Maria Lehtilahti

CHARCOT-MARIE-TOOTH DISEASE

MOLECULAR EPIDEMIOLOGY IN NORTHERN OSTROBOTHNIA
MARY LEHTILAHTI

CHARCOT-MARIE-TOOTH DISEASE
Molecular epidemiology in Northern Ostrobothnia

Academic dissertation to be presented with the assent of the Doctoral Programme Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 8 of Oulu University Hospital (Kajaanintie 50), on 25 May 2022, at 12 noon

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Charcot-Marie-Tooth (CMT) disease is probably the most common inherited neuromuscular disorder with prevalence estimates of 10–82/100,000. CMT is a clinically and genetically heterogeneous group of disorders that can be classified based on their histological, clinical, electrodiagnostic, and genetic features. Reduced or absent tendon reflexes, foot deformities, distal predominance of limb muscle weakness, wasting, and sensory loss are common clinical features. Pathogenic variants have been discovered in more than 100 genes in patients with CMT.

In the present study we investigated the epidemiology of CMT and the frequency of its molecular etiologies in Northern Ostrobothnia, Finland. The patient registries at Oulu University Hospital were searched for possible CMT patients. Blood samples were requested from patients fulfilling the diagnostic criteria, and DNA was then subjected to molecular diagnostics. Selected families were examined clinically.

We found 107 patients with CMT, suggesting a prevalence 34.6/100,000 in Northern Ostrobothnia. The dominantly inherited pathogenic variant p.His123Arg in ganglioside induced differentiation associated protein 1 (GDAP1) was found in 31.5% while peripheral myelin protein 22 (PMP22) duplication in 16.9% of the patients. In addition, we found a novel variant, p.His106Arg, in myelin protein zero (MPZ) that was described in affected members of two families and that was found to cause dominantly inherited, late-onset, relatively mild, predominantly axonal motor and sensory polyneuropathy. In addition, 23 patients with p.His123Arg in GDAP1 were examined clinically and electrodiagnostically. Remarkable proximal muscle weakness of the legs and asymmetry of symptoms and findings were defining features in these patients.

Prevalence of CMT in Northern Ostrobothnia appears to be two-fold higher than the average in European populations. One reason could be the high portion of patients with heterozygous point mutation p.His123Arg in GDAP1, which may be the most common single mutation in patients with CMT in Finland, and a cluster of the mutation was detected within the country.

Keywords: Charcot-Marie-Tooth disease, epidemiology, GDAP1, MPZ, phenotype, polyneuropathy, prevalence
Tiivistelmä
Charcot-Marie-Toothin tauti (CMT) on todennäköisesti yleisin periytyvä neuromuskulaarisairaus, sen esiintyvyydeksi on arvioitu 10–82/100 000. CMT on sekä kliinisesti että geneettisesti monimuotoinen ryhmä sairauksia, jotka voidaan luokitella histologisten, kliinisten, neurofysiologisten ja geneettisten ominaisuuksien mukaan. Vaimentuneet tai puuttuvat jännevenytysteet, jalkaterien epämuodostumat, distaalisesti painnottunut lihasheikkous ja atrofia sekä sensornet puutosoireet ovat tavallisia kliinisiä löydöksiä. CMT:a aiheuttavia haitallisia muutoksia on löydetty yli 100 geenissä.


Verrattuna keskimääräiseen esiintyvyyteen eurooppalaisissa väestöissä on CMT:n esiintyvyys Pohjois-Pohjanmaalla noin kaksinkertainen, mikä voi johtua GDAP1-geenin heterotsygoottisista potilaisista ja GDAP1-mutaaatio kantajien suuresta osuudesta. Löysimme mutaatioryvyyttä Pohjois-Pohjanmaalta, mutta tämän haitallisen muutoksen esiintyvyys mualla Suomessa ei ole tiedossa.

Asiakirjat: Charcot-Marie-Toothin tauti, epidemiologia, GDAP1, ilmiasu, MPZ, polyneuropatia, prevalenssi
To Aaro, Alisa and Eemi
Acknowledgements

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With all my heart, I want to thank my amazing children Aaro, Alisa and Eemi. You are my sunshine in a rainy day. You can be and become anything you want in life; go for your dreams. I love you.

Oulu, 29.3.2022

Maria Lehtilahti
## Abbreviations

<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>AD</td>
<td>Autosomal dominant inheritance</td>
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<tr>
<td>AR</td>
<td>Autosomal recessive inheritance</td>
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<tr>
<td>CANVAS</td>
<td>Cerebellar ataxia, neuropathy, vestibular areflexia syndrome</td>
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<td>CMAP</td>
<td>Compound muscle action potential</td>
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<td>CMT</td>
<td>Charcot-Marie-Tooth</td>
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<td>CMTNS</td>
<td>CMT neuropathy score</td>
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<td>CMTX</td>
<td>X-linked CMT</td>
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<td>CTS</td>
<td>Carpal tunnel syndrome</td>
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<tr>
<td>Cx32</td>
<td>Connexin 32</td>
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<td>dHMN</td>
<td>Distal hereditary motor neuropathy</td>
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<tr>
<td>EDx</td>
<td>Electrodiagnostic</td>
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<td>FAP</td>
<td>Familial amyloid polyneuropathy</td>
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<td>FRDA</td>
<td>Friedreich’s ataxia</td>
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<td>GDAP1</td>
<td>Ganglioside-induced differentiation-associated-protein 1 (gene)</td>
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<td>GJB1</td>
<td>Gap junction b-1 (gene)</td>
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<td>HMN</td>
<td>Hereditary motor neuropathy</td>
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<td>HMSN</td>
<td>Hereditary motor and sensory neuropathy</td>
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<td>HNA</td>
<td>Hereditary neuralgic amyotrophy</td>
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<td>HNPP</td>
<td>Hereditary neuropathy with liability to pressure palsies</td>
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<tr>
<td>HSAN</td>
<td>Hereditary sensory and autonomic neuropathy</td>
</tr>
<tr>
<td>KIF1A</td>
<td>Kinesin family member 1A (gene)</td>
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<td>KIF5A</td>
<td>Kinesin family member 5A (gene)</td>
</tr>
<tr>
<td>MFN2</td>
<td>Mitofusin 2 (gene)</td>
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<tr>
<td>MELAS</td>
<td>Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes</td>
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<td>MNCV</td>
<td>Motor nerve conduction velocity</td>
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<td>MPZ</td>
<td>Myelin protein zero (gene)</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>mRS</td>
<td>Modified Rankin scale</td>
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<tr>
<td>NARP</td>
<td>Neuropathy, ataxia and retinitis pigmentosa</td>
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<tr>
<td>NCV</td>
<td>Nerve conduction velocity</td>
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<tr>
<td>NDS</td>
<td>Neuropathy Disability Score</td>
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<td>NGS</td>
<td>Next generation sequencing</td>
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<td>NSS</td>
<td>Neuropathy Symptom Score</td>
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<td>OUH</td>
<td>Oulu University Hospital</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>PMP22</td>
<td>Peripheral myelin protein 22 kDa (gene)</td>
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<td>RFC1</td>
<td>Replication factor complex subunit 1 (gene)</td>
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<tr>
<td>SCAs</td>
<td>Spinocerebellar ataxias</td>
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<tr>
<td>SFN</td>
<td>Small-fibre neuropathy</td>
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<tr>
<td>TTR</td>
<td>Transthyretin</td>
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<tr>
<td>TTR-FAP</td>
<td>Transthyretin familial amyloid polyneuropathy</td>
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<tr>
<td>WES</td>
<td>Whole exome sequencing</td>
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<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
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List of the original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


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1 Introduction

Charcot-Marie-Tooth disease (CMT) is a genetically and clinically heterogeneous group of disorders affecting the peripheral nervous system (Timmerman et al., 2014). It was first described at the end of the 19th century as peroneal muscular atrophy. Understanding of the disease and its underlying pathophysiology started to increase as the era of electrophysiological (EDx) studies began in the late 1950s. In 1968, Dyck and Lambert described CMT1 and CMT2, the two main categories of CMT that are defined according to electrophysiological properties and histopathology (Dyck & Lambert, 1968). Most of the CMT patients follow the autosomal dominant trait of inheritance, but X-linked and recessive forms of CMT are not uncommon (M. A. Saporta & Shy, 2013).

The era of genetic diagnosis of CMT began in 1991, when two groups reported the identification of a 1.4-Mb duplicated region of chromosome 17 containing the peripheral myelin protein 22 (PMP22) gene as the cause of CMT1A (Lupski et al., 1991; Raeymaekers et al., 1991). Today, more than 100 genes are known to cause Charcot-Marie-Tooth disease (Cavallaro et al., 2021). Duplication of PMP22 is still considered the most common cause of CMT, followed by point mutations in the myelin protein zero (MPZ), mitofusin 2 (MFN2), and gap junction b-1 (GJB1) genes (Murphy et al., 2012). Although, the frequencies of pathogenic variations vary between populations (Gentile et al., 2020; Sivera et al., 2013).

CMT is heterogeneous also clinically. The most common clinical features include distal predominance of muscle weakness, wasting and sensory disturbances, reduced or absent tendon reflexes, and foot deformities such as pes cavus (A. S. Saporta et al., 2011). The symptoms typically emerge in the first two decades of life, but infantile-onset as well as adult-onset forms of CMT have been described (M. A. Saporta & Shy, 2013). Usually, symptoms are slowly progressive when they start in childhood or adulthood, but can be somewhat severe and rapidly progressive in infantile onset forms of CMT (M. A. Saporta & Shy, 2013). The severity of the disease is variable, even within the same family (Pareyson et al., 2006).

The prevalence of CMT varies between populations, with the most cited prevalence being 1/2500 (Skre, 1974). It has been determined prior to the era of molecular testing. The lowest prevalence has been reported in Serbia (9.7/100,000) (Mladenovic et al., 2011) and the highest in Akershus County, Norway (82.3/100,000) (Braathen et al., 2010). Although population-based studies have been increasingly reported in recent years, the epidemiology of CMT is still poorly investigated (Barreto et al., 2016). There are no previous population-based data on
the molecular epidemiology of CMT in Finland. The present study investigates the epidemiology and clinical phenotype of CMT in Northern Ostrobothnia.
2 Review of the literature

2.1 Historical aspects and development of nomenclature

When the disease was first described and named in 1886 by the French neurologists Jean-Martin Charcot and Pierre Marie in Paris, as well as the British neurologist Howard Henry Tooth in London, it was referred to as ‘peroneal muscular atrophy’ (M. A. Saporta & Shy, 2013). The clinical definition consisted of childhood-onset, slowly progressive distal muscle weakness and atrophy, motor symptoms predominance, and foot deformities (Harding & Thomas, 1980). Later the condition has been named as Charcot-Marie-Tooth disease (CMT) or hereditary motor and sensory neuropathy (HMSN) (Skre, 1974). In 1893, Déjérine and Sottas described a hypertrophic interstitial neuritis that had onset in infancy or early childhood (Klein, 2020). The concept of a separate entity, Déjérine-Sottas disease, was disposed when it was shown that the pathophysiology did not differ from HMSN (Harding & Thomas, 1980).

The confusion was abated in 1960s, when it was found that severe slowing of motor nerve conduction in some families correlated with abnormal myelination of peripheral nerves (Dyck & Lambert, 1968). Since then, HMSN has been divided into two main groups based on nerve conduction studies. Demyelinating form (HMSN1/ CMT1) is defined by MNCV <38 m/s in the upper limbs, and axonal form (HMSN2/ CMT2) is characterized by normal or near normal MNCV in upper limbs (>38 m/s) (Dyck & Lambert, 1968). Soon after, an intermediate form between demyelinating and axonal was discovered (MNCV between 35 m/s and 45 m/s) (Madrid et al., 1977). In the recent decades, the term HMSN has been interchangeably used with CMT.

The subclassification of CMT has evolved along with the understanding of pathophysiological and neuropathological as well as genetic characteristics. The first classification was based on inheritance pattern, pathophysiology, and nerve-conduction findings (Harding & Thomas, 1980; A. S. Saporta et al., 2011), and HMSN was classified into types 1 through 7. HMSN1 (CMT1) is an autosomal dominant (AD) demyelinating form showing uniformly slowed NCV and gross nerve hypertrophy from diffuse myelin and fibroblast lamellae on nerve biopsy. HMSN2 (CMT2) is an autosomal dominant axonal neuropathy, with normal or slightly reduced nerve conduction velocities and axonal atrophy.
CMT3, initially described synonymous with HMSN3 or Déjérine-Sottas neuropathy, is to date considered an early-onset severe form and no longer a separate category (Planté-Bordeneuve et al., 2001). Depending on the genetic cause, these patients are classified as having CMT1, X-linked CMT (CMTX), or autosomal recessive (AR) form of CMT (CMT4) (Klein, 2020). HMSN5 refers to a HMSN associated with spastic paraplegia, and HMSN6 refers to HMSN associated with optic atrophy, which is today mainly understood to be mitochondrial (Shy, Lupski, et al., 2005). HMSN7 refers to HMSN associated with retinitis pigmentosa (Shy, Lupski, et al., 2005). Indeed, the terms HMSN5, HMSN6, and HMSN7 are not used anymore, as the underlying genetic cause is used for designation (Klein, 2020).

Moreover, CMT4 does not correspond to HMSN4. HMSN4 was first described as an AR inherited, phytanic acid metabolic disorder also known as Refsum disease (Weinstein, 1999). Instead, CMT4 became the term of for all AR demyelinating forms of CMT (Tazir et al., 2013).

