diagnostic follow-up.

1.5.3 Nerve biopsy and imaging studies

In the first part of 20th century, nerve biopsy and histology meant to be the best diagnostic approach of CMT. In demyelinating form, moderate to severe reduction in density of myelinated fibers, hypermyelination or demyelination, and a high number of onion bulb formations can be observed. The axonal form is characteristic of lack, loss, or preservation of nerve fibers and signs of regeneration. After identification some of the causative genes, clear genotype-phenotype correlations have been found with certain histological changes such as focal axonal alterations due to *MFN2* and *NEFL* mutations, congenital amyelination in *SOX10, EGR2, CTDP1* alterations or tomacula in HNPP (23). In the genetic era, nerve biopsy is mainly obsoleted and is required and justified only in few cases like suspected diagnosis of CIPD, amyloidosis or small fiber involvement (23).

Nerve ultrasound is widely used as a differential diagnostic application in evaluation of neuropathies. Lately, more studies attempted to describe the characterization of different CMT subtypes like CMT1A or CMT1B. Measurement of cross-sectional area (CSA) of peripheral nerves was significantly increased in CMT1A, and CSA correlated with the disease severity (CMTNS) and peripheral nerve function (102). In CMT1B, increased CSA of median and cranial nerves was observed (103). Axonal neuropathy is not characteristic for increased CSA based on several cases (102).

MRI neurography in CMT may reveal hypertrophic roots typically with onion bulb sign which represent hypertrophic demyelination (CMT1 and CMT4) and depicting also nerve entrapment or impingement occasionally (104-106). In certain cases, some enhancement in nerves may be also seen but it is infrequently a prominent feature. Quantity muscle MRI based on Dixon sequences and quantitative muscle ultrasound is suitable to measure precisely and reliably the thigh and leg muscle atrophy as well as denervation-related fatty substitution which is held as the most responsive measure available in the monitoring of CMT progression (107, 24, 108).

1.5.4 Genetic diagnostics

The development and decreasing price of molecular genetic methodologies have opened new frontiers in diagnostics of hereditary disorders. Genetic diagnosis can likely prove CMT since positive test confirms hereditary neuropathies even in uncertain cases; however, negative test results do not exclude CMT. In the last decade, numerous study attempt to define an ultimate strategy of genetic testing but these were partly unsuccessful. Each population showed divergent gene frequencies and numerous factors biased its universal application so these should be considered more likely as a rule of thumbs [Fig. 8] (51, 27).

Recently, new generation sequencing (NGS) lifted to a new level of diagnostic perspectives in hereditary disorders. Specific CMT panels may reveal other possible genetic modification factors which potentially contribute in the different biological pathway and modulate the penetrance and/or the expressivity of the overall phenotype. However, the absolute cost of NGS is still high and the quality and reliability of results are occasionally questionable. Even today, Sanger sequencing and MLPA are held the gold standards in SNV's and CNV's analyses despite their limited output but high fidelity and feasibility (109).

In regard to the inheritance, the four most common genes can be advised for analyzing before any more extensive screening: in CMT1 *PMP22, GJB1*, and *MPZ,* and in CMT2 *GJB1, MFN2,* and *MPZ* in order, based on the following scheme (51).

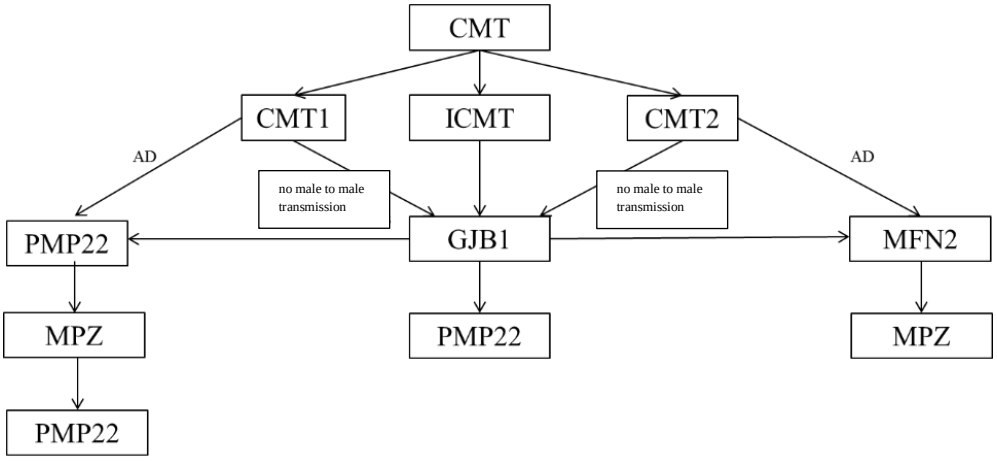


Fig. 8*. A decision tree of successive analysis of suggested genes in CMT (51).*

1.6 Management of hereditary neuropathies

1.6.1 Genetic counseling

Since the patients' genetic diagnosis is irrevocable during his/her entire life, genetic counseling has an enormous liability in the management of hereditary disorders such as CMT. The process of genetic counseling is dedicated to inform the patient about the purpose and possible outcomes of genetic testing just as about the expected psychological and physical burdens of the diagnosis and how can it affect the offsprings and other relatives (110).

The Hungarian Parliament enacted the state law about human genetics in 2008 (2008. évi XXI. törvény) which determine the major points of genetic counseling, testing, and research. Even so, in absence of implanting regulations (31.§), some crucial details of its execution are still undefined thus fundamental medical consensus and best practice should be supreme in these cases.

Major points of genetic counseling by the law:

- A patient, who has the capacity, owns the right for a private counseling.
- Patients must be informed about the purpose of testing, potential consequences, methods of processing and storing the genetic material, and data protection, and possible benefits and disadvantages of refusal, (6.§ (2)).
- Genetic counseling is necessary before and after a genetic testing (6.§ (2)).
- Prognosis, therapeutic possibilities must be communicated and psychological support must be offered in appropriate cases (6§ (4)).
- Patients have the freedom to know or not to know the genetic result (6.§ (7)).
- The genetic result must not be shared with a third party unless in possession of a permission document representing conclusive evidence, or in a case of potentially affected family members with clinical relevance (conception, prevention, therapy, etc.) related to the result (7.§)
- Patients must give their written informed consent before sample is taken (8.§)

Although Charcot-Marie-Tooth disease does not affect the life expectancy in general, presymptomatic counseling and testing require one or more sessions (predecision and

pretest counseling). Here, the clinicians need to be more prudential regarding the potential impact of diagnosis, benefits, and disadvantages. The freedom to not know of the genetic result must be kept in sight. Every case is unique and requires experience in leading the patient to the right choice. The councelor has to evaluate the possible impact and may evade the telling of diagnosis but always the patient decides finally.

Very important that presymptomatic screening is strictly forbidden and unethical under the age of legal competence unless its necessity proved vital regarding a close relative (111).

Another relevant question is the person of the counselor. In absence of implanting regulations, the law does not specify who can provide genetic counseling. Reckon with the fundamental principle of the law, best practice and medical ethics, no other professional should give a genetic counseling but a trained medical doctor with extensive knowledge of the field, otherwise the right to information (6.§ (2)) can be easily compromised.

1.6.2 Therapy and treatment

Until now, no effective treatment was developed in CMT although there are some efforts to moderate the overall disease burden. It is very important staying active and keeping up the strength with physiotherapy or regular exercises (112). Occupational therapy can help in coping with daily tasks (113). Orthoses, orthopedic shoes or canes can support the walking and orthopedic surgeries can correct the feet deformities and Achilles contractures if needed (114).

Many promising compounds are in different phases of drug research. PXT3003, which is a combination of naltrexone, baclofen, and sorbitol, is currently in phase III (NCT02579759). The encouraging results of the animal model showed its beneficial effect in lowering *PMP22* overexpression (115). Downregulation of *PMP22* with progesterone antagonist (ulipristal-acetate) is right in phase II (NCT02600286) (116). There are novel efforts as well aiming the gene silencing of *PMP22* by lowering its expression with antisense oligonucleotides and small interfering and hairpin RNAs (117). Gene therapy with intrathecal lentiviral vectors is also in scope in CMTX1 treatment since

stable Cx32 proteins were produced and maintained in treated *Gjb1*-knocked out mice (118). L-serine supplementation is promising in SPTLC1-2 defects (NCT1733407) (119).

1.6.3 Avoid of medications

There is little information about drugs which should be ultimately avoided. Unambiguously, neurotoxic medication can progress the neuropathy so the application of these drugs should not be used in general. There is a clear evidence that administration of vinca alkaloids and taxols may cause rapid and severe progression and nerve injury in CMT patients even in mild or asymptomatic CMT (120). In higher percentages of cases, nitrous oxide (50%), metronidazole (23%), nitrofurantoin (20%), phenytoin (11%), statins (10%) and sertraline (9.5%) can initiate an exacerbation of neuropathy. Other drugs have moderate to doubtful risk and should be considered the treatment individually and assess the improvement or worsening of disease helping in decision whether benefits surpass risks (121).

1.6.4 CMT and pregnancy

There are only a few publications about pregnancy risks and CMT and results are controversial. A publication from 2012 reviewed the natural history of pregnant women with neuromuscular disorders (NMD), including CMT. The study concluded that the worsening of status was present in 31% which did not remit in 22% of patients. Very important that CMT did not influence significantly pregnancy outcome, labor and delivery, mode of delivery, preterm birth and neonatal outcome (122). Another study found that pregnancy with CMT indicated a higher occurrence risk of presentation anomalies and bleeding postpartum. The rate of caesarean section was doubled and forceps was used three times as often in the CMT group as in individuals without NMD. Most of the CMT operative deliveries were emergency sections (123).

2. Objectives

In the last decades, the successive exploration of CMT made the diagnostics more successful and exact than ever. Furthermore, numerous studies proved that a well-characterized population and gene specific epidemiological data can enhance the efficiency and lower the costs of diagnostics. In this study, we aimed to investigate Hungarian CMT patients, unravel the genetic cause of the disease even in still unsolved cases and assess the phenotypical variability and spectrum of different causative genes.

The aims were the followings:

1. To estimate the frequency of most common neuropathy genes – *PMP22, GJB1, MPZ, MFN2, EGR2, CTDP1* and *NDRG1* – in an extensive cohort of Hungarian CMT patients.

2. To assess the disease features and atypical signs and symptoms of CMT in this cohort and detailed descriptions of the phenotype of novel pathogenic and likely pathogenic alterations.

3. To highlight various genotype-phenotype correlations between different CMT subgroups, especially between clinically well-characterized female and male CMTX1 patients.

4. To analyze patient for rare variants with high- throughputgenetic methods and identify the causative gene.

3. Methods

3.1 Clinical and electrophysiological characterization of the cohort studied

531 Hungarian CMT patients were enrolled (242 females and 289 males; mean age of 39.3±17.6, CI95% (37.78 to 40.76)). All individuals were born in Hungary, whereof 55 patients (10.4%, CI95% (0.78-0.13)) were likely of Roma origin. Some of the novel alterations were tested in 350 healthy control individuals (209 female, 141 male; mean age 39.88±14.87; CI95% (38.32 to 41.44)) as well. All individuals were born in Hungary and descended from Hungarian ancestors. Written informed consent was obtained from all individuals. Molecular genetic analysis was performed for diagnostic purposes in all investigated patients. The study was approved by the Ethical Committee of Semmelweis University (119/PI/12, 7891/2012/EKU).

Patients routinely underwent neurological examination. Age of onset and family history was taken in all cases by asking about other affected relative and first neuropathy related symptoms, respectively. Distal hereditary motor neuropathy (dHMN) and hereditary sensory and autonomic neuropathy (HSAN) were excluded as well.

Nerve conduction studies (NCS) were performed by standard techniques (Dantech Keypoint, Denmark) with superficial registration and stimulation of sensory, motor and mixed nerves. Routinely investigated nerves: sural sensory, peroneal and tibial motor nerve conduction and F-waves, ulnar and median nerves sensory and motor conduction inclusive F-waves. Demyelinating neuropathy was diagnosed if the distal latency and/or F-latency was prolonged and/or the conduction velocity was reduced. The increased temporal dispersion was taken as a sign of demyelination as well. Focal conduction block was ruled out. Diffuse amplitude-reduction and no evidence of demyelination indicated an axonal loss. Nerve lesion was diagnosed as intermediate type if amplitude-reduction was present with decreased nerve conduction velocity but the criteria of primary demyelination have not been fulfilled (124). We also categorized patients based on nerve conduction velocity exclusively, as follows: NCV of ≤38m/s indicated CMT1 while NCV of >38m/s indicated CMT2. Normal values in reference to patients' height and gender were calculated using the Dantec Keypoint Software (Keypoint Software v.3.03).

Patients were considered to suffer from CMT if the clinical and electrophysiological signs of motor and sensory neuropathy were present and the family history revealed other affected family members. First and second degree relatives with similar characteristics of neuropathy were considered to carry the same pathogenic variant. In sporadic cases, the causes of acquired neuropathy (e.g. metabolic, toxic, inflammatory, infectious and tumor associated polyneuropathies) were excluded using extensive differential diagnostic workup. Further diagnostic tests or procedures (e.g. additional laboratory investigation, audiological evaluation, brain and/or spinal MRI or CSF analysis) were performed if required based on the clinical picture. The severity of the disorder was assessed using the CMT examination and/or neuropathy score part of this retrospectively (125).

3.2 Genetic testing

DNA was isolated from whole blood using the QIAamp DNA mini kit (QIAGEN®). Quantitative changes in the *PMP22* gene was analyzed with multiplex ligation-dependent probe amplification assay (MLPA) (SALSA MLPA 33 CMT1 probemix, MRC Holland). Copy number variation of the *GJB1* gene was screened by real-time PCR methodology with SYBR Green (ThermoFisher®) staining. Copy number was determined using the ddCt method and compared to human serum albumin. The total coding region of *GJB1* (ENST00000374022, NM_001097642), *MPZ* (ENST00000533357, NM_000530), *EGR2* (ENST00000242480, NM_000399), *MFN2* (ENST00000235329, NM_014874), *PMP22* (ENST00000395938, NM_153321) were analyzed using Sanger sequencing with specific primers (Suppl. 3) and compared to the human reference genome using NCBI's Blast® application. Hotspot mutations in *CTDP1* and *NDRG1* (TaqI) genes were tested with the PCR-RFLP (CTDP1-NlaIII, NDRG1-Taq1) methodology.

CMT1 patients were first screened for *PMP22* duplication. If this was negative, successive analysis of the coding region of *GJB1, MPZ, EGR2,* and *PMP22* was performed. In CMT2 cases, the sequence of *GJB1* was first analyzed followed by *MFN2* and *MPZ* genes. In both CMT1 and CMT2 patients, *GJB1* deletion analysis was also performed if other tests did not identify the causative gene. *GJB1* was only tested if male to male transmission was absent. Patients with likely Roma origin were screened for founder mutations as well.

In control cohort novel alterations were also screened with PCR-RFLP: GJB1: c.38 T>A - Hpy166II (New England Biolabs® R0616S); c.557 A>G - NciI (New England Biolabs® R0196S); c.582 G>C – BmtI (New England Biolabs® R0658S))

Exome Capture was performed in Miami University, Hussman Institute. The procedure was executed according to the manufacturer's protocol. For genomic DNA library preparation, TruSeq® DNA Sample Prep Kit v2-Set A (Illumina) and NimbleGen SeqCap EZ Human Exome Library v3.0 Kit exome enrichment (Roche) was used. Crude pre-captured genomic library was analyzed by Agilent 2100 Bioanalyzer 1000 DNA chip to assess library quality. It was followed by exome enrichment, preparation of hybridized libraries, purification and library quality and quantitative control of qPCR. All library pools were sequenced on the HiScanSQ Illumina sequencing platform, using 2 × 95-bp pair-end sequencing protocol, with an extra 9-bp index sequencing run. 95-bp paired-reads were aligned to the human reference genome (hg19). The alignment was executed with Burrows–Wheeler aligner (BWA) software. For variation calling, Samtools software was used. We screened genes which are associated with neuropathies and likely involved in homeostasis of peripheral neurons. This gene set was compilated based on UNIPROT, NextProt, OMIM, and NCBI databases (Suppl. 3.). The variants were checked in Clinvar, dbSNP and Ensembl database. We focused on non-synonymous variants, splice acceptor and donor site mutations, and short, frame shift coding insertions or deletions (indel). Exonic frameshift and stop mutations were considered as damaging. Missense mutations were prioritized, which was based on the protein prediction score annotations given by Polyphen2, SIFT, MutationTaster and GERP software (SIFT < 0.1, Polyphen2 >0.5 > 3, MutationTaster: disease causing). Using ACMG guideline, the pathogenic and likely pathogenic variants were confirmed by Sanger sequencing.

Target sequencing of patients with predominant motor function impairment was performed using Illumina MiSeq platform and an in-house compiled Agilent Haloplex capture panel of following genes: *AARS, AIF1, ALS2, ANG, ASAH1, ATP7A, BICD2, BSCL2, C9ORF72, CHCHD10, CHMP2B, DCTN1, DNAJB2, DNMT1, DYNC1H1, EXOSC3, EXOSC8, FBXO38, FIG4, FUS, GAN, GARS, GEMIN2, GLE1, HEXA, HEXB, HINT1, HSPB1, HSPB3, HSPB8, IGHMBP2, LAS1L, LMNA, MAPT, MEGF10, MT3, NAIP, NEFH, OPTN, PFN1, PLEKHG5, PNPLA6, PRDX3, REEP1, SCO2, SETX, SIGMAR1, SLC52A2, SLC52A3, SLC5A7, SMNDC1, SOD1, STMN1, TARDBP, TFG,*

TRPV4, *UBA1*, *UBQLN2*, *VAPB*, *VCP*, *VIM*, *VRK1*. Bioinformatic algorithm, variant calling and variant analys was identical to the exome capture.

3.3 In silico, pathogenicity and statistical analyzes

In silico analyses were performed with PolyPhen2, MutationTaster and SIFT softwares. The significance of detected alterations was checked with HGMD (www.hgmd.cf.ac.uk), dbSNP (www.ncbi.nlm.nih.gov/SNP/), ClinVar (www.ncbi.nlm.nih.gov/clinvar/) and CMT database (http://www.molgen.ua.ac.be). The nature of novel alterations was assessed based on the ACMG guideline.

The group comparisons were performed with independent sample t-test and Mann Whitney U test regarding means. Percentages were compared with Chi square test. p values of <0.05 was considered statistically significant. Odds ratio (OR) in case control studies and the 95% confidence intervals (CI95%) for proportions and means were calculated using standard formulas.

4. Results

4.1 Clinical and electrophysiological assessment

From the 531 studied CMT patients, 409 (77.0% (CI95% (0.734 to 0.805)) were classified as CMT1 and 122 (23.0% (CI95% (0.194 to 0.265)) as CMT2. Family history was positive in 148 cases (51.0% CI95% (0.453 to 0.568)) while 142 patients (49.0% CI95% (0.433 to 0.548)) were sporadic. The inheritance pattern was autosomal dominant in 123 cases with a mean patient per family ratio (P/F) of 2.6. X-linked dominant and autosomal recessive inheritance were present in 13 (P/F: 3.4) and 12 (P/F: 1.8) cases, respectively.

CMTES and CMT related additional features could be assessed in 309 cases (58.2% CI95% (0.540-0.624)). The mean CMT examination score was 8.9±4.3 (CI95% (8.42-9.38), with a minimum of 0 and a maximum of 22. Symptoms began before the age of 30 in 69.3% of CMT cases; however, the age of onset ranged between the first and seventh decade of life. Additional features were found in a total of 22.3% of patients (CI95% (0.177-0.270)) as follows: CNS involvement in 7.8% (24), facial, glossopharyngeal and recurrent laryngeal nerve palsy in 5.2% (9, 4 and 3 respectively), bilateral sensorineural hearing impairment in 4.9% (15) (<40 dB and age at onset <40 years), immune dysfunction in 2.9% (9), autonomic nervous system (ANS) involvement in 1.6% (5), cataract in 1.3% (4) and optic atrophy in 0.7% (2) of the cases.

4.2 Genetic testing and distribution of genetic subtypes

Within the studied cohort, genetic testing confirmed the causative gene in 276 CMT1 (67.2% CI95% (0.617 to 0.728)) and in 42 CMT2 (34.4% CI95% (0.260 to 0.428)) patients. Altogether 318 CMT patients (59.9% CI95% (0.557 to 0.641)) received a genetic diagnosis, while in 213 individuals (40.1% CI95% (0.359 to 0.443)) we could not detect any pathogenic alterations within the genes studied.

Regarding the entire CMT cohort, the most frequent causative gene alteration occurred in *PMP22* (40.1%) followed by *GJB1* (9.6%), *MPZ* (4.5%), *MFN2* (2.4%) *NDRG1* (1.5%), *EGR2* (0.8%) and *CTDP1* genes (0.8%) [Table 2]. Homozygous founder mutations in *NDRG1* and *CTDP1* genes were present in 21.8% of investigated Roma patients, with eight and four cases respectively.

