Neurobiology of Disease 130 (2019) 104505

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Charcot-Marie-Tooth 2F (Hsp27 mutations): A review

Nicholas U. Schwartz

Health Science Center, L-15, 023, Stony Brook University Medical Center, Stony Brook, NY 11794-8430, United States of America

ARTICLE INFO

Keywords:
Charcot-Marie-Tooth disease
Charcot-Marie-Tooth 2F
CMT2F
CMT2F (Hsp27 mutations)
Distal hereditary motor neuropathy II
DHMN II
Neuropathy
Hsp27
HspB1

ABSTRACT

Charcot-Marie-Tooth disease is a commonly inherited form of neuropathy. Although named over 100 years ago, identification of subtypes of Charcot-Marie-Tooth has rapidly expanded in the preceding decades with the advancement of genetic sequencing, including type 2F (CMT2F), due to mutations in heat shock protein 27 (Hsp27). However, despite CMT being one of the most common inherited neurological diseases, definitive mechanistic models of pathology and effective treatments for CMT2F are lacking. This review extensively profiles the published literature on CMT2F and distal hereditary motor neuropathy II (dHMN II), a similar neuropathy with exclusively motor symptoms that is also due to mutations in Hsp27. This includes a review of case reports and sequencing studies detailing disease course. Included are tables listing of all known published mutations of Hsp27 that cause symptoms of CMT2F and dHMN II. Furthermore, pathological mechanisms are assessed. While many groups have established pathologies relating to defective chaperone function, cellular neurofilament and microtubule structure and function, and mitochondrial and metabolic dysfunction, there are still discrepancies in results between different model systems. Moreover, initial mouse models have also produced promising results with similar phenotypes to humans, however discrepancies still exist. Both patient-focused and scientific studies have demonstrated variability in phenotypes even considering specific mutations. Given the clinical heterogeneity in presentation, CMT2F and dHMN II likely result from similar pathological mechanisms of the same general disease process that may present distinctly due to other genetic and environment influences. Determining how these influences exert their effects to produce pathology contributing to the disease phenotype will be a major future challenge ahead in the field.

1. Introduction

The other sesquipedalian name of Hereditary Motor and Sensory Neuropathy, Charcot-Marie-Tooth (CMT) Disease, owes substantially to its coincidentally concurrent discovery. While earlier descriptions had been reported by other clinicians, Professor Jean Martin Charcot, his student Pierre Marie, and medical student Howard Henry Tooth, are credited for simultaneously identifying the disease in 1886. Tooth, in fact, correctly surmised was CMT was a neuropathy (Charcot and Marie, 1886; Pearce, 2000; Tooth, 1886). In 1957, Gilliatt and Thomas reported reduced nerve conduction velocity (NCV) occurred in peripheral nerve pathologies (Gilliatt and Thomas, 1957). Then, in a pair of papers in *JAMA* in 1968, Peter James Dyck and Edward Lambert described two distinct clinical presentations of CMT. Each featured symptoms of neuropathy such as gait disturbance, weakness of muscles at the extremities, and physical abnormalities of the extremities such as *pes cavus*. However, upon careful inspection, a clear subset of cases featured marked decreases in nerve conduction velocities (NCVs) of peripheral nerves, while conduction velocities in the other subset were only at most mildly impaired. Symptoms were also reported to occur later in this second form of CMT (Dyck and Lambert, 1968). This division later became the basis for the categorization of CMT1 and CMT2, the former which was generally defined by a NCV below 38 m/s (Harding and Thomas, 1980). Since then, an intermediate division of CMT with NCVs above and below 38 m/s and both demyelination and axonal pathology has been described (Nicholson and Myers, 2006). Many less common subtypes of CMT have been also identified, raising the current total to nine (Ekins et al., 2015).