Later, within CMT types 1, 2, and 4, additional subclassifications based on the specific causal gene and phenotype were constructed, and the 26 letters of the alphabet were exploited (Klein, 2018). For example, AD demyelinating neuropathy due to the PMP22 duplication was named CMT1A. As long as the number of causal genes was limited, this worked well. However, recent technology has sped up the discovery of new genes, and the genetic causes have exceeded the 26 letters of the alphabet a while ago (Klein, 2020). Also, the same gene can be associated with different CMT types; for example, Ganglioside-induced differentiation-associated-protein 1 (GDAP1) has been linked to the AD axonal form (CMT2K), AR axonal form (CMT2H or AR-CMT2K), recessive demyelinating form (CMT4A), and the recessive intermediate form (CMTRIA).

Modification of this nomenclature is therefore needed. A new proposition for the classification of CMT and other inherited disorders of the peripheral nervous system has been reported (Mathis et al., 2015). It considers inheritance patterns, causative gene defects, and primary pathological phenotype (axonal, demyelinating, or intermediate) together with clinical classification (Magy et al., 2018). For example, autosomal dominant axonal CMT caused by pathogenic variant in GDAP1 would be named AD-CMTAx-GDAP1. Consensus of what kind of reformation is needed and what is suitable has not been achieved yet. Nevertheless, some renovation of the classification in inherited neurological diseases seems to be necessary, and at least the gene names should be included (Klein, 2018; Shy, 2018; Vallat et al., 2018).
2.2 CMT and other inherited neuropathies

Hereditary neuropathies include a wide range of disorders of the peripheral nervous system (PNS) and all types of Mendelian or mitochondrial inheritance (Cavallaro et al., 2021). These disorders present with a broad spectrum of motor, sensory, autonomic, and other organ system involvement. Phenotypic and genetic overlap is remarkable among these seemingly separate entities. Morbidity and mortality in these patients are variable, depending on the affected neural and non-neural organs (Klein, 2020).

Hereditary neuropathies can be divided into two clinical groups (Figure 1) (Reilly & Shy, 2009). In the first group, neuropathy is the predominant or even sole feature of the disease and results from pathogenic variants that affect selectively the peripheral nervous system (Mathis et al., 2015). This group includes Charcot-Marie-Tooth disease and other CMT-related neuropathies.

The second group consists of neuropathies in which the neuropathy represents a minor or major component in a more widespread neurological or multisystem disorder. These are complex inherited diseases that also involve the central nervous system and/or other organs and tissues (Cavallaro et al., 2021). These can be called “complex hereditary neuropathies”.

This thesis is focused on CMT, but in this chapter other types of inherited neuropathies are also reviewed. Indeed, all these should be taken into account in the differential diagnosis of CMT.

2.2.1 CMT

CMT is the most common form of inherited neuropathy (Reilly & Shy, 2009). It is classified according to inheritance pattern (autosomal dominant, autosomal recessive, or X-linked), primary nerve pathology, as reflected in nerve conduction studies, and causative gene defects (Banchs et al., 2009).
Fig. 1. Hereditary neuropathies, a simplified overview.

CMT1 stands for autosomal dominant demyelinating neuropathy, and CMT2 for autosomal dominant axonal neuropathy (Pareyson & Marchesi, 2009). CMT1 disturbs primarily the myelinating Schwann cells and is defined by slow nerve conduction velocities (NCV). In CMT2, NCVs are in normal or near normal range (>38 m/s) and compound muscle action potential (CMAP) amplitudes are decreased, reflecting axonal degeneration in motor nerves (Pareyson et al., 2006). Nonetheless, it is notable that axonal loss exists in both CMT2 and CMT1, and it is the major cause of disability in patients with CMT, even in CMT1 where axonal loss is secondary to demyelination (Juneja et al., 2019).

CMT4 is usually reserved for AR demyelinating neuropathy, but some authors also classify AR axonal neuropathy as belonging to this category, whereas others define autosomal recessive axonal forms of CMT as AR-CMT2 (Shy, 2018; Vallat et al., 2018). Autosomal recessive forms of CMT are rare, accounting for less than 10% of all CMT patients (Klein, 2020). Autosomal recessive CMT may account for 30–50% of all CMT patients in countries that have high percentage of consanguineous marriages (Dubourg et al., 2006; Tazir et al., 2013). Pathogenic
variants in *SORD* gene have recently been described to be the most common cause of AR neuropathy (Cortese et al., 2021).

The X-linked CMTX accounts for 10–15% of all CMT cases (Klein, 2020). The majority of patients (90%) with CMTX have pathogenic variants in *GJB1* (Wang & Yin, 2016). Pathogenic variants in *GJB1* cause a milder phenotype to approximately two-thirds of female patients, as compared to male patients, because of the inactivation of the other X-chromosome (Abrams & Freidin, 2015; Siskind et al., 2011). NCVs are usually in the intermediate range (35–45 m/s) (Pareyson & Marchesi, 2009). Intermediate forms of CMT demonstrate the overlapping of electrophysiological and neuropathological features between CMT1 and CMT2, even within the same family (Juneja et al., 2019).

Typically, CMT-related symptoms occur in in the first two decades of life but can vary from infantile-onset to onset in late-adulthood and the disease progression tends to be slow (Mathis et al., 2015). The phenotype of CMT includes features that can be derived from length-dependent neuropathy and axonal degeneration, as the symptoms emerge first in distal lower extremities. Typical symptoms of the so-called “classical CMT phenotype” are distal predominance of muscle weakness, wasting, atrophy, and sensory loss; difficulties in walking/running, steppage gait, and distal-proximal progression of the symptoms over time (Pareyson & Marchesi, 2009; M. A. Saporta & Shy, 2013). Tendon reflexes are usually decreased or absent. However, specific features may be found in patients with CMT, such as vocal cord paresis in pathogenic recessive variants in *GDAP1* (Sivera et al., 2017) or Adie’s pupil and sensory-neural hearing impairment in pathogenic variants in *MPZ* (Callegari et al., 2019).

Overall, the heterogeneity, both clinically and genetically, and the overlapping of CMT and the entities listed below remain the main challenges regarding the development of curative treatment of CMT (Juneja et al., 2019).

### 2.2.2 CMT-related neuropathies

#### HSAN

The hereditary sensory and autonomic neuropathies (HSAN) consist of a clinically and genetically heterogeneous group of peripheral nerve disorders that are characterized by a predominant sensory presentation and usually also the autonomic nervous system is involved (Auer-Grumbach, 2013; M. A. Saporta &
Sly, 2013). The classification of the HSAN depends on the age of onset, mode of inheritance, clinical presentation, and genetic background (Houlden et al., 2004). Their classification shows quite a complexity and has led to controversies in terminology, as the originally described five HSAN subtypes do not coincide with the genetic subgroups (Verhoeven et al., 2006).

Patients with HSAN develop variable phenotypes depending on the genetic cause of the disease (M. A. Saporta & Shy, 2013). Age of onset varies from congenital to adult-onset. The characteristics include profound distal sensory loss and progressive degeneration of sensory and autonomic neurons. Also, chronic skin ulcers, injuries with no pain sensation, and other skin abnormalities are often present. The frequency of autonomic disturbances varies according to the genetic underlying cause of the disease, and motor disturbances may also be present (Auer-Grumbach, 2013).

**HMN**

Hereditary motor neuropathies (HMN) or distal hereditary motor neuropathies (dHMN) are a type of neuromuscular disorder characterized by length-dependent lower motor neuron dysfunction. The term has been used to define inherited polyneuropathies that are solely motor in nature but are otherwise analogous to CMT (M. A. Saporta & Shy, 2013). Classically, a patient with HMN presents with peroneal muscular atrophy and weakness, and relatively slow progressive wasting of the foot extensor and intrinsic muscles occurs. Involvement of the muscles in the proximal lower limbs and distal upper limbs occurs later (Beijer & Baets, 2020).

HMN are highly heterogeneous in terms of clinical presentation, age of onset, rate of progression, and genetic background. Currently 26 causal genes are known (Beijer & Baets, 2020), but there is still considerable missing heritability, and more than half of the cases cannot be genetically explained (Dohrn & Saporta, 2020).

Pathogenic variants in a given gene can cause the phenotypes known as dHMN, CMT2, and dSMA (distal spinal muscular atrophy), leading to the conclusion that dHMN and motor CMT should not be classified separately (Adam et al., 1998; Bansagi et al., 2017; Previtali et al., 2019).

**HNPP**

Hereditary neuropathy with liability to pressure palsies (HNPP) causes episodes of focal demyelinating neuropathy that are preceded by a minor trauma to the
peripheral nerve. It is almost always caused by the reciprocal deletion of the 1.4 Mb stretch of chromosome 17p11.2, which contains the \textit{PMP22} gene (Chance et al., 1993). Ten to twenty percent of patients have HNPP caused by a frameshift, splice site or point mutation of the \textit{PMP22} gene (M. A. Saporta & Shy, 2013). HNPP is inherited in autosomal dominant manner and de novo mutations occur. The prevalence of HNPP has been estimated to be 7.3/100,000 in England (Foley et al., 2012) and 16/100,000 in southwestern Finland (Meretoja et al., 1997).

HNPP is characterized by recurrent sensory and motor mononeuropathy that typically occurs at entrapment sites, such as the ulnar groove at the elbow, the head of the fibula at the knee, or the carpal tunnel at the wrist (M. A. Saporta & Shy, 2013). In most cases, the episode of mononeuropathy is preceded by minor trauma or compression to peripheral nerves (Stogbauer et al., 2000). Mild pes cavus and hypo- or areflexia can be found in HNPP. Patients with HNPP typically also have a slowly progressive symmetric polyneuropathy almost indistinguishable from the CMT type 1 disease (Pareyson et al., 2006). Age of onset is usually in adolescence or young adulthood, but sometimes the first episode occurs in infancy or later in life (Meretoja et al., 1997), as onset even after 80 years of age has been reported (Attarian et al., 2020). Focal conduction abnormalities are located at entrapment sites in nerve conduction studies, and sausage-like swellings (tomacula) of the myelin sheaths in nerve biopsy occur (Attarian et al., 2020).

It is important to identify this phenotype to avoid unnecessary nerve compressions from prolonged activities and postures (e.g., elbow, knee) (Attarian et al., 2020; Klein, 2020). The complications of HNPP encompass the development of a transient pressure palsy to a permanent one, such as foot drop, carpal tunnel syndrome, or more generally neuropathic pain. The treatment of these symptoms is mostly symptomatic (Attarian et al., 2020).

\textit{HNA}

Hereditary neuralgic amyotrophy (HNA), also known as hereditary brachial plexus neuropathy (HBPN), is another form of focal recurrent neuropathy. It is inherited in an autosomal dominant manner and exhibits incomplete penetrance (Klein, 2020). Approximately 50% of HNA patients carry a point mutation or duplication of the \textit{septin 9} gene (\textit{SEPT9}) on chromosome 17q25.3 (Kuhlenbäumer et al., 2005; Landsverk et al., 2009).

HNA is characterized by episodes of brachial plexus neuropathy containing muscle weakness, atrophy, and sometimes paresthesia in an upper limb. These
symptoms are usually preceded by severe pain in the affected arm (Kuhlenbäumer et al., 2005). Pain usually starts about one week prior to muscle weakness and is so severe that patients frequently seek medical care from an emergency department (Klein, 2020). Full or partial recovery occurs in most patients within weeks to months, but the improvement of motor dysfunction is often slow, and some patients are left with residual disabilities.

It is estimated that 50% of attacks are preceded by events such as stress, exposure to cold, infections, immunizations, surgery, or pregnancy that may change the balance of our immune system (Landsverk et al., 2009). Nerve biopsy at times of attacks supply evidence of an inflammatory component to the attacks (Klein et al., 2002). High-dose IV steroids may help to manage the pain, but neurologic deficits keep progressing. Moreover, patients with HNA may benefit from prophylactic steroid treatment at the time of surgery or child delivery as they are likely to experience attacks at these times (Klein et al., 2013).

2.2.3 Complex hereditary neuropathies

These entities include multisystem disorders, in which peripheral neuropathy can be a presenting feature (i.e., before multisystem involvement is appreciated) and/or one manifestation in a complex neurologic or multisystem disorder. Some of them are described below.

Hereditary ataxias with neuropathy

Ataxia, defined as imbalance and incoordination, results from dysfunction of the nervous system involved in the coordination of movement. Ataxia is characterized by slowly progressive incoordination of gait (gait ataxia) and limbs (limb ataxia). Incoordination of speech (dysarthria) and eye movements (nystagmus) exists (Chaudhari et al., 2020). Ataxia is a symptom, the cause of which must be resolved. A major proportion of progressive cerebellar ataxias are caused by acquired factors and are not genetic (Hadjivassiliou et al., 2017).

Hereditary ataxias are a genetically and phenotypically heterogeneous group of disorders that have been subclassified based on the phenotype, genetic cause, and mode of inheritance. Inherited ataxias include, for example, autosomal or X-linked dominant spinocerebellar ataxias (SCAs), recessive inherited Friedreich's ataxia (FRDA), and late-onset cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) (Klein, 2020).
SCAs are predominantly caused by unstable repeat expansions in either coding or noncoding region or regions of the relevant genes (Teive & Ashizawa, 2015). SCAs constitute a wide and complex group of degenerative diseases characterized by progressive degeneration of the Purkinje cells and/or spinocerebellar tracts, as well as other nervous system structures including the brain stem (Jayadev & Bird, 2013). Gait ataxia and incoordination, nystagmus, and dysarthria are the core triad of symptoms. Variable additional features are present depending on the underlying gene defect, and axonal sensory neuropathy can be a main presenting feature in prevalent types of SCAs (Sullivan et al., 2019).