Table 2 – Pathogenic and likely pathogenic variants identified in the studied cohort and electrophysiological features, age of onset and disease severity of probands. Abbr.: p.s. – present study

Gene	Alteration	AA change	Significance	Proband /patients	NCV	Gender of probands	Probands' age on onset (decade)	Probands' disease severity	Ref
PMP22	dupl.	-	pathogenic	136/212	CMT1	male/female	first to fifth	mild to severe	(126)
PMP22	c.353 C>T	T118M	pathogenic	1/2	CMT1	male	second	mild	(127, 128)
GJB1	c.38 T>A	V13E	likely pathogenic	1/2	CMT2	male	second	severe	(129)
GJB1	c.43 C>T	R15W	pathogenic	1/1	CMT1	male	first	moderate	(130)
GJB1	c.187 G>A	V63I	pathogenic	1/2	CMT2	male	second	moderate	(131)
GJB1	c.224 G>A	R75Q	pathogenic	2/5	CMT1	male/female	second	moderate	(132)
GJB1	c.265 C>G	L89V	pathogenic	1/2	CMT1	male	second	severe	(133)
GJB1	c.287 C>G	A96G	pathogenic	2/7	CMT1/ CMT2	male/female	third and fifth	moderate	(134)
GJB1	c.319 C>T	R107W	pathogenic	1/1	CMT1	female	third	mild	(135)
GJB1	**c.379 A>G**	**I127V**	**pathogenic**	**1/3**	**CMT1**	**male**	**second**	**moderate**	**p.s.**
GJB1	c.425 G>A	R142Q	pathogenic	1/2	CMT1	male	first	severe	(136)
GJB1	c.490 C>T	R164W	pathogenic	1/1	CMT1	female	first	mild	(137)
GJB1	c.491 G>A	R164Q	pathogenic	1/3	CMT1	male	first	severe	(138)
GJB1	c.514 C>G	P172A	pathogenic	1/4	CMT2	male	second	severe	(139)
*GJB1**	c.557 A>G	E186G	likely pathogenic	1/4	CMT2	male	third	moderate	(129)
*GJB1**	c.582 G>A	M194I	likely pathogenic	1/3	CMT1	male	second	mild	(129)
GJB1	c.614 A>G	N205S	pathogenic	1/3	CMT1	male	first	moderate	(136)
GJB1	c.623 G>A	E208K	pathogenic	1/2	CMT1	male	second	mild	(140)
GJB1	c.712C>T	R238C	pathogenic	1/2	CMT1	male	first	moderate	(141)
GJB1	deletion	-	pathogenic	2/4	CMT1/ CMT2	males	second	moderate	(142)

Gene	Alteration	AA change	Significance	Probands / patients	NCV	Gender of probands	Probands' age on onset (decade)	Probands' disease severity	Ref.
MPZ	**c.58 T>C**	**S20P**	**likely pathogenic**	**1/4**	**CMT1**	**male**	**< 1 year**	**moderate**	**p.s.**
MPZ	c.131 C>T	S44F	pathogenic	1/4	CMT2	female	n/a	n/a	(143)
MPZ	c.143 T>C	L48P	pathogenic	1/6	CMT1	female	fourth	mild	(144)
MPZ	**c.253 G>A**	**G85R**	**likely pathogenic**	**1/3**	**CMT2**	**female**	**sixth**	**moderate**	**p.s.**
MPZ	**c.284 T>G**	**F95C**	**likely pathogenic**	**1/1**	**CMT1**	**female**	**first**	**moderate**	**p.s.**
MPZ	c. 335 T>C	I112T	pathogenic	1/3	CMT1	female	fourth	mild	(145)
MPZ	**c.370 A>C**	**T124P**	**likely pathogenic**	**1/1**	**CMT1**	**male**	seventh	**moderate**	**p.s**
MPZ	c.371C>T	T124M	pathogenic	1/2	CMT2	male	fourth	moderate to severe	(146)
MFN2	c.314 C>T	T105M	pathogenic	1/2	CMT2	female	first	severe	(147)
MFN2	c.383 A>G	H128R	pathogenic	1/2	CMT2	female	first	moderate	(148)
MFN2	c.839 G>A	R280H	pathogenic	1/1	CMT2	female	second	mild	(149)
MFN2	c. 1403 G>A	R468H	pathogenic	4/8	CMT2	female/male	first to seventh	mild to severe	(81)
EGR2	**c.971 G>A**	**R324H**	**likely pathogenic**	**1/2**	**CMT1**	**male**	**second**	**moderate**	**p.s**
EGR2	c. 1142 G>A	R381H	pathogenic	1/2	CMT1	male	third	severe	(150)
NRDG1	c.442C>T	R148X	pathogenic	4/8	CMT1	female/male	first to second	moderate-severe	(93)
CTDP1	IVS6+389 C>T	-	pathogenic	2/4	CMT1	female/male	first	severe	(151)

4.3 Novel alterations in our CMT patients

In this study, nine novel mutations have been identified which were present neither in literature nor in mutation databases. All of the amino acid changes are located at highly conserved protein residues and the *in silico* analyzes qualified them as disease causing alterations. None of the tested variants were present in our control cohort (*GJB1* c.38T>A,

c. 557 A>G and c.582 G>C as well as *MPZ* c.58 T>C, c.253 G>A and c.284 T>G – OR: 5.02, CI95% (0.2 – 123.8), p=0.17) or in the Exom Aggregation Consortium (http://exac.broadinstitute.org), in the 1000 Genomes Project (http://1000genomos.org) and in the Exome Sequencing Project (http://evs.gs.washington.edu/EVS). According to the American College of Medical Genetics and Genomics' (ACMG) guideline, our newly described variants should be considered as *likely pathogenic* or *pathogenic* [Table 3].

Table 3. Unpublished novel alterations detected in the study cohort.
Abbreviation: AA – amino acid; NCV – nerve conduction velocity; 1000G – 1000Genome Database

Gene	Alteration	AA change	Sex	1000G AF (N)	Proband/ patients	Type of PNP	Age/Age at onset	*In silico* analysis		ACMG
								Polyphen2	Mutation Taster	
MPZ	c.58 T>C	S20P	m	0% (0)	1/4	ax	12/0	0.989	disease causing	Likely pathogenic
MPZ	c.253 G>A	G85R	f	0% (0)	1/3	de	67/55	1.000	disease causing	Likely pathogenic
MPZ	c.284 T>G	F95C	f	0% (0)	1/1	de	57/31	1.000	disease causing	Likely pathogenic
MPZ	c.370 A>C	T124P	m	0 % (0)	1/1	de	75/69	0.990	disease causing	Likely pathogenic
GJB1	c.38 T>A	V13E	m	0% (0)	1/2	ax	35/15	1.000	disease causing	Likely pathogenic
GJB1	c.379 A>G	I127V	m	0% (0)	1/3	in	26/12	0.998	disease causing	Pathogenic
GJB1	c.557 A>G	E186G	m	0% (0)	1/4	ax	47/35	1.000	disease causing	Likely pathogenic
GJB1	c.582 G>C	M194I	m	0% (0)	1/3	in	24/18	0.992	disease causing	Likely pathogenic
EGR2	c.971 G>A	R324H	m	0% (0)	1/1	ax	22/11	1.000	disease causing	Likely pathogenic

4.3.1 Clinical description of patients with novel alterations

MPZ c.58 T>C (p.Ser20Pro) variant

The Ser20Pro variant was found in a 5-year-old patient with severe neuropathy. Male relatives with similar symptoms were known in his family who died as an adolescent. The

overall clinical picture was SMA like, but neither SMN1 nor dystrophin gene deletion or duplication was present. Familial segregation analysis was not possible in other relatives.

The proband's symptoms started at age of 3 months with hypotonic muscles. At age of 5, he was not able to sit or walk and his muscles were severely hypotonic. Due to lack of cooperation, precise investigation of muscle weakness or sensory impairment was not possible. The ophthalmological examination found a pale optic disc and congenital nystagmus on both sides. Creatine phosphokinase laboratory test was normal.

The electrophysiological investigation found severe demyelinating neuropathy with moderate amplitude reduction. Sural nerve biopsy revealed profound nerve degeneration with both demyelinating and axonal components.

MPZ c.253 G>A (p.Gly85Arg) variant

An unknown SNV has been found in a 50-year-old female whose symptoms started in the first decade of life with gait disturbance. Longitudinal progression was minimal for 40 years, however, in the last couple of years, the symptoms notably worsened. She mentioned two relatives with neuropathy but more prudent medical history was not available.

Her status indicated moderately severe distal paresis and atrophy where proximal muscles were mildly affected. Tip toe and heel walking could not be executed and feet had high arches on both sides. No prominent sensory disturbances were detectable. The electrophysiological investigation has shown severe sensorimotor demyelinating neuropathy with marked amplitude reduction. Since 1986, she has recurrent trigeminal neuralgia on the left side and complained about tinnitus as well.

MPZ c.284 T>G (p.Phe95Cys) variant

The Phe95Cys likely pathogenic alteration was found in a 75 -year-old female patient whose symptoms became definitive at age of 69. Before that, she complained about frequent myalgia, muscle cramps and some postural instability for decades. Her mother and elder brother also suffered from severe neuropathy with prominent walking difficulties.

Currently, her gait disturbance progressed so far that she regularly falls and suffers serious traumas. Tip toe and heel walking is not possible and she needs help standing up from crouch. Hand and feet muscles, dorsal- and plantarflexion are moderately paretic. Loss of tactile, algetic, vibration, heat and cold perceptions are present distally and symmetric from knee and elbow.

MPZ c.370 A>C (p.Trp124Pro) variant

A new *MPZ* variant was detected in a 75 -year-old male CMT patients whose symptoms started at age of 69 with myalgia, limited walking distance and sensory disturbance of lower limbs. At the date of examination, his status indicated moderate paresis of dorsal- and plantarflexion and feet atrophy, paretic gait, areflexia, impaired tactile and vibration perception and spinal ataxia. Both upper limbs were spared from neuropathic symptoms.

Electroneurography indicated a severe demyelinating neuropathy with seconder axonal loss. No affected relatives were mentioned by the proband.

EGR2 c.971 G>A (p.Arg324His) variant

A missense nucleotide substitution of *EGR2* was identified in a 22 -year-old patient whose symptoms started at age of 20 with gait disturbances and multiple ankle subluxation. None of his relatives were affected with symptoms of neuropathy. Familial segregation analysis was not possible to perform.

His status indicated mild paresis of both peroneal muscles with normal knee and Achilles reflexes. Atrophic quadriceps and spared sensory qualities were present as well. Electrophysiological study revealed reduction of nerve conduction but it was more pronounced in motor nerves which are congruent with clinical findings. Interestingly, the autonomous nervous system was also affected, the patient complained about nocturnal enuresis and incontinency where no urological etiology could be explored.

GJB1 c.38 T>A (p.Val13Glu) variant

We identified the p.Val13Glu novel amino acid change in a 35 -year-old male patient whose symptoms started at age of 15 with gait disturbance and lower limb weakness. Her mother (72) suffers from similar but milder neuropathy where genetic analysis confirmed the presence of the same alteration.

Neurological examination of the proband at age of 35 has shown paretic gait, the absence of reflexes, paresis, and atrophy of lower and upper limbs – 4/5 and 3/5 in MRC scale, respectively. Tactile and vibration loss was present only on the lower limbs under the line of knees. ENG has revealed axonal neuropathy while EMG showed neurogenic lesion of tested muscles. As atypical signs and symptoms, postural tremor of hands and Babinski sign with spasticity of lower limbs were also accompanied to the phenotype.

His mother's symptoms started a bit later at age of 26. Her symptoms were less severe in spite of her advanced age but she shared her son's symptoms of paretic gait, weak hands, areflexia and sensory disturbance. None of the atypical features were present. Interestingly, both patient's hand were more severely affected with atrophy and paresis than feet or upper limbs.

GJB1 c.379 A>G (p.Ile127Val) variant

The hemizygous p.I127V alteration in *GJB1* was found in a 25 -year-old male whose definitive symptoms started at age of 11; however his motor development was little delayed and also shown less dexterity in sports and in manipulating small objects. His maternal grandfather has severe gait disturbances.

The neurological status at age of 25 indicated severe motor and sensory neuropathy associated with bilateral Achilles contractures, horizontal nystagmus and spasticity. Electrophysiological examination at age of 14 has shown axonal polyneuropathy but at age of 25 it revealed more intermediate type. Her mother also underwent electrophysiological study and neither CMAP reduction nor conduction slowing was present. Segregation analysis confirmed the carrier status of the mother.

GJB1 c.557 A>G (p.Glu186Gly) variant

The c.557 A>G nucleotide substitution in *GJB1* was found in a 47 -year-old male patient. His mother, maternal grandmother and his two daughters suffered from a milder form of CMT.

The proband's symptoms started at the age of 12 with gait disturbances but progression started later at age of 25 years. At the age of 47, the severity of paresis and atrophy were mild and moderate in the upper and lower limbs (MRC: 4-/5), respectively. All the

reflexes were absent, and distal loss of palpation and vibratory sensation on a lower limb has been found as well. Electrophysiology has registered severe axonal neuropathy. Sural nerve biopsy was also executed where axonal and demyelinating features were simultaneously present with thickened nerve, onion bulb formation, and severe axonal degradation. His comorbidities included hypertonia, non-ST elevated myocardial infarct, and three-vessel coronary disease.

All the affected relatives were female. There is little information about his mother and grandmother but about his daughters. Both daughters were relatively young at the time of examination (8 and 12) whose symptoms started under the age of 5 with clumsiness of hands and mild paresis in lower limbs with impaired tip toe and heel walking. The symptoms not influenced their daily tasks although the elder girl was less able to play violin due to the stiffness of hand muscles. ENG indicated an axonal lesion in both children. Genetic analysis confirmed the presence of an alteration in both of them.

GJB1 c.582 G>C (p.Met194Ile) variant

A novel alteration of *GJB1* was also identified in a 23 -year-old male patient who has a positive family history. While his mother was symptomless, his maternal grandfather suffered from severe neuropathy with wheelchair dependency.

The proband's symptoms started at age of 18 years with paretic gait and distal muscle weakness of lower limbs. Neurological examination at age of 23 indicated moderately severe muscle weakness and atrophy of extremities together with areflexia. The distal sensory loss was present on all limbs. Proximal muscle strength and sensory modalities were spared. Unusually, cardiomyopathy and accessory electrical conduction pathway between left atrium and ventricle were present as well. ENG registration indicated intermediate neuropathy as well as prolonged temporal dispersion with 30%. Muscle biopsy was also performed where numerous angular fibers were present. In many of the fibers targetoid sign was present indicating severe neurogenic alteration. Genetic analysis of *GJB1* identified a novel alteration [Fig. 9]. Family segregation analysis verified the presence of c.582 G>C nucleotide substitution in his mother and maternal grandfather, too. His mother was without any clinical or ENG signs of neuropathy. In spite of that, his

grandfather was affected by very severe neuropathy with a disease onset in the early twenties.

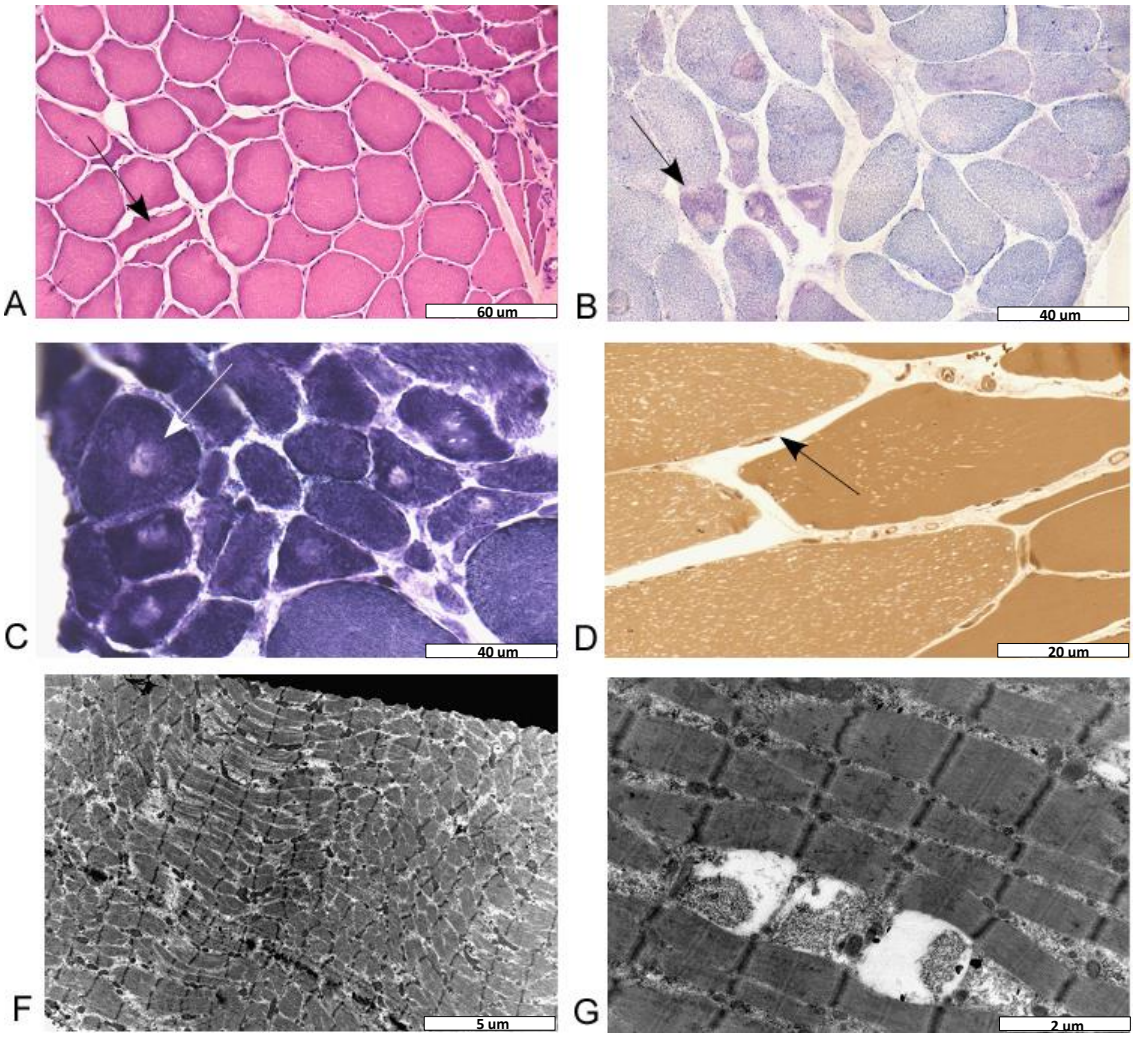


Figure 9. Muscle biopsy has shown moderate neurogenic damage with numerous targetoid fibers. (A) Hematoxylin-eosin staining with angular fibers (arrow) and a prominent difference between fiber diameters (B, C) NADH and mSDH staining show the targetoid pattern (central parts of some fibers are clearly lighter stained (arrows), which is common is neurogenic atrophy. (D, G) On the semithin sections and on EM picttures numerous vacuoles were present. (F, G) EM pictures have shown myofibrillar disorganization with slightly increased mitochondria.

4.4 Investigation of the phenotypic spectrum in CMT subtypes

Age, age of onset and disease duration did not significantly differ between the studied subtypes of CMT Males carrying the *GJB1* pathogenic alteration had the highest CMTES ($p<0.01$). Female CMTX1 patients, who had the mildest disease burden ($p<0.001$), were frequently symptomless (23.1%, $p<0.05$) and had a delayed onset of disease ($p<0.05$) [Table 4, Fig. 10].

Table 4.The distribution of CMTES, age, age of onset and disease duration in the most common genetic subtypes and in the general CMT cohort.

	N	CMTES	Age	Age of onset	Disease duration
CMT cohort	309	8.9±4.6 (8.4-9.4)	39.9 ± 17.6 (37.9-41.9)	25.5 ± 16.8 (23.6-27.4)	15.9 ± 13.6 (14.4-17.4)
PMP22 CMT1A	92	8.1± 5.3 (7.0-9.2)	39.8 ± 16.5 (31.3-48.3)	23.1 ± 16.8 (19.7-26.5)	15.4 ± 14.1 (12.5-18.3)
GJB1 (m) CMTX1	16	12.9± 3.6 (11.1-14.7)	35.6 ± 14.3 (28.6-42.6)	14.8 ± 8.00 (10.9-18.7)	20.8 ± 14.0 (13.9-20.8)
GJB1 (f) CMTX1	16	3.8± 3.4 (2.1-5.5)	41.8 ± 15.8 (34.1-49.5)	28.0 ± 14.8 (20.8-35.3)	13.9 ± 7.20 (10.4-17.4)
MPZ CMT1B CMT2I&J	12	8.0± 5.3 (5.0-11.0)	30.6 ± 19.8 (19.4-41.8)	17.4 ± 22.0 (5.0-29.9)	12.0 ± 10.7 (5.95-18.05)
MFN2 CMT2A2	10	8.2± 4.6 (5.4-11.1)	38.7 ± 23.2 (24.3-53.1)	28.5 ± 23.7 (13.8-43.2)	10.2 ± 12.3 (2.68-17.92)

The Spearman test and linear regression analysis were performed in CMT, *PMP22*, *GJB1*, *MPZ* and *MFN2* cohorts. There was a positive correlation between CMTES and age (<0.05) as well as between CMTES and disease duration (<0.01) in each group. No potential association was apparent with linear regression analysis other than a moderately weak correlation between CMTES and disease duration [Fig. 10]. CMT1A and CMTX1 patients showed a somewhat stronger correlation between disease severity and duration. However, it is important to note that the *MFN2* and *MPZ* cases represent only a narrow range of the variable, thus the extrapolated regression lines are unreliable for making such predictions [Fig. 10].