As genetic sequencing technologies have developed, it became clear that even CMT1 and CMT2 were genetically heterogeneous disorders

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth disease; CMT2F, Charcot-Marie-Tooth type 2F; dHMN, distal hereditary motor neuropathy; DRG, dorsal root ganglia; HAT, histone acetyltransferases; HDAC, histone deacetylase; Hsp27, heat shock protein 27; HspB1, heat shock protein β-1; iPSCs, induced pluripotent stem cells (iPSCs); KO, knockout; MT, microtubule; NCV, nerve conduction velocity; NF, neurofilament; NF-L, neurofilament light; PNS, peripheral nervous system; ROS, reactive oxygen species; sHSP, small heat shock protein; SNAP, sensory nerve action potentials; TSA, trichostatin A; WES, whole exome sequencing; WGS, whole genome sequencing; WT, wild-type

E-mail address: Nicholas.schwartz@stonybrookmedicine.edu.

https://doi.org/10.1016/j.nbd.2019.104505
Received 22 May 2019; Received in revised form 11 June 2019
Available online 15 June 2019

owing to a diverse cast of distinct mutations. The first genetic etiology of CMT was identified in 1991 as a DNA duplication causing CMT1A (Lupski et al., 1991), leading the way to the discovery of many distinct types of CMT. Generally speaking, genes mutated in CMT1 have been shown to be involved in myelin function, and often contain "myelin" or "Schwann" in their names. Genes that are mutated in CMT2, on the other hand, tend to be expressed in neurons, although they often subserve fundamental cellular functions in a variety of intracellular locations and tissue types. Examples include amino-acyl tRNA synthases, heat shock proteins, and enzymes involved in lipid metabolism (Ekins et al., 2015). Determining how the dysregulation of these proteins contributes to a purely peripheral nervous system (PNS) phenotype remains one of the greatest challenges in CMT research. As such, the biological consequences of CMT2 mutations resulting in pathology are not as well understood as CMT1. Many recent reviews have served to update research on CMT (Baets et al., 2014; Ekins et al., 2015; Gutmann and Shy, 2015; Mathis et al., 2015; Pareyson et al., 2017; Rossor et al., 2016; Saporta, 2014), however there is a lack of a comprehensive review of CMT2F. This review will focus specifically on examining, comparing, and analyzing the accumulated knowledge we have developed on CMT2F.

2. CMT Clinical overview

The prevalence of CMT is estimated at 1 in 2,500 (Skre, 1974; Szigeti and Lupski, 2009). CMT typically presents similarly to neuropathy, except that motor weakness is more common as an early symptom of CMT (Asadi, 2019; Callaghan et al., 2015). Decreases in sensation and balance can lead to ulcerations, amputations, and fractures due to falls (Callaghan et al., 2015). Cross-sectional studies have determined a significant relationship between age and severity of symptoms, suggesting a worsening of phenotype with age. However, despite great disability, lifespan is unaffected (MacMillan and Harper, 1994; Skre, 1974). Most types and cases of CMT are inherited in an autosomal dominant manner, although some cases exhibit X-linked or autosomal recessive inheritance. The recessive cases are thought to often present with a more severe disease course and potential mild central nervous system involvement (Skre, 1974). The differential diagnosis includes the most common cause of distal symmetric polyneuropathy, diabetes mellitus, which often initially presents with loss of pain and temperature elicited upon testing. Further history and laboratory testing can rule out other etiologies of neuropathy such as alcohol, Vitamin B12 deficiency, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, and chemotherapy, although the differential is large (Callaghan et al., 2015; England et al., 2009).

The first prospective clinical study of CMT2 in 2003 followed 43 previously identified Dutch CMT2 patients for 5 years. The results supported the traditional clinical picture of CMT. Patients most often incurred moderately increased motor degeneration, incidence of clawed toes, requirement of aids for ambulation, and disability in daily activities. Earlier disease onset correlated with specific physical deformities, while clawed toes increased in incidence over the course of the disease. Interestingly, these data suggested that pain and muscle cramps may present with onset but remit during the course of the disease (Teunissen and Notermans, 2003).