FRDA is associated with homozygous triplet GAA expansions in the FXN gene (Laurà et al., 2019). FRDA is characterized by early-onset cerebellar gait and limb ataxia, areflexia, dysarthria, positive Babinski sign, loss of position and vibration senses, and progressive motor weakness due to an axonal neuropathy (Metz et al., 2013).

The gene defect responsible for CANVAS is a biallelic intronic repeat expansion in the replication factor complex subunit 1 gene (RFC1) (Cortese et al., 2019). Axonal, predominantly or purely sensory polyneuropathy is the core feature of CANVAS, as all patients have at least subclinical neuropathy and 94% have symptomatic neuropathy (Cortese, Tozza, et al., 2020). The biallelic intronic expansion in RFC1 with typical CANVAS phenotype was found in five ataxia patients in Northern Ostrobothnia, which was 15% of all genetically confirmed ataxia syndromes (Lipponen et al., 2021).

**Hereditary Spastic Paraplegia with neuropathy**

Hereditary spastic paraplegia (HSP) consists of a genetically heterogeneous group of neurodegenerative disorders that affect primarily the central corticospinal tract and dorsal columns. Patients with HSP present with distinct progressive spasticity and variable weakness and atrophy of the lower limbs (Chrestian et al., 2016; Murala et al., 2021). HSPs are inherited in an autosomal dominant, autosomal recessive, X-linked recessive manner, or maternally, and more than 80 loci or genes have been identified (Murala et al., 2021). The estimated prevalence of HSP worldwide is 1.8/100,000 (Ruano et al., 2014). However, the prevalence varies between populations and according to the definition used for patients’ inclusion, diagnosis, and classification (de Souza et al., 2017).

Clinically HSP presents as a pure or complex form (Harding, 1981). The pure form of HSP consists of isolated pyramidal signs presenting as lower extremity
weakness and spasticity, brisk tendon reflexes, and extensor plantar responses. Pyramidal signs can be associated with decreased vibration and proprioception senses as well as sphincter disturbances (de Souza et al., 2017). Weakness and spasticity can be slowly progressive or non-progressive (de Souza et al., 2017).

In addition to spastic paraplegia, the complex HSP form may consists of cerebellar signs (including ataxia, tremor, and nystagmus), cognitive impairment (including mental retardation and dementia), epilepsy, extrapyramidal signs (including chorea, parkinsonism, and dystonia), and axonal or demyelinating peripheral neuropathy (including dysautonomia and sensory disturbances) (Murata et al., 2021). Non-neurological manifestations of complex HSP are broad and heterogeneous and may include, for example, dysmorphic features (microcephaly, macrocephaly, facial dysmorphisms, or short stature) and ophthalmological abnormalities (like optic neuropathy, optic atrophy, or retinitis pigmentosa) (Lo Giudice et al., 2014).

Moreover, HSP may be classified according to inheritance pattern, age of onset of spasticity, or intracellular pathophysiological mechanisms (de Souza et al., 2017).

HSP with neuropathy is one of the complex HSP types. The overlap between CMT and HSP is noticed phenotypically as well as genetically. Silver syndrome/HSP17 is characterized by lower limb spasticity in addition to motor or motor and sensory neuropathy and atrophy of the intrinsic hand muscles and sometimes foot (Ishihara et al., 2020; Rohkamm et al., 2007). The kinesin group of genes including kinesin family member 1A (KIF1A) and kinesin family member 5A (KIF5A) are often associated with the complex phenotype, not just pyramidal signs. Patients with dominant pathogenic variants in KIF1A present with developmental delay, autism spectrum disorder, cerebellar ataxia, spasticity, and sensory-motor axonal peripheral neuropathy. (J. R. Lee et al., 2015; Tomaselli et al., 2017). Patients with dominant pathogenic variants in KIF5A have varying degrees of axonal neuropathy and pyramidal track signs, and some specific pathogenic variants of KIF5A can cause pure CMT2 or pure HSP (Liu et al., 2014).

**Transthyretin familial amyloid polyneuropathy**

The hereditary amyloid polyneuropathies are a group of autosomal dominant multisystem disorders that consists of varying severity of autonomic and peripheral neuropathy and cardiovascular or other systemic manifestations (Adams et al., 2012). Transthyretin familial amyloid polyneuropathy (TTR-FAP) is the most prevalent subtype among hereditary amyloid polyneuropathies. The underlying
cause of TTR-FAP is an amyloidogenic pathogenic variant in transthyretin (TTR), which results in misfolding of the TTR protein, leading to protein aggregation and the formation of amyloid fibrils and, finally, to amyloidosis (Benson & Kincaid, 2007).

TTR-FAP was first reported in Portugal and was thought to be endemic in the northern part of the country. Since then, it has been reported throughout the world, but particularly in Europe, with marked phenotypic heterogeneity (Plante-Bordeneuve, 2018). The most common pathogenic variant in the TTR gene is p.Val30Met, which was generally considered to be concentrated in geographically restricted areas of Japan, Portugal, and northern Sweden (Andersson & Blom, 1972; Hellman et al., 2008; Planté-Bordeneuve et al., 2003). Today, it is known that p.Val30Met is the most common variant in Latin America and all Europe. On the other hand, the pathogenic variant p.Val122Ile in TTR is the most common genotype in the United States of America (Plante-Bordeneuve, 2018). Historical connections between the endemic population in northern Sweden and the population in northern Finland have been substantial but, so far, only one TTR-FAP patient has been reported in Finland (Druge et al., 1992), and, recently, another patient with p.Val30Met has been identified (personal communication, M. Lindroos). Screening for pathogenic variants of transthyretin in patients with idiopathic small-fiber neuropathy (SFN) or combined SFN and large-fiber sensorimotor axonal polyneuropathy has not revealed additional TTR-FAP patients in Finland (Samuelsson et al., 2019).

TTR-FAP typically manifests with length-dependent small fiber sensorimotor polyneuropathy, autonomic neuropathy, loss of temperature and pain sensation, heart failure and cardiac arrhythmias due autonomic dysfunction, and sometimes nephrotic disease (Adams et al., 2012). Some variants in TTR, such as p.Val30Met, are associated primarily with neuropathy, while others, such as p.Val122Ile, present with cardiomyopathy as the main feature. In other pathogenic variants of TTR, both manifestations occur in different proportions (Ando et al., 2013). Patients typically decease within 7–12 years from the onset of symptoms, commonly because of infection, cardiac dysfunction, or cachexia (Adams et al., 2014; Koike et al., 2012). In Sweden, and in non-endemic areas like Italy and France, patients with p.Val30Met typically have their first symptoms late in the fifth or sixth decade of life, with frequent cardiac involvement (Parman et al., 2016; Rapezzi et al., 2013). On the other hand, Portuguese as well as Japanese and Brazilian patients with p.Val30Met typically present the symptoms by the third decade, and this early-onset disease is associated almost solely associated with neuropathy (Klein, 2020).
The genetic factors influencing the phenotypic expression and variability of age of onset have been under interest (Plante-Bordeneuve, 2018).

The accurate and early diagnosis of TTR-FAP is important because delay in diagnosis prevents the timely initiation of appropriate treatment (Adams et al., 2016). Indeed, incorrect diagnosis and diagnosis delay are quite common, especially when family history is lacking. Phenotypic heterogeneity, rarity of the disease, and late onset of the symptoms form the main challenges (Said & Planté-Bordeneuve, 2011).

Liver transplantation has been the choice of treatment for TTR-FAP patients (Ando et al., 2013). However, liver transplant does not prevent the progression of the cardiac disease because the wild-type TTR may still expand the existing amyloid deposits in the heart (Adams et al., 2016). Indeed, continued cardiac system evaluation must be ensured. Pharmacologic disease-modifying treatment include small-molecule inhibitors, such as tafamidis and diflunisal. They may prolong the time from disease onset to progression (Magrinelli et al., 2020).

**Mitochondrial disorders with neuropathy**

Mitochondrial diseases are genetically and especially phenotypically heterogeneous group of disorders that result from structural and functional modifications of the mitochondria (McFarland et al., 2010). The disorder can result from pathogenic variants within the mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) of genes encoding the mitochondrial proteins (Schapira, 2012). The length and high energy demand of the axon emphasize the importance of adequate mitochondrial function in nerves.

Chronic axonal polyneuropathy is often associated with defects in mitochondrial DNA maintenance and replication or defects in the respiratory chain complex. Many genes that cause CMT are directly linked to mitochondrial dynamic function (e.g., *MFN2* and *GDAP1*) (Klein, 2020). Above that, peripheral neuropathy is a key symptom in many complex mitochondrial disorders, such as neuropathy, ataxia, and retinitis pigmentosa (NARP), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE); sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO); mitochondrial cerebellar ataxia, renal failure, neuropathy, and encephalopathy (MCARNE) and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) (Lu & Tarnopolsky, 2021; Luigetti, Sauchelli, et al., 2016; Pareyson et al., 2013).
MELAS is one of the most common mitochondrial syndromes, and it affects various organs. Manifestations of the central nervous system include stroke-like episodes, headaches, seizures, partially reversible aphasia, cortical vision loss, and motor weakness as well as developmental delay, depression, psychosis, and personality changes (Anglin et al., 2012; Boggan et al., 2019; DiMauro, 2013). Non-neurological manifestations include cardiomyopathy, conduction deficits of the heart, gastrointestinal symptoms such as constipation or diarrhea, diabetes, anemia, proteinuria, and myopathy (Boggan et al., 2019). Peripheral nervous system is also affected, leading to peripheral polyneuropathy.

The pathogenic variant m.3243A>G accounts for 80% of the patients with MELAS syndrome (Goto et al., 1990). However, many patients with m.3243A>G do not present with the full MELAS phenotype. Instead, they may be asymptomatic or express a broad spectrum of multiorgan clinical symptoms that vary in clinical severity from mild to severe (Kaufmann et al., 2009). The full MELAS phenotype includes stroke-like episodes, but this feature is missing from patients with m.3243S>G (Damian et al., 1995; Kaufmann et al., 2009). Typical manifestations of m.3243A>G include sensorineural hearing loss, diabetes, exercise intolerance, gastrointestinal disorders, and short stature (Kaufmann et al., 2009, 2011). The frequency of polyneuropathy among patients with m.3243A>G has been estimated, and it varies at least from 22% (Kärppä et al., 2003) to 77% (Kaufmann et al., 2006) and 92% (Luigetti, Sauchelli, et al., 2016). The most frequent electrodiagnostic abnormality is a sensory axonal neuropathy (Kaufmann et al., 2006; Luigetti, Sauchelli, et al., 2016), but also mixed axon loss and demyelinating and uniform demyelinating forms are seen (Kärppä et al., 2003).

Neuropathy has been reported in 12–45% of patients with various mitochondrial disorders (Luigetti, Sauchelli, et al., 2016; Mancuso et al., 2016). In an Italian nationwide cohort of 1200 patients with mitochondrial disorders, polyneuropathy was one of ten most common phenotypic features of the patients (Mancuso et al., 2016). Pathogenic variants in polymerase gamma (POLG) cause axonal or axonal and demyelinating, mainly sensory polyneuropathy, usually in the clinical context of SANDO, while pathogenic variants in TYMP lead to a demyelinating sensory and motor polyneuropathy, mainly in the clinical context of the MNGIE syndrome (Mancuso et al., 2016).
2.3 Epidemiology of CMT

Peripheral neuropathy is a common neurologic disorder, with an estimated prevalence of 1 to 3% in the general population and up to 7–8% in the elderly (Hanewinckel et al., 2016; Shy, 2020). The causes of peripheral neuropathy are numerous, extending from rare to frequent and often remaining unknown (Shy, 2020).

The historically most cited estimated prevalence of CMT is 1/2500 (Skre, 1974). Since then, multiple studies have estimated the prevalence throughout the world (Barreto et al., 2016). Population-based studies on the prevalence of CMT have been rare, but in recent years the interest towards the field seems to be rising (Table1). A systematic review on the epidemiology of CMT found only 12 studies that met the inclusion criteria and 10 of them reported the prevalence of CMT (Barreto et al., 2016). A population prevalence of 82.3/100,000 has been reported in Norway, (Braathen et al., 2010b), which is the highest known prevalence of CMT. The lowest prevalence of CMT seems to be in Serbia (9.7/100,000) (Mladenovic et al., 2011) and Japan (10.8/100,000) (Saiko et al., 2002). Recent figures on CMT prevalence have been estimated from New Zealand (15.2/100,000) (Theadom et al., 2019), Gran Canaria (24.8/100,000) (Lousa et al., 2019), Ireland (10.5/100,000) (Lefter et al., 2016), and northern Norway (29.9/100,000) (Müller et al., 2021). In other Nordic countries, the prevalence estimates range from Iceland (12.0/100,000) (Gudmundsson et al., 2010) to Sweden (20.1/100,000) (Holmberg, 1993), and Denmark (22.5/100,000) (Vaeth et al., 2017). There are no population-based studies of CMT epidemiology in Finland (Meretoja et al., 1997; Silander et al., 1998).

Subsequent epidemiological studies give a median prevalence of 17.5/100,000 for CMT in European populations.