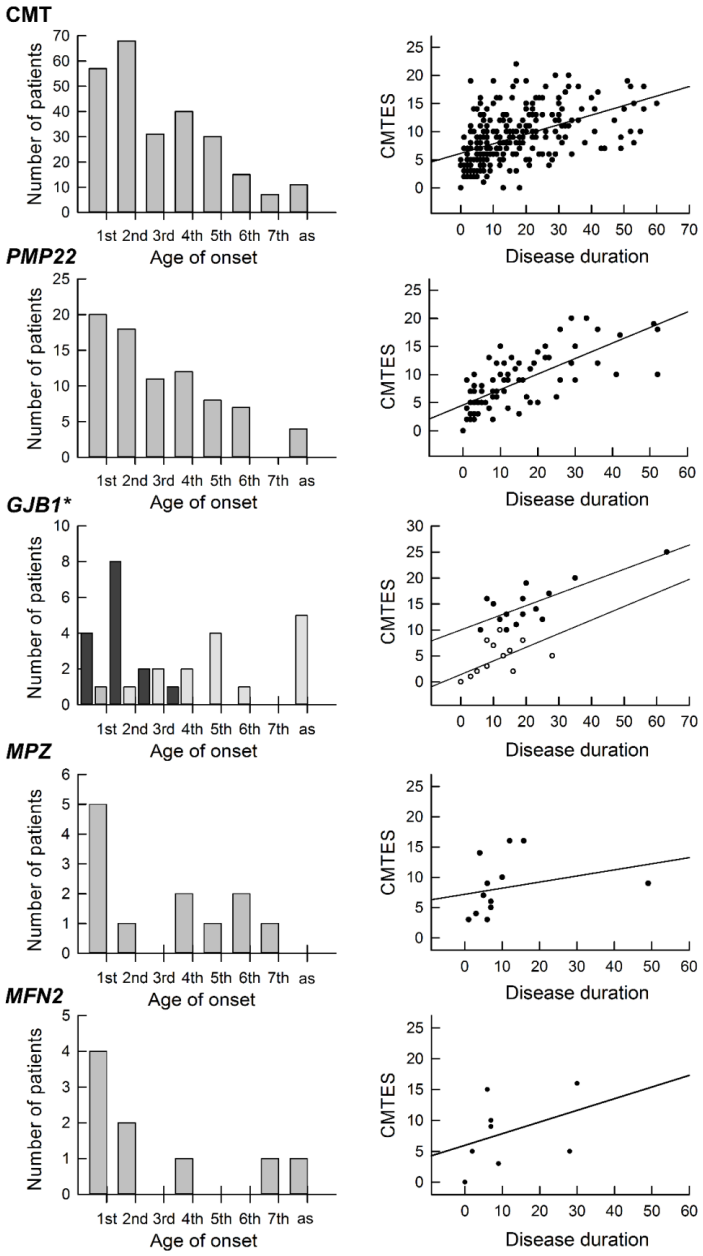


Fig. 10. Statistical analysis of disease progression in different CMT groups. The first column represents the number of patients and the age of onset (in decades) while the second column shows the regression lines fitted on the scatter plots. Values of scatter plots: CMT: r2=0.248, adjusted r2=0.245, p<0.001; PMP22: adjusted r2=0.503, p<0.001; GJB1 male: adjusted r2=0.595, p<0.001; GJB1 female: adjusted r2=0.398, p<0.01; MPZ: adjusted r2=0.000, not significant; MFN2: adjusted r2=0.024, not significant. *Dark bars and dots as well as the upper regression line indicates the male GJB1 patients. Female GJB1 patients are represented by bright bars, dots and the lower regression line.

The frequency of atypical CMT features were also statistically analyzed. Here we list only the significantly correlating features and genes.

Dysimmune mechanisms (6.5%, N=6, 5 unrelated patients) were more frequently associated with *PMP22* duplication ($p<0.01$) compared to the non-CMT1A patients. Three patients had inflammatory polyneuropathy, as well, where ENG signs of conduction block and/or temporal dispersion, elevated liquor protein, immunological markers and a good therapeutic response to steroid, plasmapheresis or IVIg indicated the diagnosis. One patient with systemic lupus erythromatosus (SLE) also carried a *PMP22* duplication where the polyneuropathy antedated the SLE. Other two patients suffered from autoimmune thyroiditis and in one case autoimmune vasculitis was also associated with CMT1A. Six patients with *NDRG1* mutations had early onset hearing impairment ($p<0.001$), and *PMP22* duplication was also commonly present with deafness (6.5%, N=6). Hearing impairment of >40 dB, starting before age of 40 indicated the early onset hearing impairment cohort of the study.

The frequency of CNS involvement was higher in *MFN2* (50%, N=5) and male *GJB1* patients (37.5%, N=6) (both $p<0.01$). These two genes were responsible for half of the cases with CNS symptoms. In CMTX1, central nervous system involvement and pathological MRI findings were present with a male prevalence (males 42%; females 9%; $p=0.57$). One individual suffered from acute episodes of CNS dysfunction accompanied by dysphagia, dysarthria and upper limb paresis at the age of 6, 10 and 14 years. Chronic corticospinal tract dysfunction was present in one patient who had Babinski sign and spasticity in lower and upper limbs. Horizontal nystagmus was observed in one male and in one female patient. Brain MRI was available in four CMTX1 cases and two patients' MRI showed (1) increased white matter signal intensity on T2 weighted and FLAIR sequences in the right hemisphere, and (2) in the periventricular area on the left side without relevant clinical symptoms or history of CNS involvement.

Interestingly, four from five *MFN2* patients with p.R468H mutation (3 female and 1 male, mean age of onset: 27.3 ys) showed CNS involvement, although the severity of neuropathy was mild in general. A female patient without any classical neuropathic feature or electrophysiological changes presented horizontal nystagmus, spasticity, cerebellar ataxia and chronic cortical tract lesion. Another female patient showed

prominent cerebellar ataxia with mild sensory involvement, normal muscle strength and moderate axonal neuropathy. Two additional cases with p.R468H mutation were complicated with spasticity and with a more prominent motor dysfunction than sensory loss.

4.5 Clinical features and gender comparison of a set of patients carrying GJB1 mutation

Clinical features of a subgroup of well-characterized CMTX1 patients (12 males and 11 females – proband and relatives as well; mean age at examination was 36.5±15.4 years and 43.8±15.5 years, respectively) showed various classical symptoms characteristic of neuropathy [Table 5] but the severity showed a broad range depending on gender.

Table 5. Male and female probands' clinical features of the subset of CMTX1 patients studied.

Abbreviations: AA – amino acid; AoO – Age of Onset, DD – disease duration, NCS – nerve conduction study, m – male, f – female, ax – axonal, in – intermediate, de – demyelinating, (-) none, (+) mild, (++) moderate, (+++) severe,

						Upper limb		Lower limb		
Patient	**Sex**	**AA change**	**AoO**	**DD**	**NCS**	**Muscle weakness**	**Sensory impair.**	**Muscle weakness**	**Sensory impair.**	**Other findings**
A-GJB1	m	V13E	15	20	ax	+++	-	+++	++	Pyramidal sign, spasticity, postural tremor
B-GJB1	m	R15T	12	14	in	++	-	++	-	migraine with aura
C-GJB1	m	V63I	11	25	ax	++	+	+	-	-
D-GJB1	m	L89V	14	8	de	+	++	+++	++	horizontal nystagmus, postural tremor
E-GJB1	m	A96G	36	10	ax	++	++	+++	+++	-
F-GJB1	f	A96G	49	12	in	+	++	+	++	-
G-GJB1	f	R107W	30	10	de	+	+	+++	++	-
H-GJB1	m	R142Q	7	21	de	+	+	++	++	transient CNS dysfunction in childhood
I-GJB1	f	R164W	5	8	in	-	-	++	+	nystagmus
J-GJB1	m	R164Q	9	63	in	++	+	+++	+	MRI abnormality
K-GJB1	m	P172A	26	27	ax	++	++	++	++	postural tremor, hepatic lesion
L-GJB1	m	E186G	12	35	ax	+	+	++	++	-
M-GJB1	m	M194I	18	6	in	-	+	++	+	postural tremor, cardiomyopathy, left ventricular arrhythmia, temp. dispersion
N-GJB1	m	N205S	3	21	de	++	++	+++	++	MRI abnormality, postural tremor

Age at onset was 15.5±8.4 for males and 30.7±16.0 for females ($p<0.05$). The disease duration did not differ significantly between genders, with a value of 21±15.4 in males and 14.5±7.1 in females. Females were frequently asymptomatic (total 17%; male 0%; females 36%; $p<0.05$). Distally prominent muscle weakness was the most common symptom (total 83%; male 100%; female 64%; $p=0.03$) which was followed by sensory disturbances (total 78%; male 100%; female 55%; $p=0.01$). The lower limbs were more often affected (total 83%; male 100%; female 64%; $p=0.03$) than the upper limbs (total 74%; male 92%; female 55%; $p=0.04$). Mean CMTNS was 16.5±6.6 for males and

4.5±4.3 for females (p<0.001) while mean CMT neuropathy subscores ranged 1.3 - 2.9 in males and 0.2 - 0.8 in females) [Table 6].

Table 6. This table shows the statistical correlation of CMTX1 phenotype between males and females.

	Male	Female	p-value
CMT neuropathy subscores			
Sensory symptoms	2.3±1.1	0.8±0.8	<0.01
Motor symptoms (legs)	1.8±1.2	0.6±0.7	<0.01
Motor symptoms (arms)	1.3±1.0	0.2±0.4	<0.01
Pinprick sensibility	2.0±1.0	0.8±0.8	<0.01
Vibration	1.8±0.9	0.5±0.7	<0.001
Strength (legs)	2.9±0.8	0.7±1.1	<0.001
Strength (arms)	1.9±1.2	0.4±0.5	<0.001
Ulnar CMAP	2.4±1.0	0.5±1.0	<0.001
Radial or Ulnar SAP	2.2±0.9	0.6±1.1	<0.01
Tremor	5 (42%)	0 (0%)	<0.05
Pes cavus	8 (67%)	2 (18%)	<0.05
Decreased/absent reflexes	11 (75%)	3 (27%)	<0.01
CNS involvement	5 (42%)	1 (9%)	not significant

The distribution of probands' electrophysiological changes was the following: 5 primarily axonal (35.7%), 5 intermediate (35.7%), 4 demyelinating (28.6%). The type of neuropathy was identical in the same families. ENG features also revealed increased temporal dispersion in 5 patients where definite temporal dispersion was found exclusively in one patient (>30% at least in two nerves). None of the CMTX1 patients reported hearing impairment.

Previously reported *GJB1* cases were also collected and compared with out findings based on different objective parameters – gender, motor and sensory symptoms, NCS [Table 7].

Table 7. Comparision of previously described mutations and our cases. *Axonal type was found in younger patients (6 and 8 ys old) while adult patients have shown intermediate nerve damage.

Mutation	Features	Patients of this study	Reported patients
Arg15Trp	Gender and reference	male	female (152)
	Age of onset	12	9
	Motoric symptoms	UL and LL severly affected, normoreflexia	UL and LL severly affected, areflexia
	Sensory symptoms	loss of sensation	loss of sensation
	Other symptoms	pes cavus	pes cavus
	NCS	intermediate	intermediate
Val63Ile	Gender and reference	male	male (66)
	Age of onset	11	14
	Motoric symptoms	severe paresis and atrophy of UL, mildly affected LL	UL mildly affected, gait disturbance
	Sensory symptoms	-	-
	Other symptoms	-	hypacusis,cognitive deficiency, nystagmus, pes cavus
	NCS	axonal	axonal
Arg142Gln	Gender and reference	male	female and male (65)
	Age of onset	6	2-8
	Motoric symptoms	atrophy and paresis of LL areflexia	atrophy and paresis of LL areflexia
	Sensory symptoms	lack of vibration sense	lack of tactile and vibration sense
	Other symptoms	-	hypacusis
	NCS	demyelinating	deminalinating and axonal*
Arg164Trp	Gender and reference	female	female and male (153)
	Age of onset	5	2-15
	Motoric symptoms	increased muscle tone, hypertrophy, hyperreflexia	moderate and severe paresis and atrophy of UL and LL, areflexia
	Sensory symptoms	-	loss of sensation
	Other symptoms	pain, pes cavus, Achilles contracture	pes cavus, hypacusis
	NCS	intermediate	intermediate
Pro172Ala	Gender and reference	male	no data (154)
	Age of onset	26	no data
	Motoric symptoms	moderate atrophy and paresis of UP and LL, hyporeflexia	distal atrophy and paresis of UL and LL, areflexia
	Sensory symptoms	deep sensory disturbances	no data
	Other symptoms	pes cavus	pes cavus
	NCS	axonal	axonal
Asn205Ser	Gender and reference	female and male	female and male (155)
	Age of onset	3	13, 19
	Motoric symptoms	paresis and atrophy of LL, female symptomless	paresis and atrophy of LL, female symptomless
	Sensory symptoms	hypaesthaesia	no data
	Other symptoms	static hand tremor, pes cavus,	pes cavus, transient CNS involvment
	NCS	demyelinating	demyelinating

4.6 Analysis of rare variants with high-througput methodology.

From the studied CMT2 cohort, 15 well-characterized CMT patients have been analyzed – 6 with whole exome sequencing and 9 dHMN-HMSN overlapping phenotype with target sequencing – (7 males and 8 females, mean age at examination was 29.5±18.2 years and 26±16.5 years, respectively). In these cases, no pathogenic variants were found in the screened genes (*PMP22*, *GJB1*, *MFN2*, *MPZ*, *CTDP*1, *NGDR*1). Through a detailed analysis of data, pathogenic or likely pathogenic alterations were found in 5 patients (33.3%). The identified alterations are summarized in Table 8.

Table 8. List of identified pathogenic or likely pathogenic alterations

Patient	Method	Sex	Gene	Mutation	ACMG	Reference
1-CMT	WES (Centogene)	female	*TRPV4*	c.806G>A p.R269H	pathogenic	(156)
2-CMT	WES	male	*MME*	c.1946T>C p.I649T	VUS	**p.s.**
	WES	male	*POLG*	c.3244G>A p.A1082T	likely pathogenic	p.s.
3-CMT	target sequencing	female	*HINT1*	c.110G>C p.R37P	pathogenic	(185)
4-CMT	target sequencing	male	*HINT1*	c.110G>C p.R37P	pathogenic	(185)
5-CMT	target sequencing	female	*HINT1*	c.110G>C p.R37P	pathogenic	(185)

4.6.1 *TRPV4* pathogenic alterations

The c.806G>A, p.R269H known pathogenic mutation of *TRPV4* was indentified with whole exome sequencing performed by Centogene AG, Rostock. Raw data of sequences were not available for further analysis, only the finding given by the company.

The alteration was found in a two and half -year-old girl whose symptoms started at age of 8 months with clumsiness and delayed motor development. The parturition was spontaneous and the perinatal period was uneventful, only congenital club-feet and mild hypotonia were already present. Her status indicated distally prominent and moderate

paresis (4/5 by MRC scale) and spared tactile and vibratory sensation of all limbs. Scapular muscles were not affected and otolaryngologist also excluded laryngeal palsy. Her body weight and height were between 25 and 50 percentile and mental development was age-appropriate, too. Expect the persisting club-feet, no other orthopedic malformation were present. Electrophysiological investigation revealed a severe reduction of CMAP amplitude in peroneal, tibial ulnar and median nerves. SAP amplitude reduction of sensory nerves were minimal. NCV was spared of all nerves. Both parents have not shown any clinical or electrophysiological signs of neuropathy and genetic analysis proved that they do not carry the *TRPV4* alteration.

4.6.2 *POLG* and *MME* likely pathogenic alterations

A 35 -year-old patient was analyzed with whole exome sequencing. Cavus feet were present since early childhood but definitive symptoms started at age of nine with gait disturbances. At the time of neurological examination he had moderate distal muscle weakness which was more pronounced in lower than in upper limbs. (4-/5 and 4/5 by MRC scale, respectively). Below the line of knee and wrists, the sensory functions were also deprived. He also barely recognized somatosensory stimuli in these areas. Electrophysiological evaluation revealed axonopathy of motor, sensory and mixed nerves with spared nerve conduction velocities. It seems to be a sporadic case but the patient has minor twins (7 ys old) who are symptomless. Genetic analysis of parents and offsprings was not possible.

Exome capture sequencing generated ~8.9 billion bases of sequence, and ~8.7 billion bases were then mapped to the target regions based on SeqCap_EZ_Exome_v3 Kit. 95% of the target regions had at least 10× coverage. After identification of variants, the synonymous variants, splice acceptor and donor site mutations, and short, frame shift coding insertions or deletions (indel) in neuropathy linked genes were screened. For this purpose we used Variant Analyser software and after filtering out synonymous SNPs, 23 heterozygous variants remained, Further narrowing based on protein prediction scores and ClinVar and allele frequency data, resulted in two variations. No rare homozygous or compound heterozygous variants were found in the analyzed neuropathy-linked genes. Finally, two mutations were kept in and validated with Sanger sequencing. The *POLG* c.3244 G>A missense nucleotide substitution (p.A1082T) was not reported yet while the

MME c.1946 T>C alteration (p.I649T) was recently described as a variant with unknown significance (ClinVar, rs184666602). Both amino acid changes are located in a taxonomic well-conserved region of the proteins and in silico analysis predicted its likely pathogenic nature. Only the *MME* alterations were present in ExAc database with an allele frequency of 0.00005. Based on ACMG guideline, the *POLG* variant is likely pathogenic alteration while the *MME* mutation was declared as variant with unknown significance.

4.6.3 *HINT1* pathogenic variant

Target sequencing of dHMN and HMSN genes was performed in 5 female and 4 male patients (mean age at examination was 20.2±13.5 years and 28.25±24.9 years, respectively) All patients had more dominant muscle involvement than sensory disturbance. Three patients had delayed muscle relaxation, two CNS involvement (cerebellar ataxia and pyramidal sign, respectively), and one patient suffered from prominent scapular weakness. All the patients data were carefully checked for pathogenic and likely pathogenic alterations using ClinVar database, PubMed and ACMG guideline.

The same homozygous pathogenic mutation was found in three patients (*HINT1*, c.110 G>C, p.R37P, rs149782619). Three variants with unknown significance were also present in CMT-linked genes (*HSPB8* c.233 G>T, p.R78M, rs55826713; *DCTN1* p.D762N and *ATP7A* p.T818A). Based on segregation analyses, these variants were found in symptomless relatives as well.

Patients, carrying *HINT1* mutation, presented progressively decreasing muscle strength, delayed muscle relaxation, elevated CK levels, impaired mental performance, and mild sensory disturbance. Age of onset ranged between 9-14 years. Interestingly, beside axonal neuropathy, EMG registered neuromyotonia which was characterized by prolonged muscle relaxation after voluntary muscle contractions, causing by the hyperexcitability of lower motor nerves. Further findings are collected in table 9.

Table 9. Clinical feature of HINT1 patitents. Abbr.: UL – upper limb; LL – lower limb, N/A – no data; ; (-) – none; (+) – mild; (++) – moderate; (+++) – severe

	D-HINT1 (f)	**E**-HINT1 (m)	**F**-HINT1 (f)
Phenotype	AR-CMT2	AR-CMT2	dHMN
Presenting symptoms	progressive feet deformity (early childhood), later hand weakness (5 ys old)	walking on tip toe (18 months), unable to run or crouch	impaired hand dexterity, hyperkinesia, and paresis at age of 11
Motor development	normal	delayed, walking on tip toes, gait disturbances	normal
Delayed muscle relaxation after contraction	++	+++	+
Muscle tone	normal	normal	normal
Muscle strength	LL: proximal and distal paresis (++)	LL: proximal and distal paresis (++)	LL: proximal and distal paresis (++); UL: distal paresis (+)
Muscle atrophy	LL (++)	LL (++)	LL (+)
Tendon reflexes	absence	absence	decreased
Pyramidal sign	-	-	-
Sensory disturbance	+	++	+
Cerebellar sign	-	-	dysdiadochokinesis
Contractures	symmetric contractures in hands and feet, Achilles contractures	symmetric contractures in hands, elbow, and feet (hammer toe), Achilles contractures	hyperflexible joints without any contractures or deformity
Dystonia	focal dystonia (writing cramp)	focal dystonia (writing cramp)	-
Brain MRI	normal	-	adenoma in the hypophysis
Laboratory features	CK: 270 U/L, LP: 0.25 g/L	CK: 1411-1806 U/L	CK: 410 U/L, Vitamin D 11.9 ng/ml
Neuromyotonia on EMG	Yes (9 ys old)	No (14 ys old)	Yes (13 ys old)
Muscle biopsy	neurogenic features, rounded, atrophic fascicules and COX negative fibers	neurogenic featuers, sural biopsy shows moderate axon reduction	-
Cognitive functions	impaired mental performance	impaired mental performance	impaired mental performance
Other disorders	depression, suicide attempt, hypothyresosis	-	anxiety

5. Discussion

5.1 Frequency of CMT genes in Hungary

This study is the first comprehensive genetic epidemiology study of CMT in Hungary. Our findings indicate, that the *PMP22* alterations (40.1%) were the most frequent which were followed by *GJB1* (9.6%), *MPZ* (4.5%) and *MFN2* (2.4%) mutations. Rare variants in *EGR2*, *CTDP1*, *NDRG1*, and *PMP22* were found only in a small percentage of our cohort (3.4%). These findings mean a 59.9% success rate which is in accordance with other recent studies (157). The distribution of electrophysiological subtypes (158) and the prevalence of the most common gene alterations was also consistent with previous observations, but the frequencies of genetic subtypes deviate with respect to other epidemiological data (Table 10).