As CMT has became more well known, diagnosis has rapidly changed as genetic testing has continuously become more prevalent and cost-effective. Traditionally, diagnosis was achieved with a thorough medical history and neurological exam. Electrodiagnostic measures could also be performed if suspicion of CMT was high. However, genetic testing is becoming more common as it becomes increasingly cost-effective (Callaghan et al., 2015). Advances in whole-genome sequencing (WGS) and whole-exome sequencing (WES) have exponentially augmented our understanding of the genetic underpinnings of CMT (Choi et al., 2012; Lupski et al., 2010; Timmerman et al., 2014). While increased utilization of genetic testing modalities may lead to further discovery of novel genetic causes of CMT, its usage alone is not always diagnostic. Estimations made as late as 2011 show that only half of CMT patients possess a causative mutation in a known gene (Braathen et al., 2011). Genetic abnormalities were identified in less than 20% of patients in a cohort of 17,880 individuals with peripheral neuropathy in 2014 (DiVincenzo et al., 2014). In another recent study, only around one half of families with a history consistent of CMT were found to possess a CMT-causing mutation using whole-exome sequencing (Gonzaga-Jauregui et al., 2015).

Nevertheless, genetic testing may hold most promise in screening many of the more common types of CMT. Among a set of 14 common genes tested, copy number variations (CNVs) in *PMP22*, comprising CMT1A, accounted for approximately 75% of genetically identifiable caused CMT cases. When combined with data from the genes *GJB1*, *MFN2*, *MPZ*, and *PMP22*, that number jumped to approximately 95% of CMT, suggesting that physicians might be best served beginning clinical assessment by screening this panel of genes (DiVincenzo et al., 2014). Over 80 genes and 1000 specific mutations have been associated with CMT (Timmerman et al., 2014), and many clinical sequencing studies have failed to identify genetic causes for many cases (Ylikallio et al., 2014), necessitating more extensive genetic to identify patients with less common subtypes of CMT2.

3. CMT2F Clinical presentation

3.1. Human genetics

CMT2F was initially discovered in 2001 through linkage analysis in a Russian family as a novel mutation mapping to a region in Chromosome 7 (Ismailov et al., 2001). In 2004, missense mutations in heat shock protein 27 (Hsp27) were identified as the cause of CMT2F (Evgrafov et al., 2004). The vast majority of CMT2F cases are due to point mutations, although a premature stop codon in the C-terminus of Hsp27 has also been reported to present similarly (Rossor et al., 2012a). Clinical evidence points almost exclusively to an autosomal dominant mode of inheritance (Echaniz-Laguna et al., 2017; Szigeti and Lupski, 2009; Tang et al., 2005a). However, very rarely in some families, CMT occurring from Hsp27 mutations (L99M) presents in a autosomal recessive manner (Echaniz-Laguna et al., 2017; Houlden et al., 2008).

Hsp27 is a member of the class of small heat shock proteins, which are capable of binding with partially denatured proteins to prevent aggregation. Many recent reviews have extensively documented functional roles of small heat shock protein (sHsp) function (Bakthisaran et al., 2014; Haslbeck and Vierling, 2015; Mogk and Bukau, 2017). As chaperones, they sequester proteins until ATP-dependent chaperones are available to assist in refolding or targeting the misfolded protein for degradation (Carra et al., 2012; Dierick et al., 2005; Hickey et al., 1986). These proteins also subserve many other functions including modulation of cytoskeletal elements, promotion of cell growth and differentiation, and apoptosis (Dierick et al., 2005).

Hsp27 itself performs many of these functions. In brief, Hsp27 is ubiquitously expressed and able to protect cells, including peripheral neurons, from various cellular stresses and apoptosis (Benn et al., 2002; Wagstaff et al., 1999). Hsp27 is thought to bind to targeted proteins and serve, at least temporarily, to prevent their degradation in an ATP-independent process (Cox and Ecroyd, 2017; Stromer et al., 2003; Wilhelmus et al., 2006; Yerbury et al., 2013). By preventing protein aggregation and regulating proteasomally mediated protein degradation, as well as regulating caspase activity, redox state, proliferation, and cytoskeletal integrity, Hsp27 and other sHsps are important regulators of cell death (Acunzo et al., 2012). Hsp27 has been implicated as a contributing and protective factor in disease states ranging from neurodegenerative disease to cancer to cardiovascular disease (Vidyasagar, 2012).