2.3.1 Molecular etiology of CMT

More than 100 genes have been associated with CMT (Cavallaro et al., 2021). The most frequent molecular etiology for CMT worldwide is the PMP22 duplication, accounting for more than half of all CMT cases and about 70% of CMT1 cases (A. S. Saporta et al., 2011). Another three genes commonly associated with CMT include MPZ, MFN2, and GJB1. Pathogenic variants in these four genes are responsible for about 90% of all genetically defined CMT (DiVincenzo et al., 2014; Gess et al., 2013; Manganelli et al., 2014; Murphy et al., 2012). These four genes
and GDAP1 are described here in more detail with a focus on pathological features, clinical phenotypes, and frequencies of pathogenic variants.

Table 1. Population based studies on prevalence of CMT in European populations.

<table>
<thead>
<tr>
<th>Geographic area</th>
<th>Population</th>
<th>Prevalence of CMT (1/100,000)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>5,600,000</td>
<td>22.5</td>
<td>Vaeth et al. 2017</td>
</tr>
<tr>
<td>England, Newcastle upon Tyne</td>
<td>259,500</td>
<td>11.8</td>
<td>Foley et al. 2012</td>
</tr>
<tr>
<td>Iceland</td>
<td>307,627</td>
<td>12</td>
<td>Gudmundsson et al. 2010</td>
</tr>
<tr>
<td>Ireland</td>
<td>3,439,565</td>
<td>10.5</td>
<td>Lefter et. al. 2016</td>
</tr>
<tr>
<td>Italy, Molise</td>
<td>332,155</td>
<td>17.5</td>
<td>Morocutti et al. 2002</td>
</tr>
<tr>
<td>Norway, Eastern Akershus county</td>
<td>297,539</td>
<td>83.3</td>
<td>Braathen et al. 2011</td>
</tr>
<tr>
<td>Northern Norway</td>
<td>484,546</td>
<td>29.9</td>
<td>Müller et al. 2020</td>
</tr>
<tr>
<td>Serbia, Belgrade</td>
<td>1,576,124</td>
<td>9.7</td>
<td>Mladenovic et al. 2011</td>
</tr>
<tr>
<td>Spain, Gran Canaria</td>
<td>851,157</td>
<td>24.8</td>
<td>Lousa et. al 2018</td>
</tr>
<tr>
<td>Sweden, Norrbotten and Västerbotten</td>
<td>518,742</td>
<td>20.1</td>
<td>Holmberg et al. 1994</td>
</tr>
<tr>
<td>Cyprus</td>
<td>794,000</td>
<td>16</td>
<td>Nicolaou et al. 2010</td>
</tr>
</tbody>
</table>

### 2.3.2 PMP22

Epidemiological studies show that 19.6% (Braathen et al., 2010) to 78.5% (Lousa et al., 2019) of all genetically defined CMT patients carry the 1.4 Mb duplication on chromosome 17p11.2 that includes the PMP22 gene (Lupski et al., 1991; Raeymaekers et al., 1991). The 22-kDa peripheral myelin protein (PMP22) is a transmembrane myelin glycoprotein component (Jetten & Suter, 2000). It belongs to the family of cellular membrane proteins defined by four transmembrane domains (Sakakura et al., 2011). Multifunctional PMP22 plays a role in Schwann cell development and proliferation, as well as in myelin formation and maintenance (Jetten & Suter, 2000). PMP22 is upregulated during proliferation of Schwann cell and may be involved in DNA metabolism and cell-cycle regulation (Giambonini-Brugnoli et al., 2005).

The disease-causing mechanism of PMP22 copy number variants or point mutations is still not fully understood (Pantera et al., 2020). Gene dosage of PMP22 is the proposed underlying mechanism of PMP22 duplication, upheld by detection of increased PMP22 protein (Gabriel et al., 1997) and increased PMP22 messenger RNA in CMT1A patients in nerve biopsy (Yoshikawa et al., 1994). Moreover, patients harboring PMP22 deletion on one copy of chromosome 17 and a
duplication on the other have no symptoms of neuropathy, as they have the correct
gene dosage (Hirt et al., 2015).

Pathogenic alterations of the PMP22 gene are associated with a variety of
inherited neuropathies (Cavallaro et al., 2021).

The age of onset of CMT1A is mainly in the first two decades of life, but in
most cases before the age of 10, often between 3 to 5 years of age (Klein, 2020;
Marques et al., 2005; Thomas et al., 1997). Still, patients with infant-onset to very
late-onset disease have been reported (van Paassen et al., 2014). CMT1A usually
manifests as a “classical phenotype of CMT” with distally accentuated muscle
weakness and atrophy, with or without sensory loss (van Paassen et al., 2014). Foot
deformity is a common feature, with up to 90% of patients presenting with pes
cavus (Bienfait et al., 2006). Deep tendon reflexes are usually decreased or absent
(Thomas et al., 1997). The severity of the disease shows great variability even
within the family. Overall, 1–7% of patients become wheelchair dependent, and a
walking aid is needed by 3–14% of patients (van Paassen et al., 2014).

Point mutations in PMP22 are detected in a small proportion of patients
suspected to have CMT or HNPP. CMT1 caused by point mutations of PMP22 can
be classified as CMT1E (Li et al., 2013), although some authors classify it as
CMT1A (Reilly, 2007). CMT1E comprises 1–5% of all CMT1 cases. PMP22 point
mutations can cause a wide range of clinical phenotype from mild HNPP to severe
dysmyelinating neuropathy reminiscent of Déjérine-Sottas syndrome or even an
overlap between the two phenotypes (Rosso et al., 2012). In general, the phenotype
of the PMP22 missense mutations tended to be more severe than that of the PMP22
duplication (Nelis et al., 1999).

The third group of inherited neuropathy associated with PMP22 is HNPP,
which is discussed in the previous chapter of CMT-related disorders.

2.3.3 GDAP1

Ganglioside-induced differentiation-associated-protein 1 (GDAP1) is an integral
membrane protein of the mitochondrial outer membrane, containing 358 amino
acids (Cuesta, Pedrola, Sevilla, Garcia-Planells, et al., 2002). It is encoded by a
17kb GDAP1 gene on chromosome 8q21.1. The gene is expressed in neural cells,
including both motor and sensory neurons as well as Schwann cells and in various
regions of the central nervous system (Niemann et al., 2005). GDAP1 has been
suggested to play a role in several mitochondrial functions, including dynamics of
mitochondria, mitochondrial transport, energy production, and calcium homeostasis.

Pathogenic variants in the GDAP gene are associated with several subtypes of Charcot-Marie-Tooth disease and show a wide range of severity and various inheritance patterns (Cassereau et al., 2011). According to the current classification, autosomal recessive GDAP1 mutations can cause demyelinating (CMT4A), axonal (AR-CMT2K), and intermediate (CMTRIA) forms of CMT, while autosomal dominant mutations cause the axonal (CMT2K) form of the disease (Baxter et al., 2002; Claramunt et al., 2005; Cuesta, Pedrola, Sevilla, Garcia-Planells, et al., 2002; Senderek et al., 2003; Sivera et al., 2010).

CMT caused by pathogenic variants in GDAP1 was first reported in families with recessive demyelinating (CMT4A) neuropathy (Baxter et al., 2002) or recessive axonal neuropathy with vocal cord paresis (Cuesta, et al., 2002), which has been named AR-CMT2K (Klein, 2020), CMTH (Adam et al., 1998), or CMT4A (Claramunt et al., 2005) afterwards. Recessive forms of CMT are rare, as they comprise less than 10% of all cases (Klein, 2020). CMT4A caused by pathogenic variants in GDAP1 is the most frequent recessive subtype of demyelinating CMT (Tazir et al., 2013). AR forms of GDAP1-related polyneuropathy are characterized by severe, early-onset disease with weakness and wasting in foot and hand. Disability occurs at the end of the first decade, and as the proximal muscles become affected, it leads to wheelchair-dependency in the second or third decade (Baxter et al., 2002; Sivera et al., 2017). Vocal cord paresis is developed for most of the patients, and diaphragmatic weakness occurs in the latter stages of the disease (Sevilla et al., 2008).

Autosomal dominant CMT caused by pathogenic variants in GDAP1 has been described (Cassereau et al., 2011; Claramunt et al., 2005; Crimella et al., 2010). The phenotype of CMT-2K is clearly milder than CMT4A. It is characterized by mostly adult onset, predominantly distal involvement, and slow progression. The age of onset may be as early as the end of first decade (Zimon et al., 2011), but most of these patients remain ambulatory their whole lives (Sivera et al., 2010; Zimon et al., 2011). Specific clinical characteristics, like dysphonia or dysautonomia, have been described in some patients, but no clear genotype-phenotype correlation has been established in AD-inherited variants (Pezzini et al., 2016). Moreover, the variability in the severity of the disease is prominent in patients with CMT-2K, even between family members (Sivera et al., 2017). The factors contributing to this variability remain unclear, but genetic modifiers have been suggested. For example, a pathogenic variant in the junctophilin-1 (JPH1)
gene has been described to modify the phenotype and GDAP1 function in a family with the p.R120W in GDAP1 (Pla-Martín et al., 2015). However, the pathogenesis behind the diversity between phenotypes caused by different pathogenic variants in GDAP1, AD or AR, is not fully understood (Binięda et al., 2021).

The frequency of GDAP1-related CMT varies between populations. In Spanish clinical series, the frequency of any pathogenic variant in GDAP1 is as high as 11.1% of all genetically confirmed CMT (Sivera et al., 2013). This is in part due to a dominant p.Arg120Trp variant, which was found in 57% of the cases. In that population the p.Arg120Trp is probably a consequence of the founder effect (Sivera et al., 2010, 2013). In an Italian cohort of 566 CMT patients, only four (0.7%) harbor a pathogenic variant in GDAP1 (Gentile et al., 2020), and in Brasilia, a cohort of 503 CMT patients include seven with pathogenic variants in GDAP1, which is 3.55% of all genetically defined CMT (Uchôa Cavalcanti et al., 2021). A study of a cohort of 17,000 individuals tested for CMT in commercial genetic testing laboratories revealed that only 0.7% of all genetically confirmed CMT patients harbor a pathogenic variant in GDAP1 (DiVincenzo et al., 2014). Likewise, a cohort of 1652 CMT patients were evaluated in 13 International Inherited Neuropathy Consortium centers. Only 0.7% of all genetically defined CMT were associated with pathogenic variants in GDAP1, six CMT4A, and three CMT2K (Fridman et al., 2015).

2.3.4 MPZ

Myelin protein zero (MPZ), a transmembrane protein of 219 amino acids, is the major structural protein of the PNS myelin and the simplest member of the immunoglobulin gene superfamily (Grandis et al., 2008; Shy et al., 2004). MPZ consists of three structural domains: a single immunoglobulin-like extracellular domain of 124 amino acids, a single transmembrane domain of 25 amino acids, and a single cytoplasmic domain of 69 amino acids (Hayasaka et al., 1991; Lemke, 1998). MPZ is necessary for normal myelin compaction, probably by holding adjacent wraps of myelin membrane together through homotypic interactions (Grandis et al., 2008; Shy et al., 2004). MPZ is encoded by a 7 kb MPZ gene in chromosome 1q23.3. MPZ is expressed exclusively by myelinating Schwann cells and it is not found in other tissues, including neurons or CNS myelin (Hayasaka et al., 1991). Moreover, in recent years, there has been evidence that MPZ localizes also at the
paranode and node of Ranvier, not only in compact myelin, participating in the maintenance of the nodal structure (Brügger et al., 2015).

More than 200 different disease-causing variants in MPZ have been identified (Howard et al., 2021). Patients with pathogenic variant in MPZ usually present with one of three distinct phenotypes. The infantile onset group have symptoms before the age of five and the nerve conduction velocities are very slow (Shy et al., 2004). Their motor development is delayed, as they seldom learn to walk independently before age of 15 months (Sammaneechai et al., 2015). The second group is called childhood-onset (Sammaneechai et al., 2015) or childhood–adolescence onset (Callegari et al., 2019) with normal early development, symptoms beginning gradually within the first two decades of life, and slow conduction velocities similar to those found with CMT1A (Callegari et al., 2019; Sammaneechai et al., 2015). The third group is adult-onset group, with symptoms developing after the age of 20–30 years, the mean age being 40 years (Sanmaneechai et al., 2015; Shy et al., 2004). Family history can be negative up to 33% of the late-onset cases (Callegari et al., 2019). They have normal or near normal nerve conduction velocities and reduced CMAP amplitudes in their lower extremities conforming to axonal neuropathy (Sanmaneechai et al., 2015).

2.3.5 MFN2

Pathogenic variants in the 35 kb MFN2 gene on chromosome 1p36.22 are associated with CMT type 2A (CMT2A) (Züchner et al., 2004), which has been reported to account for 2.5–7% of all genetically diagnosed CMT and 30–40% of genetically diagnosed CMT2 (Fridman et al., 2015; Miller et al., 2011; Murphy et al., 2012; Volodarsky et al., 2021).