Table 10. Frequency of genes in different populations

	Hungary *Recent study*	Norway (158)	USA (27)	Spain (159)	Germany (160)	USA[a] (161)	International INC [13]
	N=531	N=187	N=787	N=438	N=589	N=425	N=1652
PMP22 duplication	39.7% (211)	19.8% (37)	36.9% (290)	42.0% (184)	35.6% (180)	39.5%(168)	37.2% (614)
GJB1	9.6% (51)	4.8% (9)	10.2% (80)	12.8% (56)	8.5% (43)	10.8% (46)	6.5% (107)
MPZ	4.5% (24)	3% (6)	5.7% (45)	4.3% (19)	4.1% (21)	3.1% (13)	4.1% (67)
MFN2	2.4% (13)	1.1% (2)	2.7% (21)	1.6% (6)	2.4% (12)	2.8% (12)	4.2% (70)
EGR2	0.8% (4)	-	0.1% (1)	-	-	0.0% (0)	0.06% (1)
PMP22 point mutation	0.4% (2)	0% (0)	0.6% (5)	0.5 (2)	0.4% (2)	1.4% (6)	6.5% (107)
NDRG1 (LOM)	1.6% (8)	-	-	1.9% (7)	0.3% (2)	-	-
CTDP1 (CCFDN)	0.8% (4)	-	-	-	-	-	-
Cumulative hit rate of further genes	0% (0)	0% (0)	10.8% (85)	22.1% (97)	4.5% (23)	9.2% (21)	7.3% (121)
Number of tested genes	7	7	11	22	12	13	20
No genetic diagnosis	40.3% (214)	71% (133)	36.9% (290)	15.3% (67)	43.5% (220)	37.4% (159)	39.6% (655)

Nine novel alterations were found in the investigated patients. According to ACMG guidelines, the variants are pathogenic or likely pathogenic alterations. The clinical phenotype of patients definitely fits into the CMT spectrum; however SMA-like

phenotype (*MPZ* p.S20P) was present in one case which was an unusual feature regarding this gene.

The localization of mutant amino acids was also diverse compared to international data. In *GJB1*, the alterations of mutational hotspot codon 164 were responsible for the 14.3% of CMTX1 in our index patients, which is slightly lower than international findings (18-27.8%) (162, 163). We have also found that the occurrence of pathogenic alterations in cytoplasmic loop domain (21.4%) was the second most frequent cause of CMTX1 in our cohort which domain is usually underrepresented in other CMT studies (0-14.8%) (164, 162, 163). The p.R468H mutation of *MFN2* seems to be a mutational hotspot in Hungarian patients. A similar high proportion of this variant (42.9%) was reported from Spain (81). These observations may indicate a preferred mutational and hotspot regions among Hungarian patients compared to other cohorts and can raise the possibility of a connection between ethnicity and localization of pathogenic alterations. Notably, founder mutations were detected in 21% of Roma patients, which draws further attention to the relevance of origin and targeted screening of these founder mutations in patients with Roma ancestors.

5.2 Phenotypic spectrum of CMT genes in Hungarian patients

Clinical and electrophysiological evaluation of patients along with the genetic result was further analyzed in order to unravel possible genotypic and phenotypic connections. Regarding the age of onset, the initial symptoms manifested in the first three decades of life in 70% of patients (mean age of 25.5 ys) where asymptomatic cases were included. Due to the small number of similar investigations, it proved difficult to compare disease severity with other studies. Fridman et al. evaluated the CMTES of 462 CMT1A cases and their findings indicated a slightly higher mean CMTES than our patients' mean score (8.5 and 8.1, respectively) (157).

The progression of CMT during a patient's lifetime is well-known (165). Based on our data, the change in CMTES shows a linear correlation with age and disease duration, while the course of worsening could be estimated in the quarter of the CMT population with the regression line. Nevertheless, disease progression can be predicted 10-35% more accurately in CMT1A and CMTX1 cases. This difference may come from the fact that hereditary neuropathies show variable penetrance, wide range of clinical symptoms and

severity even with the same causative gene or in same affected family due to distinct genetic features of the causative genes and the different modifying factors, such as gene-gene interactions (simultaneous presence of one or more pathogenic allele mutation load), epigenetic modification effects, co-morbidities (diabetic neuropathy, alcoholism, autoimmune disease etc.) or disease management. These all have a cumulative impact on disease severity leading to a less predictable course of progression (25-29). The combinatorial influence of these suspected factors may interact in the same biological pathways and affect the penetrance and/or the expressivity of the phenotype.

Additional symptoms or diseases occurred in more than one fifth of our patients. CNS abnormality was the most frequently associated feature and it was present mainly with *GJB1* (see 6.3.) and *MFN2* pathogenic alterations. Both of these CMT genes were reported with CNS involvement occasionally resulting in diagnostic uncertainty (166, 167, 31). Several studies have linked *PMP22* and *NDRG1* gene variants to a higher occurrence of sensorineural hearing impairment starting in the early decades of life (96, 168, 169) which is in accordance with our results. Interestingly, *MPZ* and *GJB1* pathogenic alterations have not appeared with deafness in spite of their frequent co-occurrence (31). Our findings also suggest a higher incidence of autoimmunity in CMT1A. Few observations have raised the possibility of superimposed dysimmune mechanisms in CMT1A (170, 43) and a higher proportion of antibodies against PMP22 has been detected in CMT1A patients (171). However, the processes of immunological abnormalities are still indistinct and need to be further studied for a deeper insight.

5.3 Clinical and electrophysiological analysis of a set of CMTX1 patients

Our clinical observations and previous CMT studies have emphasized the different phenotypical appearance of *GJB1* pathogenic alterations between the two genders. The inheritance of CMTX1 is usually considered as dominant since females and males both have clinical symptoms; however, female are affected with a less severe neuropathy. To analyze this phenomenon, comparative statistical analyzes were used to investigate whether there are clear differences between Hungarian CMTX1 males and females. We found that most of the females were older at disease onset, had milder CMTNS, CMT examination scores and the frequency of certain additional signs and symptoms were rarer. Our findings are in accordance with previously performed analyzes where female

had been found less affected. (61, 163). Along with these studies, it can be presumed that CMTX1 is indeed an X-linked dominant disease but with an incomplete penetrance in females. A connection between the distribution of X inactivation and symptom severity in female CMTX1 patients can be a potential explanation for this phenomenon. However, the clinical phenotype has not shown any correlation with X inactivation pattern in blood according to a previous study (172). This raises the possibility that the X inactivation distribution may differ between Schwann cells and leukocytes.

The clinical and electrophysiological data of other previously reported cases with same mutated codon pointed out intriguing similarities regarding the age of onset, type of neuropathy and CNS involvement. Due to the limited number of data, it is not enough to reflect an unambiguous conclusion but this observation draw the attention to the possibility that same mutation can inflict highly similar phenotype and is mildy affected by other factors.

In CMTX1, the type of neuropathy frequently differs within the same family (61, 173). Usually, females had only amplitude reduction while males had intermediate or demyelinating neuropathy. Here, the electrophysiological features of neuropathy did not differ in the same family. Furthermore, the excessive temporal dispersion was present only in one case (p.M194I), while in previous electrophysiological studies it was found to be more frequently associated with CMTX1 and causing difficulty in distinguishing CMTX1 from chronic inflammatory demyelinating polyneuropathy (CIDP) (174, 175). In our case, based on the family history, clinical features, and laboratory data CIDP could be excluded (175).

GJB1 mutations have associated with CNS involvement significantly more often than CMT alone (see 6.2) but CNS symptoms can evolve in female as well. The alterations of codon 142, 164 and 205 were previously linked to CNS symptoms (www.molgen.ua.ac.be/CMTMutations) while codon 13 and 89 have not been described with CNS symptoms to date. It is not exactly known why some mutations cause CNS signs and symptom and some why not. Recently, the gap junction channel function was studied and compared between PNS and PNS+CNS mutants (CNS symptoms were absent or present, respectively). Interestingly, gap junction function was spared or slightly impaired in PNS mutants while PNS+CNS mutations lacked functional junctions leading

to complete loss of homotypic channels (68). Moreover, the penetrance of symptoms differs widely between female CMTX1 patients which also might connect to the type of protein dysfunction. Clinical observations also presumed the effect of domain localization of mutations. Highly conserved cysteine rich regions seem to be attached to more severe disease burdens and CNS involvement whereas mutations in CL loop refers to a more benign clinical picture (176); however, Shy et al confuted this hypothesis (67).

5.4 Rare variants identified with high-throughput methods

To further analyze the unsolved CMT2 cases, we applied high-throughput method for screening of pathogenic or possible pathogenic alterations. We identified pathogenic or likely pathogenic mutations in one third of patients. Based on the experience of other research groups, between a 31-46% success rate was expected with NGS methodology (177, 178). Our rate of 35.5% fits in the middle range but lower than our results with standard methodologies.

A pathogenic TRPV4 variant was found in a two and half -year-old girl. This mutation affects the intracellular N-terminal ankyring domain of the TRPV4 causing the altered function of the ion channel and leading not only to CMT2C but to scapuloperoneal spinal muscular atrophy (SPSMA), congential distal spinal muscular atrophy (CDPSMA) and autosomal dominant skeletal dysplasias (179, 180). Clinical appearance also frequently associates with vocal cord palsy and respiratory distress due to diaphragm weakness (181). The identified p.R269H variant was already reported multiple times with SPSMA phenotype. Interestingly, diverse severity of clinical picture was observed even with same mutation and in same family. Biasini et al have found that the index patient's father had much milder muscular weakness than his son whose phenotype was characterized by progressive scapuloperoneal atrophy and weakness as well as bilater congenital clubfoot (182). Zimon et al also found this alteration in association with SPSMA, skeletal dysplasia and vocal cord paresis (181). Overlapping CMT2 and SPSMA phenotype was also described in Greece with a p.R232C mutation. Our patient had a predominantly motor neuropathy with spared motor functions in scapular muscles. No major skeletal dysplasia, vocal cord palsy and respiratory distress was present. Most likely, similarly to the Greek patient, these clinical features refers to an overlapping phenotype of CMT2 and SPSMA.

However, taking her young age into consideration, the clinical picture can rapidly change therefore longitudinal follow-up is especially required in this case.

We identified two distinct non-synonym mutations of *MME* and *POLG* genes in one CMT patient. *POLG* has a crucial role in maintaining the integrity of mitochondrial DNA and in mitochondrial replication. The identified p.A1082T mutation is located in the highly conserved polymerase domain causing altered protein features and splice site changes. Several pathogenic mutations were already described in this protein region. *POLG* does not belong traditionally to CMT genes, despite the fact, that it is usually associated with axonal neuropathy. *POLG* can cause a wide range of disorders including progressive external ophtalmoplegia with mitochondrial deletions (PEO), mitochondrial recessive ataxia syndrome (MIRAS), sensory ataxia with neuropathy, dysarthria and ophtalmoplegia (SANDO), Alpers syndrome, mitochondrial neurogastroinstinal encephalopathy (MNGIE), Parkinson disease, dementia and other psychiatric disorders (183). It worth to note that the patient does not have further comorbidities. Mitochondrial DNA deletion was also found which is a sensitive marker of mutated POLG protein further prove the pathogenicity of this alteration.

MME encodes the metalloendopeptidase neprilysin. The identified p.I649T amino acid change is presumably a non-conservative alteration, which is likely to impact secondary protein structure resulting in impaired functions. In silico analysis predicted its damaging nature as well. *MME* mutations were first described with autosomal recessive inheritance (184) but Auer-Grumbach et al reported that *MME* also inherits dominantly causing a late-onset neuropathy (185). Autosomal dominant MME mutations were also linked to spinocerebellar ataxia (186). Interestingly, AD inheritance has an age-related incomplete penetrance and its patomechanism is tightly linked to reduced tissue neprilysin activity. The *MME* coded neprilysin is dedicated to degrading neuron-specific proteins including amyloid ß (Aß). Dysfunctioning Aß, among others, is involved in impaired mitochondrial dynamics, transport and Ca^{2+} homeostasis in neuronal cells (187). Since heterozygous *MME* mutations are linked to age-dependent penetrance, it has presumably no primary causative role but may modify the effect of POLG through shared functional pathways in mitochondrial dynamics it further worsens the mitochondrial dysfunction. Gonzaga-Jauregui et al described a similar phenomenon regarding the effect of mutation load. They have found that CMT patients had increased number of non-synonymous alterations in

CMT genes versus healthy controls and suggested that genetic burden influences the phenotypic variability of CMT (188). The accumulation of Aß and POLG mutations together may cumulate the chance of Alzheimer dementia in this patient (189, 190).

Loss of funtion mutations of *HINT1* were first described in 2012 by Zimon et al in a set of patients with hereditary axonal neuropathy and neuromyotonia. HINT1 is a homodimeric purine phosphoramidase that is expressed ubiquitously in different cells and participates in different apoptotic pathways, transcriptional suppression and RNA metabolism where interact and share common pathways with causal genes of CMT (*AARS, GARS, HARS, KARS, MARS, YARS*) (191). *HINT1* mutations might take effect in three different ways: alter residues critical for the catalytic activity; lead to nonsense-mediated decay of the faulty transcript; or cause instabil protein and subsequent proteasome-mediated degradation (191). Interestingley, Mice lacking Hint1 proved to be less useful for investigating HINT1-associated neurodegeneration since they showed normal motor performance test results without any signs of nerve degeneration (192).

To date, the homozygous p.R37P amino acid change proved to be the most frequent pathogenic alteration of *HINT1*. Since it was found almost exlusively in in Central-European countries and Turkey, it is considered to be a founder mutation but more data is required to validate that hypothesis (193). *HINT1* mutations are associated with a more prominent motor involvement than sensory. Clinical phenotype of patients frequently included gait disturbances, cramps, contractures, foot deformity, elevated CK level and sensory loss (194).

HINT1 is also expressed in the central nervous system implicating further effects on the CNS (195). Previous studies found that patients suffering from major depression disorder had an increased level of HINT1 protein in dorsolateral prefrontal cortex, and, on the contrary, schizophrenic patients had decreased level of protein. These phenomenon may explain the detected psychiatric disorders of the probands (196).

6. Conclusions

Regarding the aims of the study, the current research established following novelties and conclusions:

1. In Hungary, we have conducted the first genetic epidemiology study of CMT. Analysis of the most common CMT genes in Hungarian patients has revealed the genetic etiology almost in 60% of cases which is a satisfactory result compared to international findings. Our data also enhance the importance of ethnicity since certain minorities frequently carry founder mutations and certain mutational hotspot regions seems to be overrepresented in Hungarian patients. Based on that, the screening of frequent alterations should precede other gene tests in selected cases.
2. We identified nine novel pathogenic or likely pathogenic variants of *GJB1, EGR2* and *MPZ* genes. The detailed clinical phenotypes of patients harboring presumably disease causing alterations further broaden the accessible data which help in the further understand of distinct molecular patomechanisms and facilitate the research of new pharmaceutical agents.
3. In general, the applied prediction models of disease progression was less effective; however, the accuracy was higher if same genetic subtypes were analyzed. Our findings indicate that the genetic diagnosis is a strong predictor but family history, associated diseases, environmental factors and further genetic alterations was not included in this model. Further researches should also consider and study these factors in developing of a more precise prediction model.
4. Fifth of the patients have shown one or more non-conventional CMT symptoms or associated diseases. Central nervous system involvement was significantly more frequent with *GJB1* and *MFN2* mutations while dysimmune mechanisms and hearing impairment was associatied more commonly with *PMP22* duplication. Based on that observations, certain features can help in determining the most likely genes which can facilitate the diagnostic algorithm of CMT along with the family history. Our findings also broaden the spectrum of known *GJB1* and *MFN2* codons involved in CNS abnormalities.
5. Female *GJB1* patients had less severe phenotype and later age of onset than males and associaed rarely with further features. CMTX1 males, however, have shown

frequent CNS involvement. These difference clearly highlight the importance of precise a pedigree and deep phenotyping calling attention on CMTX1 patients in an extremely heterogenous disease group and further enhancing the cost-effective diagnostics of CMT.

6. New generation sequence platforms can be a robust diagnostic tool to unwrap sporadic cases and study overlapping syndromes. Cases, where traditional genetic analyses have not confirmed the pathogenic variant, new approach have lead to a genetic diagnosis in multiple cases. Here, we also determined a possibly frequent *HINT1* pathogenic alteration which analysis should be inserted into the CMT diagnostic workflow in Hungary, especially in cases, where predominantly axonal and motor neuropathy is present with or without neuromyotonia.

7. Összefoglaló

A Charcot-Marie-Tooth betegség egy örökletes, progesszív lefolyású neuromuscularis megbetegedés, mely körülbelül 3500-4500 beteget érint Magyarországon. A CMT diagnosztikájában bekövetkezett változások az elmúlt években a diagnózist gyorsabbá, pontosabbá és elérhetőbbé tette. Számos klinikai vizsgálat hívta fel korábban a a figyelmet arra, hogy a jól fenotipizált kohorszokban, bizonyos faktorok figyelembe vételével növelhető a diagnosztikus hatékonyság. Jelen értekezés célja, hogy megvizsgálja a magyarországi CMT betegek genetikai hátterét, és információt nyerjen a betegség klinikai variabilitásáról egyes gének esetében. Ebből a célból 409 CMT1-ben és 122 CMT2-ben szenvedő betegnél vizsgáltuk meg rutinszerűen a *PMP22, GJB1, MPZ, EGR2* és *MFN2* géneket, míg az *NDRG1* and *CTDP1* gének alapító mutációit roma betegeknél szűrtük. A CMT2 betegcsoport 15 betegének esetében újgenerációs szekvenátor platform segítségével vizsgáltuk a minor CMT gének érintettségét. A CMT1-es betegek 67.2%-nál, a CMT2 kohorsz 33.6%-nál sikerült genetikai diagnózist felállítani. A leggyakoribb érintett gén a *PMP22* (40.5%) volt, melyet a *GJB1* (9.2%), *MPZ* (4.5%), *MFN2* (2.5%), *NDRG1* (1.5%), *EGR2* (1%) and *CTDP1* (0.8%) patogén eltérései követtek. Az újgenerációs szekvenátor platformon történt vizsgálat során a *HINT1* gén alapító mutációját homozigóta formában három gyermeknél azonosítottuk panel szekvenálással, míg teljes exom analízis során egy *TRPV4* mutációt és egy feltételezetten patogén *POLG* variánst találtunk. A négy leggyakoribb gén vizsgálata az esetek 50%-ban azonosította a genetikai eltérést, míg a teljes kohorszt figyelembe véve a sikerességi ráta 59.9% volt a hagyományos metodikák alkalmazásával. A klinikai kép és a betegség súlyossága nagy variabilitást mutatott a betegeknél. Mintegy ötödüknél az örökletes neuropathia atípusos tünetekkel és társbetegségekkel szövődött, melyek több esetben is szignifikáns összefüggést mutattak egyes génekkel. A CMTX1 betegeknél a betegség súlyossága enyhébb volt a női pácienseknél. Az újgenerációs szekvenátor platformon végzett vizsgálatok a *HINT1* gén magas előfordulási gyakoriságára hívja fel a figyelmet a gyakran neuromyotoniával szövődött, korai kezdetű örökletes neuropathiánál. Jelen értekezésben közölt eredmények hasznosnak bizonyulhatnak egy általános vizsgálati stratégia felállításában magyarországi CMT betegek esetében, valamint az örökletes neuropathiák klinikai heterogenitásáról rendelkező tudásunkat is tovább bővíti.

8. Summary

Charcot-Marie-Tooth disease is a progressive, inherited neuromuscular disorder which may affect around 3500-4500 Hungarian patients. In the last decades, the genetic research of CMT made the diagnostics more successful and accessible than ever been. It has been also recognized that well-characterized populations and gene specific epidemiological data along with other factors can enhance the efficiency and lower the costs of diagnostics. This thesis aims to investigate Hungarian patients suffering from inherited neuropathy and unravel the genetic cause of the disease and assess the phenotypical variability and spectrum of different causative genes. Here we investigated 409 CMT1 and 122 CMT2 patients. The genetic testing of *PMP22*, *GJB1*, *MPZ*, *EGR2* and *MFN2* genes were performed routinely while *NDRG1* and *CTDP1* genes were screened only for founder mutations in Roma patients. In a small subset of CMT2 patients, new generation sequencing was applied to identify CMT genes with minor frequencies. 67.2% of the CMT1 and 33.6% of the CMT2 patients received a genetic diagnosis which indicates a 59.9% success rate in the study population. Considering all the affected individuals, the most frequent gene was the *PMP22* (40.5%) which was followed by the *GJB1* (9.2%), *MPZ* (4.5%), *MFN2* (2.5%), *NDRG1* (1.5%), *EGR2* (1%) and *CTDP1* (0.8%) pathogenic alterations. NGS target sequencing found *HINT1* homozygous founder mutations in three minor patients while whole exom sequencing revealed a pathogenic *TRPV4* alteration and a likely pathogenic *POLG* variant. The screening of the four most common causative genes resulted in the genetic diagnosis in more than the half of the cases and the result indicates a 59.9% overall success rate. The phenotypic spectrum and the disease severity of the studied patients also varied broadly. Fifth of the patients shown additional signs and symptoms and some features were tightly associated with certain genes. Regarding the CMTX1 patients female had a milder clinical appearance than males. NGS results also highlighted the high frequency of *HINT1* gene in Hungarian patients with early onset and neuromyotonia. Current results provide handful data to design a general diagnostic scheme of CMT in Hungary and deepen the current knowledge about the clinical heterogeneity of inherited neuropathies.