Hsp27 contains a similar structure to many sHsps with a conserved

Table 1
Known mutations in Hsp27 causing CMT2F.

Amino acid alteration	DNA mutation	Country	Special symptoms	Citation
P39L	116C > T	Italy, Japan		(Capponi et al., 2011; Tanabe et al., 2018)
G84R	250G > C	England		(Houlden et al., 2008)
K123*	367A > T	Japan		(Tanabe et al., 2018)
R127L	380G > T	Finland		(Ylikallio et al., 2015)
R127W	379C > T	China, Italy, France	Late onset, mild sensory, feet paresthesia, cramps.	(Echaniz-Laguna et al., 2017; Solla et al., 2010; Tang et al., 2005a)
S135F	404C > T	Russia, Korea, Armenia,	Relatively early onset, feet paresthesia	(Echaniz-Laguna et al., 2017; Evgrafov et al., 2004; Ismailov et al., 2001; Kim et al., 2014)
S135Y	404C > A	Finland	Very mild sensory phenotype	(Ylikallio et al., 2014)
S135C	404C > G	Italy		(Benedetti et al., 2010)
R136L	407G > T	Italy	Pyramidal signs, deafness	(Capponi et al., 2011; Gaeta et al., 2012; Stancanelli et al., 2015)
T139M	416C > T	United States	Hyperreflexia	(Amornvit et al., 2017)
R140G	418C > G	Japan, India		(Ghodasara et al., 2018; Tanabe et al., 2018)
T151I	452C > T	Japan		(Tanabe et al., 2018)
S158fs*200	476_477delCT	Italy	Potentially trigged in infancy from tetanus vaccination	(Mandich et al., 2010)
T164A	490A > G	Taiwan		(Lin et al., 2011)
M169C*fs2	505delA	Finland		(Ylikallio et al., 2015)
Q175*	523C > T	France, England	Feet paresthesia, early onset in some cases	(Bacquet et al., 2018; Echaniz-Laguna et al., 2017; Rossor et al., 2012a)
P182A	544C > G	Japan		(Tanabe et al., 2018)
R188W	562C > T	Italy		(Capponi et al., 2011)

α-crystallin domain of approximately 90 amino acids with a beta-sheet facilitating dimer formation. N-terminal and C-terminal regions flank both sides of the α-crystallin domain (Sun and Macrae, 2005). Both terminal regions are thought to have independently evolved, suggesting that they may be functionally differentiated from other sHsps (Kriehuber et al., 2010). The α-crystallin domain is thought to be capable of dimer formation while the termini may facilitate the formation of larger oligomers (Bagnéris et al., 2009; Delbecq et al., 2012). Oligomerization seems to largely be regulated by phosphorylation, which occurs at three serine residues in the N-terminus (Kostenko and Moens, 2009). By dynamic control of the rate of oligomerization and disassembly, Hsp27 can control its function as a chaperone (Aquilina et al., 2013; Sun and Macrae, 2005).

3.2. Epidemiology

Clinical studies suggest the prevalence of CMT2F and dHMN II is worldwide. Table 1 lists known Hsp27 mutations of CMT2F reported in the literature to date. CMT2F has been reported in China (Tang et al., 2005a), Russia (Evgrafov et al., 2004; Ismailov et al., 2001), Belgium (Evgrafov et al., 2004), England (Echaniz-Laguna et al., 2017; Houlden et al., 2008), France (Bacquet et al., 2018; Echaniz-Laguna et al., 2017), Finland (Ylikallio et al., 2014; Ylikallio et al., 2015), Italy (Benedetti et al., 2010; Capponi et al., 2011; Gaeta et al., 2012; Mandich et al., 2010; Solla et al., 2010; Stancanelli et al., 2015), the United States (Amornvit et al., 2017), Korea (Kim et al., 2014), Taiwan (Lin et al., 2011), and Armenia (Echaniz-Laguna et al., 2017), India (Ghodasara et al., 2018), and Japan (Tanabe et al., 2018).