The MFN2 gene encodes for MFN2 protein, which consists of 757 amino acids anchored to the outer mitochondrial membrane by two transmembrane domains (Pareyson et al., 2015). MFN2 is expressed in all tissues, but pathogenic variants have been associated only with neurological dysfunction, especially to peripheral length-dependent neuropathy (Pareyson et al., 2015). MFN2 is essential for mitochondrial fusion, but the exact mechanism by which this occurs is not fully understood. It is hypothesized that MFN2, located on both the outer mitochondrial membrane and the endoplasmic reticulum, mediates tethering through homologous interactions, thus preventing an excessive, potentially toxic, proximity between these two organelles (Filadi et al., 2015).
CMT2A, compared to CMT1A, is associated with a more severe, motor-predominant phenotype, commonly manifesting earlier in life (Bombelli et al., 2014; Feely et al., 2011). However, clinical presentations of pathogenic variants in MFN2 may vary from rapidly progressive and severe forms in early infancy to slowly progressive forms in late life (Pipis et al., 2020). Remarkable clinical variability is seen between families and within families. Interestingly, many of the patients with onset in childhood have normal early milestones, for example, they had learned to walk by one year of age. They can then progress rapidly and require walkers or wheelchairs before the age of 20 (Pisciotta & Shy, 2018). A smaller number of patients develop symptoms later in life with a milder phenotype. Age of onset and severity of the disease seem to be at least in part mutation-specific (Pipis et al., 2020).

In addition to neuropathy patients with pathogenic variants in MFN2 may have additional findings, such as optic nerve atrophy, tremor, hearing loss, vocal cord palsy, pyramidal tract involvement, and white matter lesions on MRI (Bombelli et al., 2014; Chung et al., 2010; Luigetti, Fabrizi, et al., 2016; Pipis et al., 2020; Büchner et al., 2004). This is expected, as MFN2 is ubiquitously expressed (Pisciotta & Shy, 2018).

Pathogenic variants in MFN2 are most commonly inherited in autosomal dominant manner, but autosomal recessive inheritance has also been described (Cavallaro et al., 2021). When comparing AR-CMT2A and AD-CMT2A, the age of onset does not differ significantly, and first symptoms appear most frequently before the age of 15 in both groups, usually with walking or balance difficulties (Pipis et al., 2020).

### 2.3.6 GJB1

Pathogenic variants in the 10 kb gap junction b-1 gene (GJB1) on chromosome Xq13.1 are the most common cause of X-linked Charcot-Marie-Tooth disease (CMTX) and among the four most common causes of CMT (Gentile et al., 2020; Gess et al., 2013). The GJB1 gene, also known as Connexin 32 (Cx32), encodes the transmembrane channel protein, connexin 32 (Cx32). The Cx32 protein is expressed in many human tissues, including oligodendrocytes in the central nervous system and myelinating Schwann cells in the peripheral nervous systems (Scherer et al., 1995). In the peripheral nervous system, Cx32 is located in the non-compact myelin of the paranodes and incisures (Cavallaro et al., 2021).
Pathogenic variants of \( GJB1 \) cause the X-linked dominant CMTX1 form. As Cx32 is expressed in both Schwann cells and oligodendrocytes, CMTX1 also has both central and peripheral nervous system manifestations (Abrams & Freidin, 2015; Halbrich et al., 2008). CMTX1 usually manifests more severely and earlier in hemizygous males, and heterozygous females are less affected (Kleopa et al., 2012). Most male patients present with clinical symptoms by the age of 10 (Shy et al., 2007). The phenotype includes distal muscle weakness and early progression to hand muscles, especially the thenar muscles, as well as decreased deep tendon reflexes and sensory loss (Abrams & Freidin, 2015). Approximately one-third of female patients have a phenotype resembling that seen in male patients. However, two-thirds of female patients have milder symptoms with later onset, and the symptoms show very slow deterioration with increasing age (Kleopa et al., 2012; Kovale et al., 2021; Siskind et al., 2011). This is probably due to X-inactivation, where the mutant allele is expressed only in a proportion of the cells, making the symptoms milder (Siskind et al., 2011). EDx findings are typically in the intermediate range between those seen in patients with demyelinating CMT and axonal CMT. NCV is typically 30–40 m/s in men, while the range is typically 30–50 m/s in women. CMAPs and SNAPs have reduced amplitudes in both genders (Senderek et al., 2003).

Central nervous system manifestations range in severity from subclinical constant findings to severe episodic syndromes. Some patients only show abnormalities in evoked potentials, like prolongation of visual evoked responses (VERs) and brain stem auditory evoked responses (BAERs) (Murru et al., 2006; Nicholson et al., 1996). Some patients have mild, non-progressive abnormalities. For example, spasticity, hyperreflexia and extensor plantar responses, dysarthria, and ataxia have been reported (Abrams & Freidin, 2015). Finally, a proportion of CMTX1 patients may present acutely with transient limb weakness, encephalopathy, ataxia, dysarthria, dysphagia, disorientation, or stroke-like episodes (Abrams & Freidin, 2015), often “triggered” by travelling back from high altitudes, intense physical activity or hyperventilation, fever, or minor acute infections (Kleopa et al., 2012).

2.4 Diagnosing CMT

The heterogeneity, both clinically and genetically, and the overlapping of CMT and other inherited complex or non-complex neuropathies compose a challenge when it comes to the clinical and genetic diagnosis of CMT. Moreover, some other
inherited or acquired conditions above neuropathies may present with symmetric distal muscle weaknesses, mimicking neuropathy (Liewluck & Saperstein, 2015; Savarese et al., 2020). Nevertheless, every effort should be made to find a genetic cause if the patient so wishes. Determining the genetic cause of CMT can aid in discussions of prognosis and provide genetic counselling, including family planning issues and, in the future, ensure accurate disease modifying treatment (Stavrou et al., 2021).

2.4.1 Differential diagnosis

When a patient complains of symptoms such as difficulties in walking, the list of conditions that may be found underneath is rather long. It starts from spinal stenosis and normal pressure hydrocephalus to amyotrophic lateralis sclerosis and distal myopathies. Moreover, distal spinal muscular atrophies and dHMN may even have the same genetic background as CMT, and a proposition that they are in fact same entity has been made (Bansagi et al., 2017). As soon as the EDx examination has confirmed the diagnosis of polyneuropathy, the differential diagnostic field diminishes. Laboratory tests are required to rule out possible underlying systemic causes for polyneuropathy. The most common cause for polyneuropathy is diabetes, accounting for 32–53% of the cases (Callaghan et al., 2015). However, too often we misdiagnose CMT as primary diabetic neuropathy (Klein, 2020). Unlike CMT, diabetic neuropathy is predominantly sensory and significant weakness is rare and, furthermore, diabetic neuropathy is typically accompanied by retinopathy or nephropathy (Faselis et al., 2019; Rard Said, 2013). Alcohol and other toxins, B12 deficiency, chronic kidney disease, chemotherapy, and paraproteinemia are other frequent causes of polyneuropathy. Moreover, the list of other, more rare causes of polyneuropathy is rather long as well, including various infectious, inflammatory, vascular, autoimmune, metabolic, nutritional, neoplastic, paraneoplastic, and iatrogenic causes (Callaghan et al., 2015).

2.4.2 Symptoms and clinical findings

Individuals with CMT manifest with slowly progressive, symmetric distal sensory and motor polyneuropathy of the arms and legs resulting in weakness and muscle atrophy in the feet and/or hands. The affected individual typically has weak ankle dorsiflexion, decreased or absent tendon reflexes, and pes cavus is common (Pareyson et al., 2006). Muscle weakness is often associated with mild to moderate
distal-predominant sensory loss. It can most easily be demonstrated by a diminished vibration sense but can also include decreased sensation of pain/pinprick, temperature, and joint position (Pareyson & Marchesi, 2009). The latter can cause sensory ataxia. Positive sensory symptoms such as paresthesia are rare, but pain can occur.

Hints in the medical history that plead for genetic cause over the acquired cause of polyneuropathy are long and slow progression, history of frequent ankle sprains, repetitive foot fractures during childhood, and difficulty on walking on heels and toes (Klein, 2020).

The above-mentioned symptoms and signs are typical for CMT, but the clinical diagnosis of CMT nearly always requires EDx studies to confirm the neuronal damage, provide classification, and to exclude other causes of the symptoms. Close relatives of a known CMT mutation carrier make an exception to this. It is cost-effective to determine if symptomatic relatives carry the same family variant.

### 2.4.3 Family history

A patient with possible CMT should be asked for a three-generation family history. Attention should be focused on other relatives with any neurologic symptoms. However, evaluation of the relatives based on report of the proband can be quite challenging, as many patients do not know the diseases or symptoms of their relatives. Patients with CMT may have a negative family history for many reasons, including minor symptoms or even subclinical disease in relatives, de novo pathogenic variant of the proband, or low penetrance. Moreover, in cases with autosomal recessive or X-linked inheritance, the small family size may result in seemingly sporadic disease (Rudnik-Schöneborn et al., 2016).

The reported findings on the relatives may be corroborated by either direct examination or by reviewing their medical records, including the results of molecular genetic testing and EDx studies. The latter is more practical in many cases. However, if the reported family history is negative, clinical examination of family members may be helpful. In an asymptomatic individual, the signs of a possible CMT include pes cavus, hammer toes, an inverted champagne bottle appearance of the legs and no sensory symptoms but still sensory signs (Reilly, 2007). If family history remains negative, patient is classified as “sporadic” until a genetic diagnosis is established.
2.4.4 Electrodiagnostic testing

The originally defined range for median MNCV is $<38$ m/s in the demyelinating (CMT1) and $>38$ m/s in the axonal (CMT2) forms (Harding & Thomas, 1980). Usually, NCV in CMT2 is normal or only mildly reduced in upper limbs, and CMAPs are reduced, at least in lower extremities (M. A. Saporta & Shy, 2013). If CMAPs and sensory nerve action potentials are absent distally, it would be recommended to perform NCV studies on proximal nerves to investigate the possibility of a severe demyelinating rather than an axonal pathology (Pisciotta & Shy, 2018). The term “intermediate” was introduced later in order to designate patients with dominantly inherited CMT who did not fit into either CMT1 or CMT2 (Davis et al., 1978). MCVs in the intermediate form are between 25 m/s and 45 m/s (Davis et al., 1978; Pareyson & Marchesi, 2009) or between 35 m/s and 45 m/s (M. A. Saporta & Shy, 2013) depending on the author.

The typical EDx features in demyelinating forms of CMT include a uniform slowing of conduction and lack of CMAP dispersion and conduction blocks (Tankisi et al., 2020). The uniformity of nerve conduction means that the slowing of NCVs show minimal alteration from one nerve to another and that the differences between the left and right limbs are minimal. Instead, acquired demyelinating neuropathies, such as acute or chronic demyelinating inflammatory polyradiculoneuropathy, are often asymmetric and non-uniform. Asymmetry is often seen both clinically and in EDx. Indeed, in some cases, the differential diagnosis may be difficult, and characteristics of inflammatory neuropathies may be seen in demyelinating hereditary neuropathies (Stanton et al., 2006).

2.4.5 Molecular genetic testing strategies

While the subtypes of CMT do have distinguishing phenotypic features, the majority of patients with CMT present with rather typical manifestations such as foot deformities, length dependent muscle weakness, atrophy, and sensory loss. Indeed, establishing a precise diagnosis and choosing the best candidate gene at the bedside may be challenging. Different algorithms and testing strategies for different types of CMT have been proposed (Murphy et al., 2012; A. S. Saporta et al., 2011), but next-generation sequencing (NGS) is replacing the gene-by-gene Sanger sequencing (Fridman et al., 2015).

However, CMT testing is still guided by mode of inheritance, clinical findings, and electrophysiology. At least three issues should be considered before applying
NGS for a CMT patient. First, AD-inherited or sporadic demyelinating CMT should first be tested for PMP22 duplication, usually using multiple ligation-dependent probe amplification (MLPA) (Rossor et al., 2015a; A. S. Saporta et al., 2011). This is mainly because of the high frequency of the rearrangement, and partly because the structural variants are not easily and cost effectively detectable with NGS methods (Gilissen et al., 2014; Jo et al., 2016). Secondly, ethnic background should be taken under consideration as founder mutations with or without high rates of consanguinity may alter the diagnostic approach in certain bottlenecked populations (Pipis et al., 2019). Thirdly, a decision of what kind of NGS technology to apply must be made.

NGS is used as a collective term to describe the different technologies that enable the simultaneous sequencing of large amounts of DNA. The whole genome sequencing (WGS), the whole exome sequencing (WES) covering protein-coding sequences, and sequencing disease-specific genes are the options (Rossor et al., 2013). The NGS panel for CMT may contain known or presumed CMT-associated genes and, in recent years, diagnostic laboratories have been moving towards the use of a single unified CMT panel containing various numbers of genes associated with any kind of CMT, or even expand it to include genes involved in all kinds of inherited neuropathies or all inherited neuromuscular disorders (Bacquet et al., 2018; Cortese, Wilcox, et al., 2020; Klein, 2020; Pipis et al., 2019). The latter consist also of genes that cause other inherited neuromuscular disorders like distal myopathies or motor neuron disease that may mimic CMT.

Up to 46% of CMT patients without a previous genetic diagnosis may receive one with WES (Gonzaga-Jauregui et al., 2015). Especially in syndromic cases advancing to WES can be considered (Klein, 2020). However, as WES is not able to cover 100% of the protein-coding regions, but rather only up to 96%, it continues to give false-negative results, which have limited its widespread use as a first-tier test in clinical settings (Pipis et al., 2019). Instead, WGS enabling near complete coverage of the protein-coding genome, depending on the reading depth, could serve as the single needed genetic test for CMT and other inherited syndromes in the future (Minoche et al., 2019). Furthermore, because WGS covers almost the complete genome, it is able to detect structural variations also in non-coding regions, such as intronic or intergenic areas, which is not possible with other methods (Drew et al., 2016; Gilissen et al., 2014).