9. Bibliography

1. Dyck, P., Peripheral neuropathy. 2005: Elsevier Inc.
2. Skre H. (1974) Genetic and clinical aspects of Charcot-Marie-Tooth's disease. Clinical genetics, 6: 98-118.
3. Bansagi B, Griffin H, Whittaker RG, Antoniadi T, Evangelista T, Miller J, Greenslade M, Forester N, Duff J, Bradshaw A. (2017) Genetic heterogeneity of motor neuropathies. Neurology, 88: 1226-1234.
4. Timmerman V, Strickland AV, Züchner S. (2014) Genetics of Charcot-Marie-Tooth (CMT) disease within the frame of the human genome project success. Genes, 5: 13-32.
5. Pareyson D, Marchesi C. (2009) Diagnosis, natural history, and management of Charcot–Marie–Tooth disease. The Lancet Neurology, 8: 654-667.
6. J H. (1912) Über progressive phyertrophische Neuritis. Dtsch Z Nervenheilkd 44.
7. WP H. (1888) Muscular atrophy of the peroneal type affecting many members of a family. Brain: 230-236.
8. Harding AE, Thomas PK. (1980) Genetic aspects of hereditary motor and sensory neuropathy (types I and II). J Med Genet, 17: 329-336.
9. Al-Thihli K, Rudkin T, Carson N, Poulin C, Melancon S, Der Kaloustian VM. (2008) Compound heterozygous deletions of PMP22 causing severe Charcot-Marie-Tooth disease of the Dejerine-Sottas disease phenotype. Am J Med Genet A, 146a: 2412-2416.
10. Auer-Grumbach M, Strasser-Fuchs S, Wagner K, Korner E, 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.
11. Hayasaka K, Himoro M, Sawaishi Y, Nanao K, Takahashi T, Takada G, Nicholson GA, Ouvrier RA, Tachi N. (1993) De novo mutation of the myelin P0 gene in Dejerine-Sottas disease (hereditary motor and sensory neuropathy type III). Nat Genet, 5: 266-268.

12. Plante-Bordeneuve V, Guiochon-Mantel A, Lacroix C, Lapresle J, Said G. (1999) The Roussy-Levy family: from the original description to the gene. Ann Neurol, 46: 770-773.
13. Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Lofgren A, Nelis E, Warner LE, Lupski JR, Martin JJ, Van Broeckhoven C. (1999) Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. Neurology, 52: 1827-1832.
14. Lupski JR, Chance PF, Garcia CA. (1993) Inherited primary peripheral neuropathies: molecular genetics and clinical implications of CMT1A and HNPP. Jama, 270: 2326-2330.
15. Pareyson D, Scaioli V, Laura M. (2006) Clinical and electrophysiological aspects of Charcot-Marie-Tooth disease. Neuromolecular medicine, 8: 3-22.
16. Roa BB, Garcia CA, Suter U, Kulpa DA, Wise CA, Mueller J, Welcher AA, Snipes GJ, Shooter EM, Patel PI. (1993) Charcot-Marie-Tooth Disease Type 1A--Association with a Spontaneous Point Mutation in the PMP22 Gene. New England Journal of Medicine, 329: 96-101.
17. Harding AE, Thomas PK. (1980) The clinical features of hereditary motor and sensory neuropathy types I and II. Brain, 103: 259-280.
18. Berciano J, Garcia A, Gallardo E, Peeters K, Pelayo-Negro AL, Alvarez-Paradelo S, Gazulla J, Martinez-Tames M, Infante J, Jordanova A. (2017) Intermediate Charcot-Marie-Tooth disease: an electrophysiological reappraisal and systematic review. J Neurol.
19. Szigeti K, Lupski JR. (2009) Charcot-Marie-Tooth disease. Eur J Hum Genet, 17: 703-710.
20. Hattori N, Yamamoto M, Yoshihara T, Koike H, Nakagawa M, Yoshikawa H, Ohnishi A, Hayasaka K, Onodera O, Baba M, Yasuda H, Saito T, Nakashima K, Kira Ji, Kaji R, Oka N, Sobue G. (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.
21. Kennedy R, Carroll K, Paterson KL, Ryan MM, McGinley JL. (2017) Deterioration in gait and functional ambulation in children and adolescents with

Charcot–Marie–Tooth disease over 12 months. Neuromuscular Disorders, 27: 658-666.

22. Kunovsky D, Cordier R, Bray P, Burns J. (2017) Handwriting difficulties of children with Charcot-Marie-Tooth disease type 1A. J Peripher Nerv Syst, 22: 34-38.
23. Weis J, Claeys KG, Roos A, Azzedine H, Katona I, Schroder JM, Senderek J. (2017) Towards a functional pathology of hereditary neuropathies. Acta Neuropathol, 133: 493-515.
24. Pareyson D, Saveri P, Pisciotta C. (9000) New developments in Charcot-Marie-Tooth neuropathy and related diseases. Current Opinion in Neurology, Publish Ahead of Print.
25. Abe A, Numakura C, Kijima K, Hayashi M, Hashimoto T, Hayasaka K. (2011) Molecular diagnosis and clinical onset of Charcot-Marie-Tooth disease in Japan. J Hum Genet, 56.
26. Remenyi V, Inczedy-Farkas G, Gal A, Bereznai B, Pal Z, Karcagi V, Mechler F, Molnar MJ. (2014) The modifying effect a PMP22 deletion in a family with Charcot-Marie-Tooth type 1 neuropathy due to an EGR2 mutation. Ideggyogy Sz, 67: 420-425.
27. Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME. (2011) Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Ann Neurol, 69: 22-33.
28. Vita G, La Foresta S, Russo M, Vita GL, Messina S, Lunetta C, Mazzeo A. (2016) Sport activity in Charcot-Marie-Tooth disease: A case study of a Paralympic swimmer. Neuromuscul Disord, 26: 614-618.
29. Weimer LH, Podwall D. (2006) Medication-induced exacerbation of neuropathy in Charcot Marie Tooth disease. J Neurol Sci, 242: 47-54.
30. Fridman V, Bundy B, Reilly MM, Pareyson D, Bacon C, Burns J, Day J, Feely S, Finkel RS, Grider T, Kirk CA, Herrmann DN, Laura M, Li J, Lloyd T, Sumner CJ, Muntoni F, Piscosquito G, Ramchandren S, Shy R, Siskind CE, Yum SW, Moroni I, Pagliano E, Zuchner S, Scherer SS, Shy ME. (2015) CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies

Consortium natural history study: a cross-sectional analysis. J Neurol Neurosurg Psychiatry, 86: 873-878.

31. Werheid F, Azzedine H, Zwerenz E, Bozkurt A, Moeller MJ, Lin L, Mull M, Hausler M, Schulz JB, Weis J, Claeys KG. (2016) Underestimated associated features in CMT neuropathies: clinical indicators for the causative gene? Brain Behav, 6: e00451.
32. Shy ME, Chen L, Swan ER, Taube R, Krajewski KM, Herrmann D, Lewis RA, McDermott MP. (2008) Neuropathy progression in Charcot-Marie-Tooth disease type 1A. Neurology, 70: 378-383.
33. Del Bo R, Moggio M, Rango M, Bonato S, D'Angelo MG, Ghezzi S, Airoldi G, Bassi MT, Guglieri M, Napoli L, Lamperti C, Corti S, Federico A, Bresolin N, Comi GP. (2008) Mutated mitofusin 2 presents with intrafamilial variability and brain mitochondrial dysfunction. Neurology, 71: 1959-1966.
34. Karadima G, Koutsis G, Raftopoulou M, Floroskufi P, Karletidi KM, Panas M. (2014) Four novel connexin 32 mutations in X-linked Charcot-Marie-Tooth disease. Phenotypic variability and central nervous system involvement. J Neurol Sci, 341: 158-161.
35. Kurihara S, Adachi Y, Wada K, Adachi A, Ohama E, Nakashima K. (2003) Axonal and demyelinating forms of the MPZ Thr124Met mutation. Acta Neurol Scand, 108: 157-160.
36. Lee MJ, Nelson I, Houlden H, Sweeney MG, Hilton-Jones D, Blake J, Wood NW, Reilly MM. (2002) Six novel connexin32 (GJB1) mutations in X-linked Charcot-Marie-Tooth disease. J Neurol Neurosurg Psychiatry, 73: 304-306.
37. Luigetti M, Zollino M, Conti G, Romano A, Sabatelli M. (2013) Inherited neuropathies and deafness caused by a PMP22 point mutation: a case report and a review of the literature. Neurol Sci, 34: 1705-1707.
38. Piscosquito G, Magri S, Saveri P, Milani M, Ciano C, Farina L, Taroni F, Pareyson D. (2017) A novel NDRG1 mutation in a non-Romani patient with CMT4D/HMSN-Lom. J Peripher Nerv Syst, 22: 47-50.
39. Ando M, Hashiguchi A, Okamoto Y, Yoshimura A, Hiramatsu Y, Yuan J, Higuchi Y, Mitsui J, Ishiura H, Umemura A, Maruyama K, Matsushige T, Morishita S, Nakagawa M, Tsuji S, Takashima H. (2017) Clinical and genetic

diversities of Charcot-Marie-Tooth disease with MFN2 mutations in a large case study. J Peripher Nerv Syst.

40. Gal A, Inczedy-Farkas G, Pal E, Remenyi V, Bereznai B, Geller L, Szelid Z, Merkely B, Molnar MJ. (2015) The coexistence of dynamin 2 mutation and multiple mitochondrial DNA (mtDNA) deletions in the background of severe cardiomyopathy and centronuclear myopathy. Clin Neuropathol, 34: 89-95.
41. Niyazov D, Africk D. (2015) Mitochondrial Dysfunction in a Patient with 8q21.11 Deletion and Charcot-Marie-Tooth Disease Type 2K due to GDAP1 Haploinsufficiency. Mol Syndromol, 6: 204-206.
42. Vielhaber S, Debska-Vielhaber G, Peeva V, Schoeler S, Kudin AP, Minin I, Schreiber S, Dengler R, Kollewe K, Zuschratter W, Kornblum C, Zsurka G, Kunz WS. (2013) Mitofusin 2 mutations affect mitochondrial function by mitochondrial DNA depletion. Acta Neuropathol, 125: 245-256.
43. Pal Z, Kiss E, Gal A, Csepany T, Lengyel A, Molnar MJ. (2009) Genetically determined neuropathy (CMT 1A) accompanied by immune dysfunction: a case report. Inflamm Res, 58: 359-361.
44. Sanvito L, Makowska A, Mahdi-Rogers M, Hadden RD, Peakman M, Gregson N, Nemni R, Hughes RA. (2009) Humoral and cellular immune responses to myelin protein peptides in chronic inflammatory demyelinating polyradiculoneuropathy. J Neurol Neurosurg Psychiatry, 80: 333-338.
45. Mademan I, Deconinck T, Dinopoulos A, Voit T, Schara U, Devriendt K, Meijers B, Lerut E, De Jonghe P, Baets J. (2013) De novo INF2 mutations expand the genetic spectrum of hereditary neuropathy with glomerulopathy. Neurology, 81: 1953-1958.
46. Park HJ, Kim HJ, Hong YB, Nam SH, Chung KW, Choi BO. (2014) A novel INF2 mutation in a Korean family with autosomal dominant intermediate Charcot-Marie-Tooth disease and focal segmental glomerulosclerosis. J Peripher Nerv Syst, 19: 175-179.
47. Snipes GJ, Suter U, Welcher AA, Shooter EM. (1992) Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). J Cell Biol, 117: 225-238.

48. Gillen C, Gleichmann M, Spreyer P, Muller HW. (1995) Differentially expressed genes after peripheral nerve injury. J Neurosci Res, 42: 159-171.
49. Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski JR. (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. Nat Genet, 12: 288-297.
50. Braathen GJ, Sand JC, Lobato A, Hoyer H, Russell MB. (2011) Genetic epidemiology of Charcot-Marie-Tooth in the general population. Eur J Neurol, 18: 39-48.
51. Murphy SM, Laura M, Fawcett K, Pandraud A, Liu YT, Davidson GL, Rossor AM, Polke JM, Castleman V, Manji H, Lunn MP, Bull K, Ramdharry G, Davis M, Blake JC, Houlden H, Reilly MM. (2012) Charcot-Marie-Tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry, 83: 706-710.
52. Remiche G, Abramowicz M, Mavroudakis N. (2013) Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) associated to hereditary neuropathy with liability to pressure palsies (HNPP) and revealed after influenza AH1N1 vaccination. Acta Neurol Belg, 113: 519-522.
53. Sohl G, Willecke K. (2003) An update on connexin genes and their nomenclature in mouse and man. Cell Commun Adhes, 10: 173-180.
54. Sohl G, Willecke K. (2004) Gap junctions and the connexin protein family. Cardiovasc Res, 62: 228-232.
55. Nielsen MS, Nygaard Axelsen L, Sorgen PL, Verma V, Delmar M, Holstein-Rathlou NH. (2012) Gap junctions. Compr Physiol, 2: 1981-2035.
56. Niemann A, Berger P, Suter U. (2006) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuromolecular Med, 8: 217-242.
57. Sargiannidou I, Markoullis K, Kleopa KA. (2010) Molecular mechanisms of gap junction mutations in myelinating cells. Histol Histopathol, 25: 1191-1206.
58. Scherer SS, Deschenes SM, Xu YT, Grinspan JB, Fischbeck KH, Paul DL. (1995) Connexin32 is a myelin-related protein in the PNS and CNS. J Neurosci, 15: 8281-8294.
59. www.molgen.ua.ac.be/CMTMutations.

60. Shy ME. Hereditary Motor and Sensory Neuropathies: an overview of clinical, fenetic, electrophysiologic, and pathologic features. In: PJ Dyck (szerk.), Peripheral neuropathy. Elsevier Saunders, Philadelphia, 2005: 1623-1658.
61. Dubourg O, Tardieu S, Birouk N, Gouider R, Leger JM, Maisonobe T, Brice A, Bouche P, LeGuern E. (2001) Clinical, electrophysiological and molecular genetic characteristics of 93 patients with X-linked Charcot-Marie-Tooth disease. Brain, 124: 1958-1967.
62. Nicholson G, Nash J. (1993) Intermediate nerve conduction velocities define X-linked Charcot-Marie-Tooth neuropathy families. Neurology, 43: 2558-2564.
63. Hanemann CO, Bergmann C, Senderek J, Zerres K, Sperfeld AD. (2003) Transient, recurrent, white matter lesions in X-linked Charcot-Marie-Tooth disease with novel connexin 32 mutation. Arch Neurol, 60: 605-609.
64. Stancanelli C, Taioli F, Testi S, Fabrizi GM, Arena MG, Granata F, Russo M, Gentile L, Vita G, Mazzeo A. (2012) Unusual features of central nervous system involvement in CMTX associated with a novel mutation of GJB1 gene. J Peripher Nerv Syst, 17: 407-411.
65. Stojkovic T, Latour P, Vandenberghe A, Hurtevent JF, Vermersch P. (1999) Sensorineural deafness in X-linked Charcot-Marie-Tooth disease with connexin 32 mutation (R142Q). Neurology, 52: 1010-1014.
66. Matsuyama W, Nakagawa M, Moritoyo T, Takashima H, Umehara F, Hirata K, Suehara M, Osame M. (2001) Phenotypes of X-linked Charcot-Marie-Tooth disease and altered trafficking of mutant connexin 32 (GJB1). J Hum Genet, 46: 307-313.
67. Shy ME, Siskind C, Swan ER, Krajewski KM, Doherty T, Fuerst DR, Ainsworth PJ, Lewis RA, Scherer SS, Hahn AF. (2007) CMT1X phenotypes represent loss of GJB1 gene function. Neurology, 68: 849-855.
68. Abrams CK, Goman M, Wong S, Scherer SS, Kleopa KA, Peinado A, Freidin MM. (2017) Loss of Coupling Distinguishes GJB1 Mutations Associated with CNS Manifestations of CMT1X from Those Without CNS Manifestations. Scientific Reports, 7: 40166.
69. Sutcliffe JG. (1987) The genes for myelin. Trends in Genetics, 3: 73-76.

70. Hayasaka K, Himoro M, Wang Y, Takata M, Minoshima S, Shimizu N, Miura M, Uyemura K, Takada G. (1993) Structure and chromosomal localization of the gene encoding the human myelin protein zero (MPZ). Genomics, 17: 755-758.
71. Warner LE, Hilz MJ, Appel SH, Killian JM, Kolodry EH, Karpati G, Carpenter S, Watters GV, Wheeler C, Witt D, Bodell A, Nelis E, Van Broeckhoven C, Lupski JR. (1996) Clinical phenotypes of different MPZ (P0) mutations may include Charcot-Marie-Tooth type 1B, Dejerine-Sottas, and congenital hypomyelination. Neuron, 17: 451-460.
72. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM, Vance JM. (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nat Genet, 36: 449-451.
73. Formosa LE, Ryan MT. (2016) Mitochondrial fusion: Reaching the end of mitofusin's tether. The Journal of Cell Biology.
74. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC. (2004) Structural basis of mitochondrial tethering by mitofusin complexes. Science, 305: 858-862.
75. Eschenbacher WH, Song M, Chen Y, Bhandari P, Zhao P, Jowdy CC, Engelhard JT, Dorn GW, 2nd. (2012) Two rare human mitofusin 2 mutations alter mitochondrial dynamics and induce retinal and cardiac pathology in Drosophila. PloS one, 7: e44296-e44296.
76. Cartoni R, Martinou JC. (2009) Role of mitofusin 2 mutations in the physiopathology of Charcot-Marie-Tooth disease type 2A. Exp Neurol. 218(2):268-73.
77. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, Cooper DN. (2009) The Human Gene Mutation Database: 2008 update. Genome Med, 1: 13.
78. Sebastián D, Palacín M, Zorzano A. (2017) Mitochondrial Dynamics: Coupling Mitochondrial Fitness with Healthy Aging. Trends in Molecular Medicine.

79. Suen D-F, Norris KL, Youle RJ. (2008) Mitochondrial dynamics and apoptosis. Genes & development, 22: 1577-1590.
80. Pallanck L. (2013) Mitophagy: mitofusin recruits a mitochondrial killer. Curr Biol, 23: R570-572.
81. Casasnovas C, Banchs I, Cassereau J, Gueguen N, Chevrollier A, Martinez-Matos JA, Bonneau D, Volpini V. (2010) Phenotypic spectrum of MFN2 mutations in the Spanish population. J Med Genet, 47: 249-256.
82. Nagarajan R, Svaren J, Le N, Araki T, Watson M, Milbrandt J. (2001) EGR2 mutations in inherited neuropathies dominant-negatively inhibit myelin gene expression. Neuron, 30: 355-368.
83. Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR. (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat Genet, 18: 382-384.
84. Boerkoel CF, Takashima H, Bacino CA, Daentl D, Lupski JR. (2001) EGR2 mutation R359W causes a spectrum of Dejerine-Sottas neuropathy. Neurogenetics, 3: 153-157.
85. Archambault J, Pan G, Dahmus GK, Cartier M, Marshall N, Zhang S, Dahmus ME, Greenblatt J. (1998) FCP1, the RAP74-interacting subunit of a human protein phosphatase that dephosphorylates the carboxyl-terminal domain of RNA polymerase IIO. J Biol Chem, 273: 27593-27601.
86. Varon R, Gooding R, Steglich C, Marns L, Tang H, Angelicheva D, Yong KK, Ambrugger P, Reinhold A, Morar B, Baas F, Kwa M, Tournev I, Guerguelcheva V, Kremensky I, Lochmuller H, Mullner-Eidenbock A, Merlini L, Neumann L, Burger J, Walter M, Swoboda K, Thomas PK, von Moers A, Risch N, Kalaydjieva L. (2003) Partial deficiency of the C-terminal-domain phosphatase of RNA polymerase II is associated with congenital cataracts facial dysmorphism neuropathy syndrome. Nat Genet, 35: 185-189.
87. Morar B, Gresham D, Angelicheva D, Tournev I, Gooding R, Guergueltcheva V, Schmidt C, Abicht A, Lochmuller H, Tordai A, Kalmar L, Nagy M, Karcagi V, Jeanpierre M, Herczegfalvi A, Beeson D, Venkataraman V, Warwick Carter K, Reeve J, de Pablo R, Kucinskas V, Kalaydjieva L. (2004) Mutation history of the roma/gypsies. Am J Hum Genet, 75: 596-609.