Table 2 lists known Hsp27 mutations of dHMN II reported in the literature to date. dHMN occurring due to Hsp27 mutations has been reported in England (Evgrafov et al., 2004), Ireland (Lewis-Smith et al., 2016), Japan (Ikeda et al., 2009; Kijima et al., 2005; Maeda et al., 2014; Nishibayashi et al., 2007; Tanabe et al., 2018), England (Evgrafov et al., 2004; Houlden et al., 2008), France (Bacquet et al., 2018; Dierick et al., 2008; Echaniz-Laguna et al., 2017), Finland (Ylikallio et al., 2015), Germany (Oberstadt et al., 2016), Belgium (Dierick et al., 2008; Evgrafov et al., 2004), Croatia (Dierick et al., 2008; Evgrafov et al., 2004), Austria (Dierick et al., 2008; Evgrafov et al., 2004), Korea (Chung et al., 2008), and Italy (Benedetti et al., 2010; Capponi et al., 2011; Echaniz-Laguna et al., 2017; Luigetti et al., 2010; Mandich et al., 2010; Solla et al., 2010), Portugal (Echaniz-Laguna et al., 2017), India (Houlden et al., 2008), Pakistan (Houlden et al., 2008), Algeria (Echaniz-Laguna et al., 2017), the Ivory Coast (Echaniz-Laguna et al., 2017), and Bangladesh. CMT2F and dHMNII are thought to comprise about 4% to 0.3% and 8% of CMT and dHMN, respectively (Capponi et al., 2011; DiVincenzo et al., 2014; Echaniz-Laguna et al., 2017).

3.3. Pathological and symptomatic presentation

The clinical presentation of CMT2F generally mirrors the loss of axonal function seen in CMT2. The first presenting symptom is often foot drop, initially occurring unilaterally in most patients (Echaniz-Laguna et al., 2017). Many patients often first complain of decreased ambulatory ability and often suffer from falling (Kijima et al., 2005; Tang et al., 2005a). Sometimes the degree of limb involvement may be asymmetrical (James et al., 2008). Nerve biopsy typically reveals axonal atrophy without acute degeneration nor substantial change in myelination (Benedetti et al., 2010). Nevertheless, even though CMT2 has been traditionally classified by axonal degeneration instead of demyelination, some patients exhibit decreased NCVs in the lower limbs (Stancanelli et al., 2015; Tang et al., 2005a). In concordance with denervation, lower limb muscle with fatty replacement wasting occurs most drastically distally (Gaeta et al., 2012). Serum creatine kinase levels are generally moderately elevated, presumably secondarily due to muscle degeneration resulting from neuropathy (Echaniz-Laguna et al., 2017).

Other aspects of clinical presentation are also similar to other types of CMT2. Some evidence suggests males may be affected earlier and more severely than females, although no comprehensive clinical studies have specifically addressed differences in presentation due to gender (Stancanelli et al., 2015). Age at onset appears to generally decrease over succeeding generations, often by as much as 30 years (Benedetti et al., 2010; Chung et al., 2008; Maeda et al., 2014; Tang et al., 2005a). However, this could be due to CMT's relatively insidious onset and greater surveillance from both patients and physicians for individuals with a family history. There is still great variability of onset though – patients have exhibited symptoms in their feet as early as age 6 while as others do not develop symptoms until their late 30s (Oberstadt et al., 2016; Tang et al., 2005a), mid-50s (Houlden et al., 2008) or even their 60s (Capponi et al., 2011). dHMN patients may not present with symptoms until much later in their mid-50s and fail to show changes in NCVs (Oberstadt et al., 2016; Rossor et al., 2012b).

While most identified mutations in CMT2F have been point mutations, a nonsense frameshift mutation at position S158 has been

Table 2
Known mutations in Hsp27 causing dHMN II.