In addition, a challenge of the genetic diagnosis in the era of NGS is to determine if the identified genetic variants are pathogenic and responsible for the disease phenotype. The number of detected variants may be enormous in all
technologies of NGS, but especially in WES and WGS. This problem involves the
diagnosis of any inherited condition, but the lack of easy functional assays makes it even more difficult regarding CMT (Pipis et al., 2019).

2.4.6 Nerve biopsy and skin biopsy

The pathology of different CMT subtypes detected by sural nerve biopsy has helped to recognize the functional consequences of several molecular defects on Schwann cells, myelin, and axons. In demyelinating forms of CMT, histology reveals segmental demyelination and remyelination with complex onion bulb formations. With time, the demyelination/remyelinating process generates axon loss (Duchesne et al., 2018). In axonal forms of CMT, there are signs of axonal degeneration with clusters of regenerating axons. In addition, many pathogenic variants lead to distinct lesion patterns that can be determined in nerve biopsies. The loss of certain types of nerve populations can be the distinguishing feature, as well as many distinct structural changes of axons, Schwann cells, and other peripheral nerve components (Weis et al., 2017).

Current CMT diagnosis is based on clinical and electrophysiological studies and confirmed by genetic tests. In challenging cases, nerve biopsy may still be used for differentiating demyelinating CMT from chronic immune demyelinating polyradiculoneuropathy (CIDP) or other acquired neuropathies (Cavallaro et al., 2021). CIDP causes multifocal onion bulbs and interstitial inflammation, whereas CMT1 appears as diffuse onion bulbs without inflammation (Tracy et al., 2019). In addition, sometimes histology may confirm the pathogenic role of DNA variants (Cavallaro et al., 2021).

The less invasive and longitudinally repeatable skin biopsy has appeared as a useful tool for basic and clinical research that may demonstrate molecular pathophysiological mechanisms of the underlying genetic defects and provide useful biomarkers of disease progression (Nolano et al., 2020).

2.5 Management

Treatment of CMT is still symptomatic. As the clinical symptoms and the severity of the disease vary between patients, individualized management is required. Motor symptoms, muscle weakness, and foot deformities are usually present and progressive and need attention. Muscular imbalance between the weaker anterolateral and stronger posteromedial muscles and weakness of intrinsic foot
muscles are thought to be the main causes of pes cavus in CMT (Holmes & Hansen, 1993).

Conservative measures of CMT patients include orthosis, in case of deficit in the foot muscles, and physiotherapy. Aspects of physical therapy and rehabilitation may involve therapeutic exercise and gait training, stretching, balance and postural stabilization, and education to reduce the risk of falling (McCorquodale et al., 2016). In addition, drug therapy for neuropathic pain may be needed.

Orthopedic surgery is sometimes required to correct severe foot deformities if conservative measures have proven unsuccessful. Approximately 30% of the patients need surgery (Laurá et al., 2018). The three main types of foot operation include soft tissue corrections, osteotomies, and fusions (Rossor et al., 2015b).

Genetic counseling of patients and families is highly recommended. Knowing the exact genetic diagnosis and inheritance pattern allows for better prediction of prognosis and accurate risk assessment for the patient and family members. Especially for women in reproductive age, knowledge of the genetic etiology of her disease may influence her reproductive decisions and issues such as pre-implantation and prenatal testing should be discussed when appropriate (Siskind et al., 2013). Likewise, family members with or without symptoms may be concerned and interested in genetic testing.

The discovery of diverse molecular mechanisms of CMT has provided the basis for developing a wide range of therapeutics for this incurable group of disorders. Exact genotyping of patients with CMT allows gene/pathway-specific clinical trials and, in the future, individualization of therapy according to the underlying genetic etiology (Juneja et al., 2019).
3 Aims of the study

Only a few epidemiological studies of CMT have been carried out in the era of modern molecular medicine. At the time this work commenced, there were only a few (Barreto et al., 2016). In recent years, figures from other Nordic countries have emerged, but no studies have been published on population-based Finnish cohorts. The general aim of this study was to evaluate the molecular etiology and the prevalence of CMT in Northern Ostrobothnia, and to examine the clinical phenotype of patients with two interesting pathogenic variants, one in GDAP1 and one in MPZ.

The three main aims of this study were:

1. To estimate the prevalence of CMT and the frequencies of pathogenic variants in GDAP1, GJB1, MFN2, MPZ, PMP22, MT-ATP6, and MT-ATP8 in a population-based cohort in Northern Ostrobothnia.
2. To evaluate the clinical phenotype of the heterozygous pathogenic variant p.His123Arg in GDAP1.
3. To describe a novel pathogenic variant p.Arg106Cys in MPZ and to evaluate its clinical phenotype.
4 Patients, materials, and methods

4.1 Patients

The study was conducted in Northern Ostrobothnia, a province in northern Finland. The population of the province on 31 December 2009 was 392,110, of whom 309,125 persons were at least 16 years old (men, 154,757; women, 154,368). Oulu University Hospital (OUH) is the only hospital providing neurological services in the province. Nevertheless, it is plausible that some individuals may have sought medical advice at private practitioners. In 2009, there were 8244 visits at the neurology outpatient clinic at OUH. At the same time, the Social Insurance Institution of Finland had granted compensation for 966 persons because of visiting a private neurologist.

In order to identify possible CMT patients, a search of the outpatient registry and the discharge registry at OUH was carried out, including the ICD10 diagnoses G60.0-G60.9, G62.8, G62.9, and G64. Patients that had visited the hospital between 1 November 1997 and 31 October 2009 were selected. A similar previous study covering the period from 1967 to 1 November 1997 (Remes et al., 2003) had been carried out, and the medical records of those patients were also reviewed. It is generally acknowledged that the accuracy of Finnish healthcare registries is good (Pajunen et al., 2005; Sund, 2012).

A search of the registries identified 730 patients with ICD10 codes referring to neuropathy, and 514 of them were at least 16 years old and resided in Northern Ostrobothnia on 31 December 2009 (figure 1). A review of the medical records confirmed 379 subjects with chronic polyneuropathy. The medical records of these cases were then scrutinized in order to exclude other causes of peripheral neuropathy such as endocrine, metabolic, toxic, paraneoplastic, and connective tissue disorders (England et al., 2005). The remaining cases included 275 patients with polyneuropathy from secondary causes or pure motor neuropathy or predominantly sensory and autonomic neuropathy or polyneuropathy as a part of neurological syndrome or multisystem disorder, 86 patients with probable CMT, and 18 patients with HNPP. In addition, four patients without a previous diagnosis were identified in the families of the probands and 17 patients from a previous study (Remes et al., 2003) were included. Altogether, there were 107 patients with probable CMT.
Twenty-eight subjects with heterozygous p.His123Arg in GDAP1 were identified in the above-mentioned population-based survey and were invited to the clinical study (II). Twenty subjects consented and three relatives were also included.

A novel pathogenic variant in MPZ (III) was studied in three CMT patients from a Finnish family and one CMT patient from a German family.

Samples from 95 healthy blood donors from the Finnish Red Cross were included as controls (III).

Fig. 2. Flowchart of the case selection. The chart indicates the exclusions and the selection of study population. Abbreviations: CMT, Charcot-Marie-Tooth; HNPP, hereditary neuropathy with liability to pressure palsies. Reproduced with permission from Karger.
4.2 Ethical aspects (I-III)

The research plan has been approved by The Ethics Committee of the OUH. All patients who gave blood samples or were clinically examined, gave their written informed consent. The patients’ identities were anonymized during the analysis. The patients were aware of the benefits and discomforts of the study and no financial compensation was provided. The patients were given an opportunity to genetic counseling.

4.3 Genetic investigations (I, III)

In the epidemiologic study (I), there were 107 patients with clinical diagnosis of probable CMT, and 16 of them had already received a molecular diagnosis. The remaining 91 patients were requested for blood samples, and 73 patients consented. Furthermore, PMP22 deletion had been previously found in 15 HNPP patients.

The protein coding regions of GDAP1, MPZ, MFN2, GJB1, MT-ATP6, and MT-ATP8 were analyzed by Sanger sequencing (I). MFN2 was also analyzed using conformation sensitive gel electrophoresis before sequencing. PMP22 duplication was analyzed by quantitative multiplex PCR analysis. For a detailed description of the molecular methods, see study I.

In study III the coding region of MPZ was first sequenced from the proband. A fragment covering exon 3 was then amplified from other affected and unaffected subjects of the family, as well as from 95 healthy controls. The amplified fragment was analyzed using conformation sensitive gel electrophoresis, and the presence of the pathogenic variant was verified by sequencing the fragments with altered mobility.

4.4 Clinical evaluation (II, III)

Clinical evaluation and electrodiagnostic (EDx) testing were performed for each of the 23 patients with p.His123Arg in GDAP1 (II). The Neuropathy Symptom Score (NSS) and the Neuropathy Disability Score (NDS) (Dyck, 1988; Dyck et al., 1980) were estimated as a part of the clinical examination. The symptoms of neuropathy are scored present or absent in the NSS. The maximum score is 17, and scores ≥1 were considered abnormal. The NDS is a detailed neurologic examination of deficits. The scale is 0–280, and scores ≥6 were considered abnormal. Family history study including first-degree relatives was accomplished. Modified Rankin
scale (mRS) (Rankin, 1957; van Swieten et al., 1988) and Staging Severity of Neuropathy (Rankin, 1957) were used to stage the clinical severity of the disease, but quantitative sensory and autonomic examinations were not used (Karppä et al., 2003). Two or more abnormalities among EDx examination, NDS, or NSS were required to establish a diagnosis of polyneuropathy. If the minimal criteria for polyneuropathy were not fulfilled, the patient was classified as having no neuropathy. A classification of asymptomatic neuropathy was conducted when there were at least 2 abnormalities in EDx testing or NDS, but the NSS was 0, and symptomatic neuropathy if symptoms were also present (NSS ≥1). Patient was classified as having a disabling neuropathy when he was unable to walk without assistance (Dyck, 1988).

Pedigrees of the patients with the pathogenic variant p.His123Arg in GDAP1 were established. They included 17 complete sibships and ancestors born in the 19th century. Population registry data were used to compose the pedigrees.

For the patients in MPZ study (III), a complete neurological examination was carried out by neurologist, special effort was put in testing of motor and sensory modes of the upper and lower extremities. Clinical history, clinical findings, and electrodiagnostic examination were considered when making the diagnosis of polyneuropathy. The CMT neuropathy score (CMTNS) (Shy, Blake, et al., 2005) was determined for affected members of family A. The severity of the disease was considered severe if CMTNS ≥ 21, moderate if CMTNS = 11–20 and mild if CMTNS ≤ 10.

### 4.5 Classification and electrodiagnostic testing

#### 4.5.1 Classification

In the epidemiologic study (I), EDx examination had been carried out on all patients as a part of the diagnostic workup. Patients with upper limb motor NCV <38 m/s were considered to have demyelinating neuropathy and those patients with upper limb motor NCV >45 m/s were considered to have axonal neuropathy. If the upper limb motor NCV was in the range of 35–45 m/s, patient was considered to have intermediate CMT (M. A. Saporta & Shy, 2013). Motor NCVs of the lower extremities were used for classification if the upper limb motor NCV had not been recorded in the patient chart. In cases where the exact NCVs were not available,
the definition of polyneuropathy was based on the written description by the clinical neurophysiologist. The remaining patients were unclassified.

If a patient had a history of transient palsies or sensory loss, and NCV slowing in typical sites of entrapment, they were considered to have HNPP.

### 4.5.2 Electrodiagnostic testing

Standardized sensory, motor, and compound nerve conduction studies (NCSs) with fixed electrode distances were carried out on 23 patients (II) using a Dantec Keypoint Focus workstation (Alpine Biomed ApS, Skovlunde, Denmark). Placements of cursors for the parameters and settings for measurements were checked manually. For lower extremities, skin temperature was kept above 28°C and for upper extremities it was kept above 30°C (Stalberg et al., 2019).

Bilateral medial plantar and sural sensory NCSs were performed with 14 cm fixed distance for medial plantar and 10 cm for sural sensory conduction velocity (SCV). Right ulnar and median nerve antidromic SCV was measured from the index and little fingers, and compound NCSs were measured by palmar stimulation with 8 cm fixed distances for compound conduction velocities (CCVs) in both nerves. Also, antibrachial median nerve CCV was measured by stimulating the nerve at the wrist and recording at the antecubital fossa. Bilateral tibial and peroneal motor NCSs were performed with measurements of F waves and H reflexes for the tibial nerve.

Values were adjusted for height, age, and temperature with multiple regression, and the values were compared with our reference material. Parameters which exceeded 2.5 SD of the mean were considered abnormal. Asymmetry was estimated using compound muscle action potential of peroneal and tibial nerves and sensory nerve action potential of sural nerves. The ratio of the lower amplitude to the higher amplitude was computed, and the reported cutoff values based on 95th percentiles were used to define asymmetry (Bromberg & Jaros, 1998). The cutoff was 0.37 for tibial nerve, 0.16 for peroneal nerve, and 0.39 for sural nerve. The definition of asymmetry included situations when action potentials were found on one side but not on the other side. Difference in muscle strength at the discretion of an experienced neurologist was also used to define clinical asymmetry.