88. Kalaydjieva L, Gresham D, Gooding R, Heather L, Baas F, de Jonge R, Blechschmidt K, Angelicheva D, Chandler D, Worsley P, Rosenthal A, King RH, Thomas PK. (2000) N-myc downstream-regulated gene 1 is mutated in hereditary motor and sensory neuropathy-Lom. Am J Hum Genet, 67: 47-58.
89. Hunter M, Bernard R, Freitas E, Boyer A, Morar B, Martins IJ, Tournev I, Jordanova A, Guergelcheva V, Ishpekova B, Kremensky I, Nicholson G, Schlotter B, Lochmuller H, Voit T, Colomer J, Thomas PK, Levy N, Kalaydjieva L. (2003) Mutation screening of the N-myc downstream-regulated gene 1 (NDRG1) in patients with Charcot-Marie-Tooth Disease. Hum Mutat, 22: 129-135.
90. Li LX, Liu GL, Liu ZJ, Lu C, Wu ZY. (2017) Identification and functional characterization of two missense mutations in NDRG1 associated with Charcot-Marie-Tooth disease type 4D (CMT4D). Hum Mutat.
91. Okamoto Y, Goksungur MT, Pehlivan D, Beck CR, Gonzaga-Jauregui C, Muzny DM, Atik MM, Carvalho CMB, Matur Z, Bayraktar S, Boone PM, Akyuz K, Gibbs RA, Battaloglu E, Parman Y, Lupski JR. (2014) Exonic duplication CNV of NDRG1 associated with autosomal-recessive HMSN-Lom/CMT4D. Genet Med, 16: 386-394.
92. Kalaydjieva L, Hallmayer J, Chandler D, Savov A, Nikolova A, Angelicheva D, King RH, Ishpekova B, Honeyman K, Calafell F, Shmarov A, Petrova J, Turnev I, Hristova A, Moskov M, Stancheva S, Petkova I, Bittles AH, Georgieva V, Middleton L, Thomas PK. (1996) Gene mapping in Gypsies identifies a novel demyelinating neuropathy on chromosome 8q24. Nat Genet, 14: 214-217.
93. Kalaydjieva L, Nikolova A, Turnev I, Petrova J, Hristova A, Ishpekova B, Petkova I, Shmarov A, Stancheva S, Middleton L, Merlini L, Trogu A, Muddle JR, King RH, Thomas PK. (1998) Hereditary motor and sensory neuropathy--Lom, a novel demyelinating neuropathy associated with deafness in gypsies. Clinical, electrophysiological and nerve biopsy findings. Brain, 121 (Pt 3): 399-408.
94. Misra UK, Kalita J, Nair PP. (2008) Diagnostic approach to peripheral neuropathy. Annals of Indian Academy of Neurology, 11: 89-97.

95. Murphy SaM, Herrmann DN, McDermott MP, Scherer SS, Shy ME, Reilly MM, Pareyson D. (2011) Reliability of the CMT neuropathy score (second version) in Charcot-Marie-Tooth disease. Journal of the peripheral nervous system : JPNS, 16: 191-198.
96. Burns J, Ouvrier R, Estilow T, Shy R, Laurá M, Pallant JF, Lek M, Muntoni F, Reilly MM, Pareyson D, Acsadi G, Shy ME, Finkel RS. (2012) Validation of the CMT Pediatric Scale as an outcome measure of disability. Annals of Neurology, 71: 642-652.
97. Mandarakas MR, Menezes MP, Rose KJ, Shy R, Eichinger K, Foscan M, Estilow T, Kennedy R, Herbert K, Bray P. (2018) Development and validation of the Charcot-marie-tooth Disease Infant Scale. Brain, 141: 3319-3330.
98. Padua L, Pazzaglia C, Pareyson D, Schenone A, Aiello A, Fabrizi GM, Cavallaro T, Santoro L, Manganelli F, Gemignani F, Vitetta F, Quattrone A, Mazzeo A, Russo M, Vita G. (2016) Novel outcome measures for Charcot-Marie-Tooth disease: validation and reliability of the 6-min walk test and StepWatch() Activity Monitor and identification of the walking features related to higher quality of life. Eur J Neurol, 23: 1343-1350.
99. Albers JW, Kelly JJ. (1989) Acquired inflammatory demyelinating polyneuropathies: clinical and electrodiagnostic features. Muscle & nerve, 12: 435-451.
100. Fuglsang-Frederiksen A, Pugdahl K. (2011) Current status on electrodiagnostic standards and guidelines in neuromuscular disorders. Clinical Neurophysiology, 122: 440-455.
101. Kelly Jr. JJ. (1983) The electrodiagnostic findings in peripheral neuropathy associated with monoclonal gammopathy. Muscle & Nerve, 6: 504-509.
102. Noto Y, Shiga K, Tsuji Y, Mizuta I, Higuchi Y, Hashiguchi A, Takashima H, Nakagawa M, Mizuno T. (2015) Nerve ultrasound depicts peripheral nerve enlargement in patients with genetically distinct Charcot-Marie-Tooth disease. J Neurol Neurosurg Psychiatry, 86: 378-384.
103. Cartwright MS, Brown ME, Eulitt P, Walker FO, Lawson VH, Caress JB. (2009) Diagnostic nerve ultrasound in Charcot-Marie-Tooth disease type 1B. Muscle Nerve, 40: 98-102.

104. Aho TR, Wallace RC, Pitt AM, Sivakumar K. (2004) Charcot-Marie-Tooth disease: extensive cranial nerve involvement on CT and MR imaging. AJNR Am J Neuroradiol, 25: 494-497.
105. Chhabra A, Andreisek G, Soldatos T, Wang KC, Flammang AJ, Belzberg AJ, Carrino JA. (2011) MR Neurography: Past, Present, and Future. American Journal of Roentgenology, 197: 583-591.
106. Chhabra A, Carrino JA, Farahani SJ, Thawait GK, Sumner CJ, Wadhwa V, Chaudhary V, Lloyd TE. (2016) Whole-body MR neurography: Prospective feasibility study in polyneuropathy and Charcot-Marie-Tooth disease. J Magn Reson Imaging, 44: 1513-1521.
107. Morrow JM, Sinclair CDJ, Fischmann A, Machado PM, Reilly MM, Yousry TA, Thornton JS, Hanna MG. (2016) MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. The Lancet. Neurology, 15: 65-77.
108. Shahrizaila N, Noto Y, Simon NG, Huynh W, Shibuya K, Matamala JM, Dharmadasa T, Devenney E, Kennerson ML, Nicholson GA, Kiernan MC. (2017) Quantitative muscle ultrasound as a biomarker in Charcot-Marie-Tooth neuropathy. Clinical Neurophysiology, 128: 227-232.
109. Yohe S, Thyagarajan B. (2017) Review of Clinical Next-Generation Sequencing. Arch Pathol Lab Med, 141: 1544-1557.
110. Weil J. (2002) Genetic counselling in the era of genomic medicine. As we move towards personalized medicine, it becomes more important to help patients understand genetic tests and make complex decisions about their health. EMBO reports, 3: 590-593.
111. (2003) Guidelines for genetic testing of healthy children. Paediatrics & child health, 8: 42-52.
112. Kenis-Coskun O, Matthews DJ. (2016) Rehabilitation issues in Charcot-Marie-Tooth disease. J Pediatr Rehabil Med, 9: 31-34.
113. Cup EH, Sturkenboom IH, Pieterse AJ, Hendricks HT, van Engelen BG, Oostendorp RA, van der Wilt GJ. (2008) The evidence for occupational therapy for adults with neuromuscular diseases: a systematic review. OTJR: Occupation, participation and Health, 28: 12-18.

114. Yagerman SE, Cross MB, Green DW, Scher DM. (2012) Pediatric orthopedic conditions in Charcot–Marie–Tooth disease: a literature review. Current opinion in pediatrics, 24: 50-56.
115. Attarian S, Vallat J-M, Magy L, Funalot B, Gonnaud P-M, Lacour A, Péréon Y, Dubourg O, Pouget J, Micallef J. (2014) An exploratory randomised double-blind and placebo-controlled phase 2 study of a combination of baclofen, naltrexone and sorbitol (PXT3003) in patients with Charcot-Marie-Tooth disease type 1A. Orphanet journal of rare diseases, 9: 199.
116. Kaore SN, Langade DK, Yadav VK, Sharma P, Thawani VR, Sharma R. (2012) Novel actions of progesterone: what we know today and what will be the scenario in the future? Journal of Pharmacy and Pharmacology, 64: 1040-1062.
117. Zhao HT, Damle S, Ikeda-Lee K, Kuntz S, Li J, Mohan A, Kim A, Hung G, Scheideler MA, Scherer SS. (2018) PMP22 antisense oligonucleotides reverse Charcot-Marie-Tooth disease type 1A features in rodent models. The Journal of clinical investigation, 128: 359-368.
118. Kagiava A, Karaiskos C, Richter J, Tryfonos C, Lapathitis G, Sargiannidou I, Christodoulou C, Kleopa K. (2018) Intrathecal gene therapy in mouse models expressing CMT1X mutations. Human molecular genetics, 27: 1460-1473.
119. Pareyson D, Saveri P, Pisciotta C. (2017) New developments in Charcot–Marie–Tooth neuropathy and related diseases. Current opinion in neurology, 30: 471-480.
120. Trobaugh-Lotrario AD, Smith AA, Odom LF. (2003) Vincristine neurotoxicity in the presence of hereditary neuropathy. Medical and pediatric oncology, 40: 39-43.
121. Weimer LH, Podwall D. (2006) Medication-induced exacerbation of neuropathy in Charcot Marie Tooth Disease. Journal of the Neurological Sciences, 242: 47-54.
122. Awater C, Zerres K, Rudnik-Schoneborn S. (2012) Pregnancy course and outcome in women with hereditary neuromuscular disorders: comparison of obstetric risks in 178 patients. Eur J Obstet Gynecol Reprod Biol, 162: 153-159.
123. Hoff JM, Gilhus NE, Daltveit AK. (2005) Pregnancies and deliveries in patients with Charcot-Marie-Tooth disease. Neurology, 64: 459-462.

124. Pareyson D, Scaioli V, Laura M. (2006) Clinical and electrophysiological aspects of Charcot-Marie-Tooth disease. Neuromolecular Med, 8: 3-22.
125. Murphy SM, Herrmann DN, McDermott MP, Scherer SS, Shy ME, Reilly MM, Pareyson D. (2011) Reliability of the CMT neuropathy score (second version) in Charcot-Marie-Tooth disease. Journal of the Peripheral Nervous System, 16: 191-198.
126. Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA, Chakravarti A, Patel PI. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell, 66: 219-232.
127. Russo M, Laura M, Polke JM, Davis MB, Blake J, Brandner S, Hughes RA, Houlden H, Bennett DL, Lunn MP, Reilly MM. (2011) Variable phenotypes are associated with PMP22 missense mutations. Neuromuscul Disord, 21: 106-114.
128. Shy ME, Scavina MT, Clark A, Krajewski KM, Li J, Kamholz J, Kolodny E, Szigeti K, Fischer RA, Saifi GM, Scherer SS, Lupski JR. (2006) T118M PMP22 mutation causes partial loss of function and HNPP-like neuropathy. Ann Neurol, 59: 358-364.
129. Milley GM, Varga ET, Grosz Z, Bereznai B, Aranyi Z, Boczan J, Dioszeghy P, Kalman B, Gal A, Molnar MJ. (2016) Three novel mutations and genetic epidemiology analysis of the Gap Junction Beta 1 (GJB1) gene among Hungarian Charcot-Marie-Tooth disease patients. Neuromuscul Disord, 26: 706-711.
130. Wicklein EM, Orth U, Gal A, Kunze K. (1997) Missense mutation (R15W) of the connexin32 gene in a family with X chromosomal Charcot-Marie-Tooth neuropathy with only female family members affected. J Neurol Neurosurg Psychiatry, 63: 379-381.
131. Fairweather N, Bell C, Cochrane S, Chelly J, Wang S, L.mostacciuolo M, P.monaco A, E.haites N. (1994) Mutations in the connexin 32 gene in x-linked dominant Charcot-Marie-Tooth disease (CMTX1). Human Molecular Genetics, 3: 29-34.

132. Taylor RA, Simon EM, Marks HG, Scherer SS. (2003) The CNS phenotype of X-linked Charcot-Marie-Tooth disease: more than a peripheral problem. Neurology, 61: 1475-1478.
133. Liu Y, Wei X, Kong X, Guo X, Sun Y, Man J, Du L, Zhu H, Qu Z, Tian P, Mao B, Yang Y. (2015) Targeted Next-Generation Sequencing for Clinical Diagnosis of 561 Mendelian Diseases. PLoS One, 10: e0133636.
134. Kennerson MM, Warburton T, Nelis E, Brewer M, Polly P, De Jonghe P, Timmerman V, Nicholson GA. (2007) Mutation scanning the GJB1 gene with high-resolution melting analysis: Implications for mutation scanning of genes for Charcot-Marie-Tooth disease. Clinical Chemistry, 53: 349-352.
135. Tan CC, Ainsworth PJ, Hahn AF, MacLeod PM. (1996) Novel mutations in the connexin 32 gene associated with X-linked Charcot-Marie tooth disease. Human Mutation, 7: 167-171.
136. Bone LJ, Deschênes SM, Balice-Gordon RJ, Fischbeck KH, Scherer SS. (1997) Connexin32 and X-linked Charcot–Marie–Tooth Disease. Neurobiology of Disease, 4: 221-230.
137. Ionasescu VV, Searby C, Ionasescu R, Neuhaus IM, Werner R. (1996) Mutations of the noncoding region of the connexin32 gene in X-linked dominant Charcot-Marie-Tooth neuropathy. Neurology, 47: 541-544.
138. Mersiyanova IV, Ismailov SM, Polyakov AV, Dadali EL, Fedotov VP, Nelis E, Löfgren A, Timmerman V, Van Broeckhoven C, Evgrafov OV. (2000) Screening for mutations the peripheral myelin genes PMP22, MPZ and Cx32 (GJB1) in Russian Charcot-Marie-Tooth neuropathy patients. Human Mutation, 15: 340-347.
139. Huehne K, Benes V, Thiel C, Kraus C, Kress W, Hoeltzenbein M, Ploner CJ, Kotzian J, Reis A, Rott HD, Rautenstrauss BW. (2003) Novel mutations in the Charcot-Marie-Tooth disease genes PMP22, MPZ, and GJB1. Human mutation, 21: 100.
140. Kochanski A, Kabzinska D. (2004) Molecular genetic analysis of the GJB1 gene: a study of six mutations. J Appl Genet, 45: 95-100.

141. Martikainen MH, Majamaa K. (2013) Novel GJB1 mutation causing adult-onset Charcot-Marie-Tooth disease in a female patient. Neuromuscul Disord, 23: 899-901.
142. Ainsworth PJ, Bolton CF, Murphy BC, Stuart JA, Hahn AF. (1998) Genotype/phenotype correlation in affected individuals of a family with a deletion of the entire coding sequence of the connexin 32 gene. Hum Genet, 103: 242-244.
143. Marrosu MG, Vaccargiu S, Marrosu G, Vannelli A, Cianchetti C, Muntoni F. (1998) Charcot-Marie-Tooth disease type 2 associated with mutation of the myelin protein zero gene. Neurology, 50: 1397-1401.
144. Szabo A, Züchner S, Siska E, Mechler F, Molnar MJ. (2005) Marked phenotypic variation in a family with a new myelin protein zero mutation. Neuromuscular Disorders, 15: 760-763.
145. (1998) 3rd workshop of the European CMT consortium: 54th ENMC International Workshop on genotype/phenotype correlations in Charcot-Marie-Tooth type 1 and hereditary neuropathy with liability to pressure palsies 28-30 November 1997, Naarden, The Netherlands. Neuromuscul Disord, 8: 591-603.
146. De Jonghe P, Timmerman V, Ceuterick C, Nelis E, De Vriendt E, Löfgren A, Vercruyssen A, Verellen C, Van Maldergem L, Martin J-J, Van Broeckhoven C. (1999) The Thr124Met mutation in the peripheral myelin protein zero (MPZ) gene is associated with a clinically distinct Charcot–Marie–Tooth phenotype. Brain, 122: 281-290.
147. Kijima K, Numakura C, Izumino H, Umetsu K, Nezu A, Shiiki T, Ogawa M, Ishizaki Y, Kitamura T, Shozawa Y, Hayasaka K. (2005) Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. Hum Genet, 116: 23-27.
148. Calvo J, Funalot B, Ouvrier RA, Lazaro L, Toutain A, De Mas P, Bouche P, Gilbert-Dussardier B, Arne-Bes MC, Carriere JP, Journel H, Minot-Myhie MC, Guillou C, Ghorab K, Magy L, Sturtz F, Vallat JM, Magdelaine C. (2009) Genotype-phenotype correlations in Charcot-Marie-Tooth disease type 2 caused by mitofusin 2 mutations. Arch Neurol, 66: 1511-1516.

149. Verhoeven K, Claeys KG, Zuchner S, Schroder JM, Weis J, Ceuterick C, Jordanova A, Nelis E, De Vriendt E, Van Hul M, Seeman P, Mazanec R, Saifi GM, Szigeti K, Mancias P, Butler IJ, Kochanski A, Ryniewicz B, De Bleecker J, Van den Bergh P, Verellen C, Van Coster R, Goemans N, Auer-Grumbach M, Robberecht W, Milic Rasic V, Nevo Y, Tournev I, Guergueltcheva V, Roelens F, Vieregge P, Vinci P, Moreno MT, Christen HJ, Shy ME, Lupski JR, Vance JM, De Jonghe P, Timmerman V. (2006) MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. Brain, 129: 2093-2102.

150. Pareyson D, Taroni F, Botti S, Morbin M, Baratta S, Lauria G, Ciano C, Sghirlanzoni A. (2000) Cranial nerve involvement in CMT disease type 1 due to early growth response 2 gene mutation. Neurology, 54: 1696-1698.

151. Tournev I, Kalaydjieva L, Youl B, Ishpekova B, Guergueltcheva V, Kamenov O, Katzarova M, Kamenov Z, Raicheva-Terzieva M, King RH, Romanski K, Petkov R, Schmarov A, Dimitrova G, Popova N, Uzunova M, Milanov S, Petrova J, Petkov Y, Kolarov G, Aneva L, Radeva O, Thomas PK. (1999) Congenital cataracts facial dysmorphism neuropathy syndrome, a novel complex genetic disease in Balkan Gypsies: clinical and electrophysiological observations. Ann Neurol, 45: 742-750.

152. Birouk N, LeGuern E, Maisonobe T, Rouger H, Gouider R, Tardieu S, Gugenheim M, Routon MC, Leger JM, Agid Y, Brice A, Bouche P. (1998) X-linked Charcot-Marie-Tooth disease with connexin 32 mutations: clinical and electrophysiologic study. Neurology, 50: 1074-1082.

153. Ionasescu VV, Searby C, Ionasescu R, Neuhaus IM, Werner R. (1996) Mutations of the noncoding region of the connexin32 gene in X-linked dominant Charcot-Marie-Tooth neuropathy. Neurology, 47: 541-544.

154. Huehne K, Benes V, Thiel C, Kraus C, Kress W, Hoeltzenbein M, Ploner CJ, Kotzian J, Reis A, Rott HD, Rautenstrauss BW. (2003) Novel mutations in the Charcot-Marie-Tooth disease genes PMP22, MPZ, and GJB1. Hum Mutat, 21: 100.

155. Bahr M, Andres F, Timmerman V, Nelis ME, Van Broeckhoven C, Dichgans 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.

156. Landouré G, Zdebik AA, Martinez TL, Burnett BG, Stanescu HC, Inada H, Shi Y, Taye AA, Kong L, Munns CH, Choo SS, Phelps CB, Paudel R, Houlden H, Ludlow CL, Caterina MJ, Gaudet R, Kleta R, Fischbeck KH, Sumner CJ. (2010) Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. Nature genetics, 42: 170-174.

157. Fridman V, Bundy B, Reilly M, Pareyson D, Bacon C, Burns J, Day J, Feely S, Finkel R, Grider T. (2015) CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies Consortium natural history study: a cross-sectional analysis. Journal of Neurology, Neurosurgery & Psychiatry, 86: 873-878.

158. Braathen G, Sand J, Lobato A, Høyer H, Russell M. (2011) Genetic epidemiology of Charcot–Marie–Tooth in the general population. European journal of neurology, 18: 39-48.

159. Sivera R, Sevilla T, Vilchez JJ, Martinez-Rubio D, Chumillas MJ, Vazquez JF, Muelas N, Bataller L, Millan JM, Palau F, Espinos C. (2013) Charcot-Marie-Tooth disease: genetic and clinical spectrum in a Spanish clinical series. Neurology, 81: 1617-1625.

160. Gess B, Schirmacher A, Boentert M, Young P. (2013) Charcot-Marie-Tooth disease: frequency of genetic subtypes in a German neuromuscular center population. Neuromuscul Disord, 23: 647-651.

161. Murphy SM, Laura M, Fawcett K, Pandraud A, Liu YT, Davidson GL, Rossor AM, Polke JM, Castleman V, Manji H, Lunn MP, Bull K, Ramdharry G, Davis M, Blake JC, Houlden H, Reilly MM. (2012) Charcot-Marie-Tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry, 83.

162. Casasnovas C, Banchs I, Corral J, Martinez-Matos JA, Volpini V. (2006) Clinical and molecular analysis of X-linked Charcot-Marie-Tooth disease type 1 in Spanish population. Clin Genet, 70: 516-523.