Amino acid alteration	DNA mutation	Country	Special symptoms	Citation
P7S	19C > T	Algeria		(Echaniz-Laguna et al., 2017)
G34R	100G > A	Italy		(Capponi et al., 2011)
P39L	116C > T	England, France		(Echaniz-Laguna et al., 2017; Houlden et al., 2008)
E41K	121G > A	Italy		(Capponi et al., 2011)
G53D	158G > A	Ivory Coast	Cerebellar ataxia	(Echaniz-Laguna et al., 2017)
L58Afs*105	165_171dup	France		(Echaniz-Laguna et al., 2017)
A61Rfs*100	180dup	France	Lower limb spasticity	(Echaniz-Laguna et al., 2017)
R75H	224G > A	France		(Bacquet et al., 2018)
G84R	349G > C		Asymmetrical weakness	(James et al., 2008)
L99M	295C > A	Pakistan	Reported autosomal recessive inheritance	(Houlden et al., 2008)
R127L	380G > T	Finland		(Ylikallio et al., 2015)
R127W	379C > T	Belgium, Italy, France, Japan	Lower limb spasticity, cramps	(Benedetti et al., 2010; Dierick et al., 2008; Echaniz-Laguna et al., 2017; Evgrafov et al., 2004; Solla et al., 2010; Tanabe et al., 2018)
Q128R	383A > G	France	Lower limb spasticity	(Echaniz-Laguna et al., 2017)
D129E	387C > G	Ireland	Decreased sensory conduction after progression in one patient, cramping	(Lewis-Smith et al., 2016)
S135F	404C > T	England, France, Korea, Italy, Algeria	Rare early onset	(Chung et al., 2008; Echaniz-Laguna et al., 2017; Evgrafov et al., 2004; Houlden et al., 2008)
S135C	404C > G	Germany, Japan		(Oberstadt et al., 2016; Tanabe et al., 2018)
R136L	407G > T	Italy		(Capponi et al., 2011)
R136W	406C > T	Belgium		(Evgrafov et al., 2004)
R140G	404C > T(?), 418C > G	India, Japan	Fasciculations	(Houlden et al., 2008; Tanabe et al., 2018)
K141Q	421A > C	Japan		(Ikeda et al., 2009; Maeda et al., 2014; Tanabe et al., 2018)
T151I	452C > T	Croatia, Japan, France		(Dierick et al., 2008; Echaniz-Laguna et al., 2017; Evgrafov et al., 2004; Nishibayashi et al., 2007; Tanabe et al., 2018)
S158fs*200	476_477delCT	Italy		(Capponi et al., 2011; Mandich et al., 2010)
Q175*	523C > T	France		(Echaniz-Laguna et al., 2017; Rossor et al., 2012a)
T180I	539C > T	Italy, Portugal	Early onset, fasciculations	(Capponi et al., 2011; Echaniz-Laguna et al., 2017; Luigetti et al., 2010)
P182L	545C > T	Austria		(Dierick et al., 2008; Evgrafov et al., 2004)
P182S	544C > T	Japan	Early onset	(Kijima et al., 2005)
S187L	560C > T	France		(Echaniz-Laguna et al., 2017)

identified in an Italian family, presenting in a child at 3 months of age after a tetanus vaccination and worsening after a subsequent booster dose months later (Mandich et al., 2010). The patient subsequently lost ambulatory ability and lacked motor and sensory NCVs. This suggests nonsense mutations may cause a more dramatic phenotype, however, the unvaccinated father has had a rather typical course of disease with mildly impaired motor loss and no sensory loss. Additionally, other reports of nonsense mutations have not demonstrated a particularly severe or early onset phenotype (Rossor et al., 2012a; Tanabe et al., 2018). Recently It is unknown whether truncated forms of Hsp27 can cause a much earlier onset and more severe disease, and whether immunizations or other potential stressors in susceptible patients may exacerbate the disease course.