EDx of family A (III) included measurement of sural and radial SCV, and MCV of peroneal and median nerves or the ulnar nerve in A:II-7. The proband (A: III-1) underwent needle electromyography. EDx of case B: II-1 included the
measurement of median, ulnar, and sural SCV as well as median and peroneal MCV. She also underwent needle electromyography.
5 Results

5.1 Epidemiology of CMT and frequencies of pathogenic variants in Northern Ostrobothnia (I)

Probable CMT was found in 107 subjects in 78 families. They were ascertained to be alive, resident in the area of the study, and at least 16 years of age on the prevalence day. Polyneuropathy was supposedly sporadic in 28 patients. A classification through EDx examination was possible in 99 (93%) patients, while eight patients were regarded as unclassified because of incomplete neurophysiological data. Based on EDx studies, there were there were 19 patients with CMT1, 66 patients with CMT2, and 14 patients with ICMT among these 99 individuals. In the adult population of Northern Ostrobothnia, the prevalence of CMT was calculated to be 34.6/100,000 (95% confidence interval 28.4–41.8). Furthermore, there were 18 subjects in 17 families with a clinical diagnosis of HNPP.

5.1.1 Genetic analysis

Molecular diagnosis was reached in 56% of the patients, as DNA was available from 89 patients and 50 of them were found to have a pathogenic DNA variant (Table 1). The most common molecular etiology was the heterozygous pathogenic variant p.His123Arg in GDAP1. It was found in 31.5% of the patients. The PMP22 duplication was the second most common, being found in 16.9% of the patients. Six patients (6.7%) carried a point mutation in MPZ, MFN2, or GJB1. The biallelic repeat expansion in RFC1 was found in one CMT patient. Pathogenic variants were not found in the MT-ATP6 or MT-ATP8 genes. Eighteen patients had a clinical diagnosis of HNPP. DNA was available from 15 of them, and they all harbored the PMP22 deletion. EDx testing had been performed in sufficient scope in 99 patients, and DNA was available from 82 of them (Table 2). The duplication of PMP22 was found in all the genetically defined CMTI patients, and the p.His123Arg in GDAP1 was found in 90% of the genetically defined CMT2 patients. In addition, the biallelic repeat expansion AAGGG in RFC1 was found in three patients with pure sensory axonal polyneuropathy, but without cerebellar symptoms (Lipponen et al., 2021). These three patients were ascertained among the 275 patients that had other or secondary causes of polyneuropathy (Fig 1).
Table 2. Heterozygous point mutations and DNA rearrangements in patients with CMT or HNPP in Northern Ostrobothnia.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Amino acid change</th>
<th>Phenotype</th>
<th>Patients, n</th>
<th>Families, n</th>
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<td>GDAP1</td>
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<td>p.His123Arg</td>
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<td>p.Cys217Phe</td>
<td>CMT2</td>
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<td>CMT1</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>RFC1</td>
<td>Repeat expansion</td>
<td>NA</td>
<td>CMT2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

NA, not applicable. Reproduced and updated with permission from Karger.

Table 3. Number of patients with pathogenic variants in CMT genes.

<table>
<thead>
<tr>
<th>Classification</th>
<th>No alterations</th>
<th>PMP22</th>
<th>MFN2</th>
<th>GDAP1</th>
<th>GJB1</th>
<th>MPZ</th>
<th>RFC1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT1</td>
<td>3</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>CMT2</td>
<td>26</td>
<td>0</td>
<td>2</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>56</td>
</tr>
<tr>
<td>ICMT</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>14</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>82</td>
</tr>
</tbody>
</table>

Result from EDx examination enabled CMT classification in 99 patients. DNA was available from 82 patients. Reproduced and updated with permission from Karger.

5.2 Patients with p.His123Arg in GDAP1 (II)

5.2.1 Patient demography

Thirty-one patients with the pathogenic variant p.His123Arg in *GDAP1* were detected, giving a frequency of 10/100,000 among the adult population of the province of Northern Ostrobothnia. Clinical evaluation and EDx examination were conducted on 23 patients from 8 families (1–7 patients per family). Detailed values of EDx examination are shown in the supplementary table of study II. The EDx criteria of polyneuropathy were fulfilled in all the patients, and autosomal dominant inheritance was recognized in each family. Evaluation of factors contributing to polyneuropathy revealed a previous diagnosis of diabetes mellitus in one patient. The median age of onset of the disease was 17 years (range, 5–73 years), and the diagnosis was made at median age of 40 years (range, 6–79 years). The duration of polyneuropathy was 24 years (median), (range, 1–75 years).
Table 4. Demography of Patients With p.His123Arg in GDAP1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severity of polyneuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic</td>
</tr>
<tr>
<td>Patients (men/women)</td>
<td>6/10</td>
</tr>
<tr>
<td>Duration of the disease (years)</td>
<td>17 (7–39)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 (22–61)</td>
</tr>
<tr>
<td>mRS</td>
<td>1.0 (1.0–2.0)</td>
</tr>
<tr>
<td>NDS</td>
<td>62 (33–85)</td>
</tr>
<tr>
<td>NSS</td>
<td>4.5 (1.3–6.8)</td>
</tr>
</tbody>
</table>

Abbreviations: mRS, modified Rankin Scale; NDS, Neuropathy Disability Score; NSS, Neuropathy Symptom Score. The severity of the disease was defined according to the Staging Severity of Neuropathy classification. Values are medians (interquartile ranges ranges). Reproduced from study II.

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5.2.2 Clinical features

Neuropathy was asymptomatic in 1 patient according to the Staging Severity of Neuropathy classification, whereas 16 patients had symptomatic neuropathy, and in 6 patients, neuropathy was classified as disabling (Table 3). Motor weakness in the lower limbs had been the first symptom in 18 patients, whereas 5 patients had had sensory symptoms at the onset. Pes cavus and muscle atrophy in the lower legs were common signs, as 15 patients had them both, 3 had pes cavus, and 3 had muscle atrophy.

Abnormal gait was observed in 14 patients, and it was most commonly caused by extensive muscle weakness in the legs, or foot drop (9 patients, 39%). The 6 patients who had disabling neuropathy used a walking aid. Muscle cramps were reported by 14 patients. Vocal cord palsy was not detected in any of the patients. All 23 patients had muscle weakness and decreased ankle tendon reflexes (Figure 2.). Sensory symptoms, such as prickling or sensory loss, were reported by 19 patients. The mean NSS was 4.8 (range, 0–10).

The mean NDS was 73.3 (range, 10–135), and the mean mRS score was 2.1 (range, 0–4). NDS as a dependent variable in multiple regression analysis showed that age of onset as well as current age were predictors. Sex was not a predictor. The correlation between the age at examination and NDS was weak ($R^2 = 0.268$) but alludes an increase of 8.5 points per decade. The mean mRS score was 1.5 among the 12 patients younger than the median age of 54 years, whereas the score was 2.8 among patients older than 54 years.
Fig. 3. Frequency (%) of clinical findings in patients with p.Arg123His in GDAP. Based on data shown in Table 2 (II). The table is licensed under Creative Commons BY-NC-ND 4.0 license <https://creativecommons.org/licenses/by-nc-nd/4.0/>

5.2.3 EDx testing

All patients had sensory and motor axonal polyneuropathy in EDx examination (supplementary table of study II). Fourteen patients had asymmetrical EDx findings, consisting of asymmetrical peroneal motor response or asymmetrical tibial motor response in 13 patients and asymmetrical sural sensory response in 1 patient. The weakness of the lower extremities was asymmetrical in 11 patients (48%). Clinical or EDx asymmetry was detected in 17 patients giving a concordance rate of 61%. H reflex was absent in the lower extremities in 21 patients (93%). Segmental demyelination was not suspected based on the motor responses. EDx testing showed median nerve peripheral neuropathy in median nerve, suggesting subclinical carpal tunnel syndrome (CTS) in 4 patients (17%). Laboratory tests had been carried out to rule out the common causes of CTS.
5.2.4 Pedigrees

The 23 patients identified in the study were from 8 pedigrees. Seventeen complete sibships with 1–4 generations were identifiable in the family histories reported by the patients. The sibships included 87 members, 27 of whom were with polyneuropathy, and 22 of them participated in our clinical study. At the age of 75 years, cumulative incidence of polyneuropathy in the sibships was 48.4%. The percentage is consistent with autosomal dominant inheritance and indicates full penetrance (Figure 1 in study II). Thirty-five ancestors in these 8 pedigrees were traced, and they preceded the identified patient with p.His123Arg in GDAP1 by 2 to 4 generations. The median year of birth of these ancestors was 1855 (range 1837–1882). Surprisingly, 31 of the 35 ancestors had been born in two clusters of municipalities, one on the west coast and the other in the northeast of the province (Figure 2 in study II).

5.3 Patients with p.Cys106Arg in MPZ (III)

5.3.1 Genetic analysis

Sequencing of the coding region of MFN2 revealed no pathogenic variants in the proband of family A, whereas heterozygous c.316C>T was identified in exon 3 of the MPZ gene. This variant leads to an amino acid change from arginine to cysteine (p.Arg106Cys). The heterozygous variant was also detected in the mother and the maternal uncle of the proband. The two unaffected siblings (A:III-2 and A:III-3) and the daughter (A:IV-2) of the proband were homozygous with respect to the wild-type allele. The p.Arg106Cys allele was also found in patient B:II-1. The p.Arg106Cys allele was not found in 190 Finnish control chromosomes.

5.3.2 Clinical findings and EDx testing

The proband (A:III-1) (Fig. 1 in study III) began to have weakness and paresthesia in the lower limbs at the age of 48 years (Table 1. in study III). He suffered myocardial infarct at the age of 54 because of coronary heart disease. He was diagnosed with late-onset predominantly axonal motor and sensory polyneuropathy (Table 2. in study III). The EDx examination showed more demyelination than axon loss in the motor nerves, whereas axon loss was more detectable in the sensory nerves. Blood count, blood glucose, liver enzymes, vitamin B12, thyroid hormones,
and cholesterols were in the normal range. Lumbar spine magnetic resonance imaging (MRI) did not reveal abnormalities. Better ear hearing level was 24 dB, compatible with mild hearing impairment. CMTNS was 5 at 53 years of age suggesting mild neuropathy.

The proband’s mother (A:II-4) had her first neuropathy symptoms at the age of 67 years (Table 1. in study III), and at 74 years of age, her walking distance was only 50 m. She had thyroid cancer at the age of 55 years, and since then she has had hypothyroidism and thyroxin medication. Because of hypercholesterolemia and coronary heart disease, she had used statins. She was affected with axonal and demyelinating motor and sensory polyneuropathy (Table 2 in study III). Blood count, blood glucose, thyroid hormones, liver enzymes, vitamin B12, folic acid, ACE, nuclear antibodies, protein fraction of serum, and immunofixation of urine were normal, as were abdominal ultrasound and chest X-ray. She suffered from progressive gait difficulty, and lumbar spine MRI revealed spinal stenosis. The patient was operated on at 72 years of age, but the symptoms remained. At 75 years of age, CMTNS was 9.

The proband’s maternal uncle (A:II-7) started to have difficulties in walking at 56 years of age (Table 1. in study III). He also had hypothyroidism, asthma, arthrosis in his knees, and sleep apnea. EDx examination revealed axonal motor more than sensory polyneuropathy (Table 2. in study III). Blood count, blood glucose, prostate-specific antigen, and thyroid hormones were normal. Better ear hearing level was 33 dB. At 61 years of age, CMTNS was 10.

Patient B:II-1 is a member of a German family. Her other ailments included glaucoma, hypercholesterolemia, pseudospondylolisthesis, and pneumatocele in the left lung (Table 1 in study III). Her B12 deficiency has been treated since she was 52 years old. Her mother had had peroneal paresis, but she did not seek medical care for that. Information is not available on the other relatives nor their diseases. She was affected with axonal motor and sensory polyneuropathy. Unfortunately, the exact values of her EDx examination were not obtainable. Her vitamin B12 levels, borrelia serology, electrophoresis and immunofixation of urine and serum, C3, C4, and complement system were normal. The cerebrospinal fluid was normal as were the levels of ANCA antibodies.
6 Discussion

6.1 Epidemiology of CMT in Northern Ostrobothnia

Based on our population-based survey, the prevalence of CMT is 34.6/100,000 in Northern Ostrobothnia. Genetic diagnosis could be made for 56% of the cases. The proportion of axonal CMT was higher than that of demyelinating CMT in this population. One systematic review on the epidemiology of CMT has been made, but it found only 12 studies that met the inclusion criteria and 10 of them reported the CMT prevalence (Barreto et al., 2016). Indeed, studies on the epidemiology of inherited polyneuropathies have been quite a few (Braathen et al., 2010; Foley et al., 2012; Gudmundsson et al., 2010; Nicolaou et al., 2010) and some of them have been conducted before the era of modern molecular diagnostics (MacMillan & Harper, 1994; Skre, 1974). Nonetheless, in recent years the number epidemiologic studies on CMT have been increasing (Lefter et al., 2016; Lousa et al., 2019; Theadom et al., 2019), and data from all Nordic countries are now available. (Müller et al., 2021; Vaeth et al., 2017). Instead, the studies of clinical series, describing the frequency of CMT and its genotypes, are numerous (DiVincenzo et al., 2014; Gentile et al., 2020; Gess et al., 2013; Lorefice et al., 2017; Manganelli et al., 2014; Miller et al., 2011; Milley et al., 2018; Murphy et al., 2012; Silander et al., 1998; Sivera et al., 2013; Uchôa Cavalcanti et al., 2021; Volodarsky et al., 2021).