163. Kim Y, Choi KG, Park KD, Lee KS, Chung KW, Choi BO. (2012) X-linked dominant Charcot-Marie-Tooth disease with connexin 32 (Cx32) mutations in Koreans. Clin Genet, 81: 142-149.
164. Boerkoel CF, Takashima H, Garcia CA, Olney RK, Johnson J, Berry K, Russo P, Kennedy S, Teebi AS, Scavina M, Williams LL, Mancias P, Butler IJ, Krajewski K, Shy M, Lupski JR. (2002) Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation. Ann Neurol, 51: 190-201.
165. Shy M, Chen L, Swan E, Taube R, Krajewski K, Herrmann D, Lewis R, McDermott M. (2008) Neuropathy progression in Charcot-Marie-Tooth disease type 1A. Neurology, 70: 378-383.
166. Abrams CK, Freidin M. (2015) GJB1-associated X-linked Charcot-Marie-Tooth disease, a disorder affecting the central and peripheral nervous systems. Cell Tissue Res, 360: 659-673.
167. Stuppia G, Rizzo F, Riboldi G, Del Bo R, Nizzardo M, Simone C, Comi GP, Bresolin N, Corti S. (2015) MFN2-related neuropathies: Clinical features, molecular pathogenesis and therapeutic perspectives. Journal of the Neurological Sciences, 356: 7-18.
168. Echaniz-Laguna A, Degos B, Bonnet C, Latour P, Hamadouche T, Lévy N, Leheup B. (2007) NDRG1-linked Charcot-Marie-Tooth disease (CMT4D) with central nervous system involvement. Neuromuscular Disorders, 17: 163-168.
169. Joo IS, Ki CS, Joo SY, Huh K, Kim JW. (2004) A novel point mutation in PMP22 gene associated with a familial case of Charcot-Marie-Tooth disease type 1A with sensorineural deafness. Neuromuscul Disord, 14: 325-328.
170. Gabriel C, Gregson N, Wood N, Hughes R. (2002) Immunological study of hereditary motor and sensory neuropathy type 1 a (HMSN1 a). Journal of the Peripheral Nervous System, 7: 206-206.
171. Ritz MF, Lechner-Scott J, Scott RJ, Fuhr P, Malik N, Erne B, Taylor V, Suter U, Schaeren-Wiemers N, Steck AJ. (2000) Characterisation of autoantibodies to peripheral myelin protein 22 in patients with hereditary and acquired neuropathies. J Neuroimmunol, 104: 155-163.

172. Murphy SM, Ovens R, Polke J, Siskind CE, Laura M, Bull K, Ramdharry G, Houlden H, Murphy RP, Shy ME, Reilly MM. (2012) X inactivation in females with X-linked Charcot-Marie-Tooth disease. Neuromuscul Disord, 22: 617-621.
173. Gutierrez A, England JD, Sumner AJ, Ferer S, Warner LE, Lupski JR, Garcia CA. (2000) Unusual electrophysiological findings in X-linked dominant Charcot-Marie-Tooth disease. Muscle Nerve, 23: 182-188.
174. Capasso M, Di Muzio A, Ferrarini M, De Angelis MV, Caporale CM, Lupo S, Cavallaro T, Fabrizi GM, Uncini A. (2004) Inter-nerves and intra-nerve conduction heterogeneity in CMTX with Arg(15)Gln mutation. Clin Neurophysiol, 115: 64-70.
175. Van den Bergh PY, Hadden RD, Bouche P, Cornblath DR, Hahn A, Illa I, Koski CL, Leger JM, Nobile-Orazio E, Pollard J, Sommer C, van Doorn PA, van Schaik IN. (2010) European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society - first revision. Eur J Neurol, 17: 356-363.
176. Moszyńska I, Kabzińska D, Kochański A. (2009) Mild form of Charcot-Marie-Tooth type 1X disease caused by a novel Cys179Gly mutation in the GJB1/Cx32 gene. Acta Myologica, 28: 72-75.
177. Antoniadi T, Buxton C, Dennis G, Forrester N, Smith D, Lunt P, Burton-Jones S. (2015) Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. BMC Med Genet, 16: 84.
178. Hoyer H, Braathen GJ, Busk OL, Holla OL, Svendsen M, Hilmarsen HT, Strand L, Skjelbred CF, Russell MB. (2014) Genetic diagnosis of Charcot-Marie-Tooth disease in a population by next-generation sequencing. Biomed Res Int, 2014: 210401.
179. Deng H-X, Klein CJ, Yan J, Shi Y, Wu Y, Fecto F, Yau H-J, Yang Y, Zhai H, Siddique N, Hedley-Whyte ET, DeLong R, Martina M, Dyck PJ, Siddique T. (2009) Scapuloperoneal spinal muscular atrophy and CMT2C are allelic disorders caused by alterations in TRPV4. Nature Genetics, 42: 165.

180. Nishimura G, Lausch E, Savarirayan R, Shiba M, Spranger J, Zabel B, Ikegawa S, Superti-Furga A, Unger S. (2012) TRPV4-associated skeletal dysplasias. Am J Med Genet C Semin Med Genet, 160c: 190-204.
181. Zimoń M, Baets J, Auer-Grumbach M, Berciano J, Garcia A, Lopez-Laso E, Merlini L, Hilton-Jones D, McEntagart M, Crosby AH, Barisic N, Boltshauser E, Shaw CE, Landouré G, Ludlow CL, Gaudet R, Houlden H, Reilly MM, Fischbeck KH, Sumner CJ, Timmerman V, Jordanova A, Jonghe PD. (2010) Dominant mutations in the cation channel gene transient receptor potential vanilloid 4 cause an unusual spectrum of neuropathies. Brain, 133: 1798-1809.
182. Biasini F, Portaro S, Mazzeo A, Vita G, Fabrizi GM, Taioli F, Toscano A, Rodolico C. (2016) TRPV4 related scapuloperoneal spinal muscular atrophy: Report of an Italian family and review of the literature. Neuromuscul Disord, 26: 312-315.
183. Stumpf JD, Saneto RP, Copeland WC. (2013) Clinical and Molecular Features of POLG-Related Mitochondrial Disease. Cold Spring Harbor Perspectives in Biology, 5: a011395.
184. Higuchi Y, Hashiguchi A, Yuan J, Yoshimura A, Mitsui J, Ishiura H, Tanaka M, Ishihara S, Tanabe H, Nozuma S, Okamoto Y, Matsuura E, Ohkubo R, Inamizu S, Shiraishi W, Yamasaki R, Ohyagi Y, Kira J, Oya Y, Yabe H, Nishikawa N, Tobisawa S, Matsuda N, Masuda M, Kugimoto C, Fukushima K, Yano S, Yoshimura J, Doi K, Nakagawa M, Morishita S, Tsuji S, Takashima H. (2016) Mutations in MME cause an autosomal-recessive Charcot-Marie-Tooth disease type 2. Ann Neurol, 79: 659-672.
185. Auer-Grumbach M, Toegel S, Schabhuttl M, Weinmann D, Chiari C, Bennett DLH, Beetz C, Klein D, Andersen PM, Bohme I, Fink-Puches R, Gonzalez M, Harms MB, Motley W, Reilly MM, Renner W, Rudnik-Schoneborn S, Schlotter-Weigel B, Themistocleous AC, Weishaupt JH, Ludolph AC, Wieland T, Tao F, Abreu L, Windhager R, Zitzelsberger M, Strom TM, Walther T, Scherer SS, Zuchner S, Martini R, Senderek J. (2016) Rare Variants in MME, Encoding Metalloprotease Neprilysin, Are Linked to Late-Onset Autosomal-Dominant Axonal Polyneuropathies. Am J Hum Genet, 99: 607-623.

186. Depondt C, Donatello S, Rai M, Wang FC, Manto M, Simonis N, Pandolfo M. (2016) MME mutation in dominant spinocerebellar ataxia with neuropathy (SCA43). Neurol Genet, 2: e94.
187. Spires-Jones TL, Hyman BT. (2014) The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron, 82: 756-771.
188. Gonzaga-Jauregui C, Harel T, Gambin T, Kousi M, Griffin LB, Francescatto L, Ozes B, Karaca E, Jhangiani S, Bainbridge MN, Lawson KS, Pehlivan D, Okamoto Y, Withers M, Mancias P, Slavotinek A, Reitnauer PJ, Goksungur MT, Shy M, Crawford TO, Koenig M, Willer J, Flores BN, Pediaditrakis I, Us O, Wiszniewski W, Parman Y, Antonellis A, Muzny DM, Baylor-Hopkins Center for Mendelian G, Katsanis N, Battaloglu E, Boerwinkle E, Gibbs RA, Lupski JR. (2015) Exome sequence analysis suggests genetic burden contributes to phenotypic variability and complex neuropathy. Cell reports, 12: 1169-1183.
189. Finsterer J. (2009) Mitochondrial disorders, cognitive impairment and dementia. J Neurol Sci, 283: 143-148.
190. Hellström-Lindahl E, Ravid R, Nordberg A. (2008) Age-dependent decline of neprilysin in Alzheimer's disease and normal brain: Inverse correlation with Aβ levels. Neurobiology of Aging, 29: 210-221.
191. Zimoń M, Baets J, Almeida-Souza L, De Vriendt E, Nikodinovic J, Parman Y, Battaloğlu E, Matur Z, Guergueltcheva V, Tournev I, Auer-Grumbach M, De Rijk P, Petersen B-S, Müller T, Fransen E, Van Damme P, Löscher WN, Barišić N, Mitrovic Z, Previtali SC, Topaloğlu H, Bernert G, Beleza-Meireles A, Todorovic S, Savic-Pavicevic D, Ishpekova B, Lechner S, Peeters K, Ooms T, Hahn AF, Züchner S, Timmerman V, Van Dijck P, Rasic VM, Janecke AR, De Jonghe P, Jordanova A. (2012) Loss-of-function mutations in HINT1 cause axonal neuropathy with neuromyotonia. Nature Genetics, 44: 1080-1083.
192. Seburn KL, Morelli KH, Jordanova A, Burgess RW. (2014) Lack of Neuropathy-Related Phenotypes in Hint1 Knockout Mice. Journal of Neuropathology & Experimental Neurology, 73: 693-701.
193. Peeters K, Chamova T, Tournev I, Jordanova A. (2017) Axonal neuropathy with neuromyotonia: there is a HINT. Brain : a journal of neurology, 140: 868-877.

194. Zhao H, Race V, Matthijs G, De Jonghe P, Robberecht W, Lambrechts D, Van Damme P. (2014) Exome sequencing reveals HINT1 mutations as a cause of distal hereditary motor neuropathy. European Journal of Human Genetics, 22: 847-850.
195. Zhang F, Fang Z, Wang JB. (2015) Hint1 knockout results in a compromised activation of protein kinase C gamma in the brain. Brain Res, 1622: 196-203.
196. Chen Q, Wang X, O'Neill FA, Walsh D, Kendler KS, Chen X. (2008) Is the histidine triad nucleotide-binding protein 1 (HINT1) gene a candidate for schizophrenia? Schizophrenia Research, 106: 200-207.

10. Bibliography of the candidate's publications

Relevant for the PhD thesis:

Milley GM, Varga ET, Grosz Z et al. Genotypic ad phenotypic spectrum of the most common causative genes of Charcot-Marie-Tooth disease in Hungarian patients. Neurmuscul Disord. 2017;28(1):38-43 (2.612)

Milley GM, Varga ET, Grosz Z et al. Three nove lmutations and genetic epiemiology analysis of the Gap Junction Bet 1 (GJB1) gene among Hungarian Charcot-Marie-Tooth disease patients. Neurmuscul Disord. 2016;26(10):706-711 (IF: 2.969)

Further publications:

Pentelenyi K, Remenyi V, Gal A, **Milley GM,** Csosz A, Mende BG, Molnar MJ: Asian-specific mitochondrial genome polymorphism (9bp deletion) in Hungarian patients with mitochondrial diesease. Mitochondrial DNA. 2014 Sep 22:1-4

Kecskeméti N, Szönyi M, Gáborján A, Küstel M, **Milley GM**, Süveges A, Illés A, Kékesi A, Tamás L, Molnár MJ, Szirmai Á, Gál A.: Analysis of GJB2 mtutations and the clinial manifestation in a large Hungarian cohort. Eur Arch Otorhinolaryngol. 2018;275(10):2441-2448 (IF: 1.750)

Cumulative impact factor: 7.331

Poster and oral presentation

Gal A, Bereznai B, Varga ET, Balicza P, **Milley GM**, Aranyi Z, Boczan J, Dioszeghy P, Kalaydiyeva L, Molnar MJ.: Genetic testing in hereditary neuropathies: our 10 years experience. European Human Genetics Conference 2013 Paris, European Journal of Human Genetics, Vol 21.Suppl 2 p. 218

GM Milley, A. Gal, B. Bereznai, E.T. Varga, P. Balicza, Z. Aranyi, J. Boczan, P. D Dioszeghy, MJ Molnar., Genetic epidemiology of Charcot- Marie- Tooth disease in Hungary. European Journal of Neurology, 1st EAN, Berlin Vol. 22 (Suppl. 1) p. 370

GM Milley, E Varga, Z Grosz, B Bereznai, Z Aranyi, J Boczan, P Dioszeghy, B Kálmán, A Gal, MJ Molnar. Genetic epidemiology analysis of Cx32 gene mutations in Hungary. 2nd EAN, Copenhagen 2016, European Journal of Neurology, 23 (Suppl. 1), 625–912

GM Milley, Z. Grosz, E. Varga, N.A. Varga, J. Boczan, Z. Aranyi, P. Dioszeghy, M.J. Molnár, A. Gal1 Genetic epidemiology analysis of Hungarian Charcot-Marie-Tooth patients 3nd EAN, Amsterdam 2017, European Journal of Neurology, 24 (Suppl. 1), 445–678

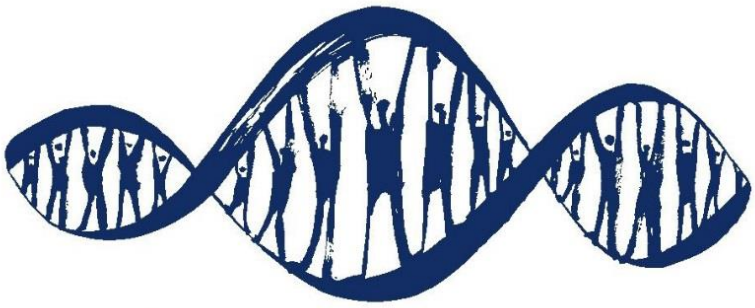
11. Acknowledgements

Firstly, I would like to express my sincere gratitude to my tutor, Dr. Aniko Gál for the support of my Ph.D study and related research, for her constant motivation, endless patience, and immense knowledge. Her guidance helped me through even the hardest part of my work.

Besides my tutor, I would like to thank Prof. Mária Judit Molnár the continuous trust, guidance and that she planted the seeds of knowledge, teached the sense of vocation and shown how to become a good physician.

My sincere thanks also goes to Dr. Zoltán Grosz, Dr. Edina Varga, Dr. Csilla Nemes, Györgyi Báthori, Dr. Péter Balicza, and Dr. Noémi Varga, who provided me essential clinical work, laboratory help and helpful, stimulating discussions. Their assistance helped to get the thesis done.

Last but not the least, I would like to thank my family. They always meant to be a solid support and shelter through the university and doctoral years.



12. Supplement

Supplement 1 - Genetic classification of Charcot-Marie-Tooth and related neuropathies. OMIM database and the Neuromuscular Database of Washington University has been used for this compilation.

Abbreviations: Inher. – Inheritance, AD – autosomal dominant; AR – autosomal recessive; XLD – X-linked dominant; XLR – X-linked recessive

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT1 - Autosomal dominant demyelinating HMSN								
CMT1A	PMP22 #601097	Peripheral myelin protein 22	17p12	AD	1991	myelin protein	early onset, pupil. abn., optic neuritis, nystagmus, facial and bulbar nerve weakness, vocal cord palsy, deafness, CNS inv., UMN sign, MRI abn., cognitive imp., diaphragmal weakness, ANS inv., tremor, scoliosis, RLS	drug induced polyneuropathy, autoimmunity, HNPP
CMT1B	MPZ/P0 #159440	Myelin protein zero	1q22-q23	AD	1993	myelin protein	early onset, pupil. abn., bulbar and facial weakness, vocal cord palsy, MRI abn., cognitive imp., ANS inv., tremor, scoliosis, respiratory imp., chest deformity, cold induced hand cramps	-
CMT1C	LITAF #603795	Lipopolysacharide induced tumor necrosis factor alpha factor	16p13.13	AD/ AR?	2003	transcriptional regulation	early onset, deafness, cognitive impairment, ANS inv., tremor, scoliosis, conduction block	carciogenesis of extramammary Paget disease, leukemia
CMT1D	EGR2 #129010	Early growth factor 2	10q21.3	AD	1998	transcriptional regulation	early onset, nystagmus, opthalmoparesis, bulbar and facial weakness, vocal cord palsy, deafness, tremor, scoliosis, hip dysplasia, resp. failure	-
CMT1E	PMP22 #601097	Peripheral myelin protein 22	17p12	AD	1991	myelin protein	see above (CMT1A)	see above (CMT1A)
CMT1F	NEFL #162280	Neurofilament protein light chain	8p21.2	AD	2000	cytoskeleton	early onset , nystagmus, bulbar and facial weakness, deafness, UMN inv., MR abn., ANS inv., cognitive impairment, stremor, scoliosis,	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT2 - Autosomal dominant and recessive axonal hereditary motor and sensory neuropathy								
CMT2A1	KIF1B #605995	Kinesin family member 1B	1p36.2	AD	2001	axonal transport	UMN inv.	neuroblastoma, phaeochromo-cytoma
CMT2A2	MFN2 #608507	Mitofusin 2	1p36.2	AD/AR	2004	mitochondrial dynamics	early onset, pupil. abn., nystagmus, ophtalmo-paresis, optic atrophy, facial and bulbar weakness, vocal cord palsy, CNS and UMN inv., spasticity, MR abn., cognitive imp., tremor, scoliosis, ANS inv., respiratory failure	-
CMT2B	RAB7 #602298	RAS associated protein	3q21.3	AD	2000	intracellular transport	more prominent sensory symptoms, scoliosis, fasciculation, pain, ANS dysf., nystagmus, cerebellar atrophy	-
CMT2B1	LMNA #150330	Lamin A/C	1q21.2-q21.3	AR	2002	nuclear envelope	scoliosis, hand deformity, proximal weakness,	muscular dystrophies, lypodystrophy, cardiomyopathy
CMT2B2	MED25 #610197	Mediator complex subunit 25	19q13.33	AR	2009	transcriptional regulation	paresthesia, odema	-
CMT2C	TRPV4 #605427	Transient receptor potential cation channel subfamily V member 4	12q24.11	AD	2010	ion channel	vocal cord palsy, early onset, tremor, skeletal abnormalities, contractures, deafness, bulbar signs, tremor, facial and progressive paresis, strabismus, ophtalmoparesis, respiratory failure,	dHMN VIII, SMA, dwarfism
CMT2CC	NEFH #162230	neurofilament protein heavy chain	22q12.2	AD	2016	nucleoskeleton	-	susceptibility to ALS
CMT2D	GARS #600287	Glycyl-tRNA synthetase	7p14.3	AD	2003	protein biosynthesis	scoliosis, cold induced hand cramps	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT2E	NEFL #162280	Neurofilament protein light chain	8p21.2	AD	2000	necleosceleton, cytosceleton	early onset, more prominent sensory symptoms, scoliosis, contractures, deafness, cognitive impairment, UMN signs, chronic vomiting, nystagmus, cerebellar atrophy, calf hypertrophy, hyperkeratosis	-
CMT2F	HSPB1 #600287	Heat shock protein B1	7q11.23	AD	2004	chaperone	more prominent motor symptoms, hand deformities, fasciculation, pain,	dHMN IIB
CMT2G	-	-	12q12-q13.3	AD	2004	-	-	-
CMT2G HMSN-P (type Okinawa)	TFG #602498	TRK-fused gene	3q12.2	AD	2012	-	tremor, deafness, UMN and bulbar signs, facial weakness, proximal> distal muscle paresis, paresthesia, respiratory failure, constipation, myotonia	HSP (SPG57)
CMT2I	MPZ/P0 #159440	Myelin Protein Zero	1q22-q23	AD	1993	myelin protein	see above (CMT1B)	-
CMT2H	-	-	8q13-q23	AR?	-	-	pyramidal sign	-
CMT2J	MPZ/P0 #159440	Myelin Protein Zero	1q22-q23	AD	1993	myelin protein	see above (CMT1B)	-
CMT2K	GDAP1 #606598	Ganglioside-induced differentiation-associated protein1	8p21.11	AD	2002	mitochondrial dynamics	early onset, vocal cord palsy, scoliosis, hand deformities, skeletal deformities, bulbar signs, facial weakenss, pain, optic atrophy, respiratory failure, ANS involvement	-
CMT2L	HSPB8 #608104	Heat shock protein B8	12q.24.23	AD	2005	chaperone	scoliosis	HMN-IIA
CMT2M	DNM2 #602378	Dynamin 2	19p13.2	AD	2005	mitochondrial dynamics	early onset, facial weakness, cataract, ophtalmoparesis, mtDNA deletion cognitive imp., low blood cells	centronuclear myopathy

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT2N	AARS #601065	Alanine-tRNA synthetase	16q22.1	AD	2010	protein biosynthesis	deafness	epileptic encephalopathy
CMT2O	DNCH1 #600112	Dynein heavy polypeptide	14q32.31	AD	2011	axonal transport	early onset, prominent proximal muscle weakness, tremor, mental retardation,	SMA
CMT2P	LRSAM1 #610933	Leucine-rich repeated and sterile alpha motif containing 1	9q33.3-q34.1	AD/AR	2010	protein degradation	deafness, fasciculation	-
CMT2Q	DHTKD1 #615025	Dehydrogenase E1 and transketolase domains 1	10p14	AD	2012	energy production	-	aminoaciduria
CMT2R	TRIM2 #614141	Tripartite motif containing protein 2	4q31.3	AR	2013	protein degradation	early onset, tracheomalacia, respiratory failure	-
CMT2S	IGHMBP2 #600502	Immunoglobulin mu-binding protein 2	11q13.3	AR	2014	transcriptional regulation	more prominent muscular paresis	SMARD1, dHMN VI
CMT2T1	DNAJB2 #120520	Heat shock protein DNAJ-like 1	2q35	AD	2016	chaperone	-	dSMA, dHMN
CMT2T2	MME #120520	Membrane metalloendopepti-dase	3q25.2	AR	2016	interaction with extracellular environment	-	motor neuron disease, SCA43
CMT2U	MARS #156560	Methionyl-tRNA synthetase	12q13.3	AD	2013	protein biosynthesis	Pain, proximal=distal weakness	Interstitial lung and liver disease

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT2V	NAGLU #609701	N-acetylglucos-aminidase	17q21.2	AD	2015	protein degradation	-	MPS type IIIB (AR)
CMT2W	HARS #142810	Hystidil-tRNA acyltransferase	5q31.3	AD	2015	protein biosynthesis	deafness and blindness	Usher syndrome
CMT2X	SPG11 #610844	Spatacsin	15q21.1	AR	2016	intracellular transport	-	SPG11, juvenile ALS, thin corpus callosum
CMT2Z	MORC2 #610661	MORC family CW type zync finger protein	22q12.2	AD	2016	transcriptional regulation	myokimia	-
I-CMT - Autosomal dominant and recessive intermediate HMSN								
DI-CMTA	-	-	10q24.1-q25.1	AD	-	-	-	-
DI-CMTB	DNM2 #602378	Dynamin 2	19p13.2	AD	2005	mitochondrial dynamics	see above (CMT2M)	centronuclear myopathy
DI-CMTC	YARS #603623	Tyrosil-tRNA synthetase	1p34-p35		2006	protein biosynthesis	-	-
DI-CMTD	MPZ/P0 #159440	Myelin Protein Zero	1q22-q23	AD	1996	myelin protein	see above (CMT1B)	-
DI-CMTE	IFN2 #610982	Inverted formin 2	14q32.33	AD	2011	cytosceleton	early onset, tremor,scoliosis, hand deformities, FSGS, deafness, cognitive imp., pain, enlarged vintricles and altered WM signal	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
DI-CMTF	GNB4 #610863	Guanine nucleotide-binding protein beta 4	3q26.33	AD	2013	interaction with extracellular environment	borth demyelinating and axonal neuropathy	-
RI-CMTA	GDAP1 #606598	Ganglioside-induced differentiation-associated protein1	8p21.11	AR	2002	mitochondrial dynamics	see above (CMT2K)	-
RI-CMTB	KARS #6041421	Lysyl-tRNA synthetase	16q23.1	AR	2010	protein biosynthesis	early onset, deafness, psychiatric disorders	-
RI-CMTC	PLEKHG5 #611101	Plechstrin homology Domain-containing protein, fam. G member 5	1p36.31	AR	2013	Rho GTPase signalling	-	dSMA IV (AR)
RI-CMTD	COX6A1 #602072	Cytochrome C oxidase subunit VIA polyptide 1	12q24.31	AR	2014	energy production	-	-
CMT4 -Autosomal recessive demyelinating hereditary motor and sensory neuropathy								
CMT4A	GDAP1 #606598	Ganglioside-induced differentiation-associated protein1	8p21.11	AR	2002	mitochondrial dynamics	see above (CMT2K)	-
CMT4B1	MTMR2 #603557	Myotubulin related protein 2	11q21	AR	2000	phosphoinositide metabolism	early onset, more prominent proximal weakness, vocal cord palsy, scolisosis, hand deformities, bulbar signs, faical weakness, severe respiratory failure, tongue atrophy, masticatory weakness	-
CMT4B2	SBF2 #607967	Set binding factor 2	11p15.4	AR	2009	phosphoinositide metabolism	early onset, scoliosis, hand deformities, glaucoma, pain, cranial nerve inv.	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMT4B3	SBF1 #603560	Set binding factor 1	22q13.33	AR	2013	phosphoinositide metabolism	microcephaly and cognitive impairment, scoliosis, strabismus, ophthalmoplegia, facial weakness, variable syndactyly of the hands and feet, dysarthria, and urinary incontinence	-
CMT4C	SH3TC2 #608206	SH3 domain and tetratricopeptide repeat domain 2	5q32	AR	2003	intracellular transport	proximal weakness, cranial nerve involvement, deafness, scoliosis, slow pupillary light reflexes, and lingual fasciculations, spine deformities, respiratory weakness	mono-neuropathy of the median nerve (AD);
CMT4D (LOM)	NDRG1 #606598	Ganglioside-induced differentiation-associated protein1	8p21.11	AR	2002	intracellular transport	early onset, vocal cord palsy, deafness, scoliosis, hand deformities, bulbar and facial weakness, nystagmus	-
CMT4E	EGR2/ MPZ	Early growth factor 2 / Myelin Protein Zero	see above	AR	see above	transcriptional regulation/ myelin protein	see above (CMT1E)	-
CMT4F	PRX #605725	Periaxin	19q13.2	AR	2000	interaction with extracellular environment	early onset CMT	-
CMT4G (HMSN type Rousse)	HK1 #142600	Hexokinase 1	10q22.1	AR	2009	energy production	hemolytic anaemia, severe and early onset neuropaty	-
CMT4H	FGD4 #611104	Fyve, RhoGEF and pH domain-containing protein 4	12p11.21	AR	2007	Rho GTPase signalling	early onset, more prominent sensory symptoms, tremor, scoliosis, abn. pupil	-
CMT4J	FIG4 #611228	SAC domain containing inositol phosphatase 3	6q21	AR	2007	phosphoinositide metabolism	early onset, more prominent proximal weakenss, tremor, scoliosis, hand deformity, facial weakness, cranial nerve involvement, respiratory failure, cerebellar and brain atrophy, dyskinesia	ALS11, Yuris-Varon syndroma
CMT4K	SURF1 #616684	Surfeit 1	9q34.2	AR	2013	energy production	scoliosis, deafness, nystagmus, cerebellar ataxia, T2 hyperintense lesions in putamen and periaquaductal, lactic acidosis	Leigh syndrome due to complex IV deficincy

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CMTX - X-linked hereditary motor and sensory neuropathy								
CMTX1	GJB1 #304040	Gap junction beta 1	X13.1	XLD	1993	intracellular transport	CNS involvement, pyramidal signs, sensorineural hearing loss, early onset, conduction block	-
CMTX2	-	-	Xp22.2	XLR	-	-	-	-
CMTX3	-	-	Xq26	XLR	-	-	-	-
CMTX4 (Cowchock syndrome)	AIFM1 #304040	apoptosis-inducing factor mitochondrion-associated 1	Xq24-q26	XLR	2012	mitochondria mediated apoptosis	deafness, cognitive impairment, COX negative fibers	deafness X-linked 5, oxidative phosphorylation deficiency 6, motor neuron disease
CMTX5	PRPS1 #311850	Phosphoribosil pyrophoshate synthetase	Xq22.3	XLR	2010	purine metabolism and nucleotide biosynthesis	sensorineural hearing loss	-
CMTX6	PDK3 #300906	Pyruvate dehydrogenase kinase 3	Xp22.11	XLR	2013	mitochondrial energy production	-	-
CMTX-I	DRP2 #300052	Dystrophin related protein 2	Xq22.1	XLR	2015	cytoskeletal protein	elevated protein in CSF, late onset	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
Hereditary sensory and autonomic neuropathy								
HSAN IA	SPTLC1 #605712	Serine palmitoyl transferase long chain subunit 1	9q22.31	AD	2010	sphyngolipid biosynthesis	-	-
HSAN IB	-	-	3p24-p22	AD	-	-	GERD and cough	-
HSAN IC	SPTLC2 #605713	Serine palmitoyl transferase long chain subunit 2	14q24.3	AD	ő	sphyngolipid biosynthesis	-	-
HSAN ID	ATL1 #606439	Atlastin GTPase 1	14q22.1	AD	2011	ER membrane shaping	neuropathic pain, upper motor neuron signs	HSP (SPG3A)
HSAN1E	DNMT1 #126375	DNA methyltransferase 1	19p13.2	AD	2013	transcriptional regulation	hearing loss, dementia	Cerebellar ataxia, deafness, and narcolepsy
HSAN IIA	WNK1 #605232	Prostate derived sterile 20-like kinase	12p13.33	AR	2004	interaction with extracellular environment	-	-
HSAN IIB	FAM134B #613114	Family with sequence similarity 134 member B	5p15.1	AR	2009	ER membrane shaping	-	-
HSAN IIC	KIF1A #601205	Kinesin family member 1A	2q37.3	AD	2011	axonal transport	-	HSP, ALS, mental retardation
HASAN IID	SCN9A #603415	Sodium channel protein type 9 subunit alpha	2q24.3	AR		ion channel		
HSAN III	IKBKAP #603722	Inhibitor of kappa light polypeptide gene enchancer in B-cells	9q31.3	AR	2001	transcriptional regulation	-	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
HSAN IV	NTRK1 191315	Neurotrophic tyrosin kinase receptor 1	1q21-q22	AR	1996	interaction with extracellular environment	-	-
HSAN V	NGFB #614653	Nerve growth factor beta	1p11.2-1p13.2	AR	2004	transcriptional regulation	-	-
HSAN VI	DST #614653	Dystonia musculorum	6p12.1	AR	2012	cytosceletal protein	-	Epidermolysis bullosa
HASN VII	SCN11A #604385	Sodium channel voltage gated type XI alpha subunit	3p22.2	AD	2013	ion channel	loss of pain perception	-
Arthrogryposis with impaired proprioception and touch	PIEZO2 #613629	Piezo-type Mechanosensitive Ion Channel Component 2	18p11.22-p11.21	AD	2016	Mechanosensitive receptor	Loss of vibration sensing, touch discrimination and proprioception	?Marden-Walker syndrome, arthrogryphosis
Distal hereditary motor neuropathy								
dHMN I	DYNC1H1 #600112	Dyenin cytoplasmic heavy chain type 1	14q32.31	AD	2013	axonal transport	-	SMA
dHMN IIA	HSPB8 #608104	Heat shock protein B8	12q.24.23	AD	2005	chaperone	see above (CMT2L)	CMT2L
dHMN IIB	HSPB1 #600287	Heat shock protein B1	7q11.23	AD	2004	chaperone	see above (CMT2F)	CMT2F
dHMN IIC	HSPB3 #604624	Heat shock protein B3	5q11.2	AD	2010	chaperone	-	-
dHMN IID	FBXO38 #608533	F box only protein 38	5q32	AD	2013	transcription regulation	-	-
dHMN III	-	-	11q13	AR	-	-	-	-
dHMN IV	-	-	11q13	AR	-	-	Severe proximal and distal weakness, diaphragm palsy	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
dHMN VA	GARS #600287	Glycyl-tRNA synthetase	7p14.3	AD	2003	protein biosynthesis	-	-
dHMN VB	REEP1 #609139	Receptor expression enhancing protein1	2p11.2	AD	2012	intracellular transport	respiratory failure	SPG31
dHMN VC	BSCL2 #606158	Seipin	11q12.3	AD	2004	ER membrane shaping	UMN involvement	SMA, SPG, progressive encephalopathy +/- lipodystrophy, congenital lypodystrophy
dHMN VI	IGHMBP2 #600502	Immunoglobulin mu-binding protein 2	11q13.3	AR	2014	transcriptional regulation	Early diaphragm weakness	diaphrahmatic SMA, CMT2S
dHMN VIIa	SLC5A7 #608761	Solute carrier family 5, member 7	2q12.3	AD	2012	intracellular transport	vocal cord palsy, sensorineural hearing loss	SMA
dHMN VIIb	DCTN1 #601143	Dynactin 1	2p13.1	AD	2003	axonal transport	vocal cord palsy	-
dHMN VIII, Scapuloperoneal SMA	TRPV4 #605427	Transient receptor potential cation channel subfamily V member 4	12q24.11	AD	2010	ion channel	see above (CMT2C)	CMT2C, dwarfism
dSMA2	-	-	9p21	AR	-	-	cortical tract involvement, patients originating from the Jerash region of Jordan	-
dSMA3	-	-	11q13	AR			diaphragmatic palsy	
dSMA5	DNAJB2 #120520	Heat shock protein DNAJ-like 1	2q35	AR	2014	chaperone	-	CMT2T1
SMARD1	IGHMBP2 #600502	Immunoglobulin mu-binding protein 2	11q13.3	AR	2014	regulation of transcription	neonatal onset, diaphragmatic palsy and respiratory failure	CMT2, dHMN VI

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
SMARD2	LAS1L	LAS1 like ribosome biogenesis factor	Xq12	XLR	2014	ribosome biosynthesis	neonatal onset, diaphragmatic palsy and respiratory failure	Wilson-Turner syndrome
dSMAX3	APT7A #300011	ATPase 7A	Xq13.1-q21	XLR	2010	intracellular transport	-	Menkes disease, occipital horn syndrome
SMALED2	BICD2 #615290	Bicaudal D homolog of 2	9q22.31	AD	2013	cytosceletal protein	severe distal weakness, UMN abn., brisk reflexes	HSP
SIGMAR1	-	-	9p13	AR	-	-	-	
dSMA with mitochondrial dysfunction	mtATP6	mitochondrially encoded ATP synthase 6	mtDNA	mat.	2012	energy production	episodic weakness	-
	mtATP8	mitochondrially encoded ATP synthase 8	mtDNA	mat.	2013	energy production	episodic weakness	-
Special forms of hereditary neuropathies								
hearing loss + neuropathy	GJB3 #603324	Gap junction Beta 3	1p34	n/a	2001	intracellulare transport	sensorineural hearing loss	erythro-keratodermia
HSAN + HSP	CCT5 #610150	Chaperonin containing T-complex polypeptide 1	5p15.2	AR	2006	chaperone	-	HSP
HMSN + ataxia	IFRD1	Interferon-related developmental regulator gene 1	7q31.1	AD		transcriptional regulation	Dysmetria, ataxia, nystagmus	-
CCFDN	CTDP1 #604927	C-terminal domain of RNA polymerase II subunit A, phosphatase of, subunit 1	18q23	AR	1999	protein biosynthesis	congenital cataracta, facial dysmorphisms, nystagmus, short stature, kyphoscoliosis, mental retardation, pyramidal signs, ataxia	-

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
CFEOM3	TUBB3 #602661	Tubulin beta 3	16q24	AD	2010	transcriptional regulation	fibrosis of extraocular muscles, cortical dysplasia	-
GAN type 1	GAN1 #605379	Giant axonal neuropathy 1	16q23.2	AR	2000	cytosceletal protein	gaint axons, kinky red hair, cerebellar ataxia, spastic paraparesis, pyramidal signs, facial diplegia, lower IQ, minor clinical signs	-
GAN type 2	DCAF8 #615820	Giant axonal neuropathy 2	1q23.2	AD	2014	cytosceletal protein, neurofilament degenartion	giant axons in nerve biopsy	-
PCWH	SOX10 #602229	SRY Box 10	22q13.1	AD	2004	transcriptioal regulation	-	Waardenburg syndrome, Hirschsprung disease
Hypo-myelination	ARHGEF10 #608236	Rho guanin nucleotide exchange factor 10	8p23.3	AD	1998	Rho GTPase signalling	demyelinating polyneuropathy	-
Hereditary disorders associated with neuropathy								
Refsum disease in childhood	PHYH #602026	Phytanoyl-CoA Hydroxylase	10p13	AR	1997	metabolic process	night blinding, iris atrophy, cataract, ataxia, cardiac failure, ichtyosis, diabetes	-
Refsum disease in adults	PEX7 #601757	Peroxin 7	6q23.3	AR	1997	peroxisome biogenesis	-	Rhizomelic chondrodysplasia punctata
Refsum disease of infants	PEX1 #602136	Peroxin 1	7q21.2	AR	2002	peroxisome biogenesis	deafness, retinitis pigmentosa, hepatomegaly, facial dysmorphisms, hypocholesterinaemia, leukodystrophy	Heimler disease, Zellweger syndrome

Classification	Gene and MIM#	Gene name	Locus	Inh.	Year	Function	Associated features to the neuropathy	Associated diseases of the gene *
PHARC	ABHD12 #613599	Abhydrolase domain- containing protein 12	20p11.21	AR	2010	metabolic process	PHARC: **P**olyneuropathy, **H**earing loss, **A**taxia, **R**etinitis pigmentosa, and **C**ataract	-
HMSN + connective tissue disorder	EMILIN1 #130660	Elastin microfibril interfacer 1	2p23.3	AD	2016	extracellular matrix glycoprotein	aortic aneurysma, bronchiectasia, arthropathy, increased elasticity of skin	-
HMSN + corpus callosum agenesis	KCC3 #604870	Solute carrier family 12, member 6	15q14	AR	2002	ion channel	Andermann syndrome, ptosis, optic atrophy	dHMN
Metilchromatic leukodystrophy	ARSA #607574	Arylsulfatase A	22q13.33	AR	1991	protein biosynthesis	leukodystrophy	-
Krabbe disease	GALC #606890	Galactosylceramide β-galactosidase	14q31.3	AR	1994	metabolic process	In childhood: mental retardation, excessive crying, optic atrophy, spasticity; in adulthood: spastisity, optic atrophy, dementia, ataxia,	-
Mitochondrial neuropathy	MT-ATP6 #516060	mitochondrially encoded ATP synthase 6	mtDNA	mat.	1990	energy production	pronounced motor neuropathy, CNS involvement, UMN sign, episodic weakness, Leigh syndrome	NARP, Leigh syndrome, cardiomyopathy
SCAN1	TDP1 #607198	Tyrosyl-DNA phosphodiesterase 1	14q32.11	AR	2002	DNA complex repair	ataxia, dysarthria	-
CMT2 + neuromyotonia	HINT1 #601314	Histidine triad nucleotide-binding protein 1	5q31.1	AR	2012	purin metabolism	neuromyotonia	SMA

Supplement 2: CMTES and CMTNS by Murphy et al (95)

Parameter	0	1	2	3	4
Sensory symptoms	None	Symptoms below or at ankle bones	Symptoms up to the distal half of the calf	Symptoms up to the proximal half of the calf, including knee	Symptoms above knee (above the top of the patella)
Motor symptoms (legs)	None	Trips, catches toes, slaps feet Shoe inserts	Ankle support or stabilization (AFOs) Foot surgery*	Walking aids (cane, walker)	Wheelchair
Motor symptoms (arms)	None	Mild difficulty with buttons	Severe difficulty or unable to do buttons	unable to cut most foods	Proximal weakness (affect movements involving the elbow and above)
Pinprick sensibility	Normal	Decreased below or at ankle bones	Decreased up to the distal half of the calf	Decreased up to the proximal half ofthe calf, including knee	Decreased above knee (above the top ofthe patella)
Vibration	Normal	Reduced at great toe	Reduced at ankle	Reduced at knee (tibial tuberosity)	Absent at knee and ankle
Strength (legs)	Normal	4+, 4, or 4— on foot dorsiflexion or plantar flexion	<3 on foot dorsiflexion or <3 on foot plantar flexion	<3 on foot dorsiflexion and <3 on plantar flexion	Proximal weakness
Strength (arms)	Normal	4+, 4, or 4— on intrinsic hand muscles**	<3 on intrinsic hand muscles**	<5 on wrist extensors	Weak above elbow
ulnar CMAP	>6 mV	4-5.9 mV	2-3.9 mV	0.1 -1.9 mV	Absent
(median)	(>4 mV)	(2.8-3.9)	(1.2-2.7)	(0.1-1.1)	(absent)
Radial SAP amplitude, antidromic testing	>15 pV	10-14.9 pV	5-9.9 pV	1 -4.9 pV	<1 pV

Supplement 3: Neuropathy gene list for NGS screening.

PMP22, MPZ, LITAF, EGR2, PMP22, NEFL, KIF1B, MFN2, RAB7, LMNA, MED25, TRPV4, NEFH, GARS, NEFL, HSPB1, TFG, MPZ, GDAP1, HSPB8, DNM2, AARS, DNCH1, LRSAM1, DHTKD1, TRIM2, IGHMBP2, DNAJB2, MME, MARS, NAGLU, HARS, SPG11, MORC2, DNM2, YARS, MPZ, IFN2, GNB4, GDAP1, KARS, PLEKHG5, COX6A1, MTMR2, SBF2, SBF1, SH3TC2, NDRG1, PRX, HK1, FGD4, FIG4, SURF1, AIFM1, PRPS1, PDK3, DRP2, SPTLC1, SPTLC2, ATL1, DNMT1, WNK1, FAM134B, KIF1A, SCN9A, IKBKAP NTRK1, NGFB, DST, SCN11A, POLG, OPA1, MFN1, DNMT2, ATX2, PIEZO2, DYNC1H1, HSPB8, HSPB1, HSPB3, FBXO38, REEP1, BSCL2, IGHMBP2, SLC5A7, DCTN1, TRPV4, LAS1L, APT7A, BICD2, mtATP6, mtATP8, GJB3, CCT5 IFRD1, CTDP1, TUBB3, GAN1, DCAF8, SOX10, HINT1, ARHGEF10, PHYH, PEX7, PEX1, MICU1, ABHD12, EMILIN1, KCC3 ARSA, GALC, MT-ATP6, TDP1, SETX, SCO2, TRIM2, GALC