The prevalence of CMT in Northern Ostrobothnia is two-fold higher than the median prevalence in European populations (see chapter 2.3, Table 1). The highest prevalence has been reported in Akershus County, Norway, being 82.3/100,000 (Braathen et al., 2010b), whereas in northern Norway the estimated prevalence is 29.2/100,000 (Müller et al., 2021). Elsewhere in the Nordic countries the prevalence has been reported to be 20.1/100,000 in Sweden (Holmberg, 1993), 12.0/100,000 in Iceland (Gudmundsson et al., 2010), and 22.5/100,000 in Denmark (Vaeth et al., 2017). These differences are interesting, especially the difference between the two Norwegian regions that show a three-fold difference in the prevalence. Such a difference may suggest an underestimation of the prevalence, as patients with milder symptoms do not seek medical care (Müller et al., 2021). The prevalence in Denmark is considered a minimum estimation for the same reason (Vaeth et al., 2019). Vaeth and colleagues included only patients with a primary CMT diagnosis from the Danish National Patients Registry (DNPR) (Vaeth
et al., 2019). Likewise, Müller and colleagues included only patients with previous CMT diagnoses in specified patient hospital records and other registries (Müller et al., 2021). Instead, Braathen and colleagues identified patients from a search in hospital registries, including diagnosis of CMT as well as other neuropathic disorders, and then established the CMT diagnosis according to determined classification (Braathen et al., 2010a). We performed our study (I) in the same fashion. Some do not report the exact way of case definition (Lousa et al., 2019). So, variable case definition may be one cause for the eight-fold difference between the lowest and highest prevalence. On the other hand, history has shaped the genetic background of certain populations like the Icelanders as a result of geographic isolation and the Finns as a result of geographic and linguistic isolation. The differences may therefore partly result from different genetic backgrounds.

EDx examination is used in the diagnostics of CMT, and the division of CMT to CMT1 and CMT2 is based on upper limb NVCs (Harding & Thomas, 1980; M. A. Saporta & Shy, 2013). Still, retrospective data are inherently incomplete, and all the required data may not be available. Among the 66 patients with CMT2 in our cohort, there were 27 patients with definite axonal polyneuropathy in upper limbs. In addition, there were 31 patients with a definite axonal polyneuropathy in lower limbs, while EDx examination of the upper limbs was normal in 27 cases and had not been performed in four cases. In eight cases, axonal polyneuropathy was mentioned in patient files, but the results of EDx examination were not available.

We found that the pathogenic variant p.His123Arg in GDAP1 was the most common molecular etiology of CMT in our cohort. The frequency of this variant was 31%, while the combined frequency of pathogenic variants in the GJB1, MFN2, and MPZ genes was only 7%. The p.His123Arg variant has previously been found in four families that could be traced to Northern Ostrobothnia and that shared the same disease haplotype suggesting a founder effect (Auranen et al., 2013). In cohorts of CMT patients in clinical settings, the frequency of any pathogenic variant in GDAP1 has varied from 0.7% (DiVincenzo et al., 2014) to 11.1% (Sivera et al., 2013). In our cohort, the patients with p.His123Arg had axonal CMT, except for one. Indeed, the patients with p.His123Arg in GDAP1 comprise 48% of CMT2 in our cohort. Likewise, patients with the PMP22 duplication comprise 17% of all cases and 81% of CMT1 in our cohort. A previous population-based cohort in Norway included PMP22 duplication in 20% of the patients (Braathen et al., 2010b), a proportion that is close to what we found. The frequency of pathogenic variants in MFN2 in our cohort was 2.2%, being lower than that in many other studies. Especially in clinical cohorts, MFN2 mutation rates are higher and
mutations in the *MFN2* have been estimated to be the most common molecular diagnosis in CMT2 patients (Cortese, Wilcox, et al., 2020; Gess et al., 2013; Murphy et al., 2012; A. S. Saporta et al., 2011). However, in a population-based study from Norway, 3.2% of the affected harbored a *MFN2* point mutation (Braathen et al., 2010b). Frequencies of pathogenic variants in *GJB1* and *MPZ* are very low also in other population-based studies (Braathen et al., 2010b; Theadom et al., 2019).

Since the completion of this study, next generation sequencing techniques have taken a larger role in the molecular diagnostics of CMT (Pipis et al., 2019). However, when it comes to the demyelinating forms of CMT, the duplication of *PMP22* should be investigated before conducting NGS (Cortese, Wilcox, et al., 2020). In Finnish patients with axonal CMT, the analysis of p.His123Arg in *GDAP1* before NGS could be justified as well.

### 6.2 Phenotype of p.His123Arg in *GDAP1*

The age of onset in CMT associated with p.His123Arg in *GDAP1* was in young adulthood, but it varied clearly. The proportion of affected subjects increased almost linearly between 5 and 73 years of age. Motor symptoms frequently outlined the onset of the disease, but eventually the patients developed sensory and motor axonal polyneuropathy. Of interest, EDx testing revealed a significant number of asymmetrical findings. Disease progression was relatively slow, as the mean duration of the disease was 41 years among patients with mRS ≥ 3 and 21 years among patients with mRS ≤ 2. We found that the frequency of the pathogenic variants p.His123Arg in *GDAP1* was 10/100,000 in the adult population of Northern Ostrobothnia, and a founder effect was observed.

We found that muscle weakness was predominantly distal, but 17 patients (74%) also had proximal muscle weakness in the lower limbs. Proximal weakness in lower extremities has been reported in 13% of patients with CMT2 (H. M. Bienfait et al., 2007a) which is a markedly lower proportion than that in our patients (p < 0.0002 for difference). Muscle cramps have been described to be a frequent symptom in patients with CMT (Johnson et al., 2015; Lousa et al., 2019). Patients with pathogenic variants in *GDAP1* have also been described with muscle cramps (Garcia-Sobrino et al., 2017). We found that the frequency of muscle cramps in our cohort (14 patients, 61%) was similar to that in the cohort of 225 patients with polyneuropathy (Maxwell et al., 2014). EDx testing revealed a notable number of asymmetrical findings, as 70% of the patients showed asymmetry. Furthermore, in
clinical examination, muscle weakness was asymmetrical in 11 patients (48%) including five patients with asymmetrical sensation as well. The phenotype of CMT2 is considered symmetrical, although 10% of patients with CMT2 have been reported with asymmetrical muscle weakness in the lower limbs (H. M. Bienfait et al., 2007b). Our findings together with previous data (Auranen et al., 2013b) suggest that many patients with p.His123Arg in GDAP1 show asymmetrical features.

NDSs suggested that the neuropathy was slow in progression. Severe disability was rare, as all our patients remained ambulatory. Similarly, previous studies have demonstrated that dominant pathogenic variants in GDAP1 lead to a rather mild and late-onset axonal polyneuropathy (Pakhrin et al., 2018; Pezzini et al., 2016; Sivera et al., 2010, 2017).

The p.His123Arg variant in GDAP1 is the most frequent etiology of CMT in Northern Ostrobothnia with a prevalence of 10/100,000 (I). It is higher than the prevalence of the PMP22 duplication in this population, which is 4.8/100,000. In clinical cohorts of CMT patients, the frequency of any GDAP1 mutation has varied from 0.7% (DiVincenzo et al., 2014; Gentile et al., 2020) to 11.1% (Sivera et al., 2013), whereas in our population-based cohort the frequency of p.His123Arg in GDAP1 was 31.5% (I). Such a high frequency may indicate a founder effect. Indeed, we found that the ancestors of each family were from two regions in Northern Ostrobothnia, one of which seemed to be the founder location (Figure 2 in study II). The genealogic relationship between the families in these two regions could not be ascertained, however. Some diseases belonging to the Finnish disease heritage show clustering of cases within the country (Norio et al., 1973). CMT caused by the p.His123Arg in GDAP1 may be a true cluster in Northern Ostrobothnia, but this suggestion needs to be substantiated in a study covering the entire Finnish population. Such clustering of diseases in Finland is partly due to the founder effect and partly to the isolation of the Finnish population. Population genetic studies have demonstrated several geographical clusters in the population of Finland that are related to settlement history (Kerminen et al., 2017). Indeed, Northern Ostrobothnia is one of the clusters.

6.3 The p.Arg106Cys variant in MPZ

We found a family with CMT caused by a novel mutation in the third exon of the MPZ gene. As a result of the c.316C>T mutation, a replacement of the basic amino acid arginine by cysteine occurs at position p.106. The disease was inherited in an
autosomal dominant manner. EDx examination of three family members showed normal or reduced NCVs, and reduced or absent CMAPs and sensory action potentials. These EDx features suggest the diagnosis of predominantly axonal motor and sensory polyneuropathy.

The clinical features of the family members included mild to moderate distal limb weakness, especially peroneal weakness, reduced or absent deep tendon reflexes, and diminished sensation of touch or pain. The positive Romberg sign was found in three out of the four patients. The median age at onset was 53 years (range, 48–67 years). The clinical features, EDx features, and segregation of the phenotype with the genotype indicate that the p.Arg106Cys variant in MPZ is pathogenic and causes late-onset, predominantly axonal, CMT disease.

Patients with pathogenic variants in the MPZ gene fall into three main categories according to the age at onset (Sanmaneechai et al., 2015). The infantile-onset group develop symptoms before the age of five and the NCVs are very slow (Shy et al., 2004). The childhood–adolescence–onset group (Callegari et al., 2019) with symptoms beginning in the first two decades of life have slow NCVs like those found with CMT1A (Callegari et al., 2019; Sanmaneechai et al., 2015). The third group is adult-onset group with the mean age of onset being 40 years (Sanmaneechai et al., 2015; Shy et al., 2004). They have normal or near normal NCVs and reduced CMAP amplitudes in the legs, indicating axonal neuropathy. Indeed, the two families with p.Arg106Cys in MPZ obviously fall into the adult-onset group, as the most common age of onset in our cases was in the 6th decade of life. Interestingly, two of our four patients had hearing impairment. Case A:III1 had tinnitus besides hearing impairment that had been thought to be noise-induced. Case A:II-7 had an undefined deafness in the right ear since he was child, and sensorineural hearing impairment on the left. Pupillary abnormalities were absent in both cases. Pupillary abnormalities are considered a clinical feature of CMT2J in addition to hearing loss (Jonghe et al., 1999). The association of hearing loss with polyneuropathy in two patients in our families suggests that the p.Arg106Cys allele may impart to a syndromic presentation.

There are two cysteins in the extracellular domain of the MPZ protein, at positions 50 and 127. A third cysteine resides in the intracellular domain at position 182 (Sanmaneechai et al., 2015). Prediction of disulfide bonds using Disulffind (Ceroni et al., 2006) indicates a disulfide bond between Cys-50 and Cys-127. Introduction of a novel Cys to position 106 in the extracellular domain of MPZ did not change this prediction, suggesting that the variant does not lead to altered disulfide bonding. However, MPZ variants that add a cysteine residue into the
extracellular loop of MPZ are likely to be deleterious while disrupting disulfide bridging and hence likely to cause early-onset neuropathy (Sanmaneechai et al., 2015; Shapiro & Doyle, 1996). For example, variants p.Tyr82Cys, p.Arg98Cys, and p.Gly123Cys result in infantile polyneuropathy (Rouger et al., 1996; Shy et al., 2004; Silander et al., 1998). In comparison, p.Arg98His and p.Gly123Ser result in a less severe form of late-onset CMT (Y. C. Lee et al., 2008; Rouger et al., 1996; Souayah & Chong, 2010). However, p.Tyr119Cys causes late-onset disease, even though it introduces a cysteine residue, as it allows myelination to develop (Sanmaneechai et al., 2015).

Arginine at position 106 participates in head-to-head interface, and the residue is essential for cis and trans adhesion between side-by-side myelin wraps (Callegari et al., 2019). These data suggest that p.Arg106Cys is a novel pathogenic variant in MPZ. We found the p.Arg106Cys variant in affected members in two families, and in one family the allele segregated completely with CMT and showed autosomal dominant inheritance.
7 Conclusions

I The estimated prevalence of CMT is 34.6/100,000 in Northern Ostrobothnia. The epidemiology of CMT in a population-based sample was studied for the first time in Finland. The pathogenic founder variant p.His123Arg in GDAP is the most frequent cause of CMT in Finland and was found in 31% of the cases. PMP22 duplication was found in 17%, pathogenic variants of MPZ in 3.3%, MFN2 in 2.2%, and GJB1 in 1.1% of the cases. AAGGG repeat expansion in RFC1 was found in 1.1% of the cases, whereas pathogenic variants in MT-ATP6 or MT-ATP8 were not detected.

II We presented comprehensive clinical and electrophysiological data on 23 patients with p.His123Arg in GDAP1. It seems that proximal muscle weakness in the legs together with asymmetry could be the defining features in these patients. This pathogenic variant may be the most frequent variant in patients with CMT in Finland and, indeed, we identified a cluster of this variant within the country. This variant should be tested first in CMT2 patients, at least in Northern Ostrobothnia.

III We found the novel p.Arg106Cys variant in MPZ in four affected members in two families. The allele segregated completely with neuropathy in one of the families and was present in two generations. The variant was not found in non-affected family members or in 190 Finnish control chromosomes. Our findings indicate that the c.316C>T (p.Arg106Cys) in MPZ is a cause of dominantly inherited, late-onset and rather mild, predominantly axonal CMT.
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Original publications


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Original publications are not included in the electronic version of the dissertation.

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1657. Salin, Janne (2022) Luokkahuoneen sisälman mikrobien ja polyn toksisuudet lisäävät opettajien työympäristön liittyvien oireiden riskiä: poikkileikkaustutkimus

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