Other cases have also presented specific mutations that present as either CMT2F or dHMN II. It may be difficult to generalize rules for certain point mutations such as R136L presenting as CMT2F and dHMN II, even in a single study (Capponi et al., 2011). One study determined that R136L patients present with greater loss of plantarflexion than dorsiflexion in the ankles (Stancanelli et al., 2015), whereas a different mutation encoding a premature stop codon presented with equal loss of each type of flexion (Rossor et al., 2012a). Furthermore, it appears that certain mutations may present slightly differently between different families – for example, an R127W mutation in a Chinese family presents with an later age of onset but with sensory involvement in hands compared to an R136W mutation in an Italian family (Solla et al., 2010; Tang et al., 2005a), while onset of K141Q has differed in Japanese families (Ikeda et al., 2009; Maeda et al., 2014). Additionally, even cases inherited within a single family have presented with a wide spectrum of sensory symptoms, from no loss to severe failure, which also further suggests that CMT2F and dHMN exist as a continuum and other factors may affect symptom presentation (Gaeta et al., 2012; Solla et al., 2010).

Considering that many of these same mutations occur in CMT2F and dHMN II, especially the commonly reported 404C > T (S135F), the categorical distinction of these two diseases is likely more based on symptomatology than differences in cellular pathology (Capponi et al., 2011; Evgrafov et al., 2004; Kim et al., 2014). Furthermore, many CMT2F patients show relatively minor deficits in sensory function (Penno et al., 2010). However, determining why almost all patients with related Hsp27 mutations consistently develop motor systems, while only a subset develop sensory symptoms, remains a lingering question. It is still unclear whether genetic or environmental differences among individuals contribute to a varying clinical presentation. While some studies have suggested that mutations in the C-terminus of Hsp27 may cause a strictly motor phenotype (d'Ydewalle et al., 2011), mutations in other regions of the gene can produce a pure loss of motor capabilities (Capponi et al., 2011), while some cases of C-terminal mutations present with a mixed motor and sensory picture (Rossor et al., 2012a).

This level of variance suggests that if mutation location has an effect on symptomatology, it is not generalizable. Consequently, caution should be exercised when generalizing clinical findings that occur only in the presence of specific mutations as pathological mechanisms that explain symptomatology, such as observed alterations in mitochondrial transport in S135F but not P182L cells, as discussed more later (d'Ydewalle et al., 2011). As keenly pointed out by Rossor *et al.*, 2012, the lack of patient-reported sensory symptoms does not preclude sensory involvement of the disease on examination (Rossor et al., 2012a). The later onset of dHMN II may also reflect a milder disease presentation.

Finally, clinical studies have also demonstrated heterogeneity in clinical presentation beyond motor and sensory loss in limbs. A recent case report identified a novel D129E Hsp27 mutation thought to cause both motor neuropathy and a distal myopathy, although there was substantial variation in presentation, as the second generation patient had only minor symptoms of cramping (Lewis-Smith et al., 2016). Other reports have detailed patients that demonstrate hearing loss that precedes motor and sensory symptoms (Stancanelli et al., 2015), or ocular symptoms such as optic atrophy and ocular myopathy (Skre, 1974). Interestingly, some patients also present with hyperreflexia and positive pyramidal signs including a positive Hoffmann sign or Babinski sign, suggesting upper motor neuron deficits in the corticospinal tract may also be present and potentially masked by peripheral dysfunction (Amornvit et al., 2017; Capponi et al., 2011; Stancanelli et al., 2015). However, signs of pathology have generally not been detected clinically in the brain and spinal cord (Echaniz-Laguna et al., 2017).

Patient data in further clinical reports should augment our understanding of how certain mutations impart different likelihoods of developing distinct clinical phenotypes. Thus far, the low prevalence of CMT2F among patients presenting with distal symmetrical polyneuropathy has presented a challenge to producing sufficient data to assertively shape conclusions about prevalence. However, as diagnostic sequencing increases in ubiquity across the world, we should expect that our insight into the effects of specific point mutations, genetic environment, and environmental exposures will considerably increase our knowledge on CMT2F.

4. CMT2F Experimental reports

4.1. Hsp27 knockout mice

Hsp27 is commonly referred to as Hsp β-1 or HspB1 in mice. HspB1 knockout (KO) mice have been created and described in publications by three different groups to date. Huang et al., 2007 ablated HspB1 and replaced it with a knock-in of the *lacZ* reporter gene under the HspB1 promoter, allowing visualization of HspB1 tissue expression at different developmental stages (Huang et al., 2007). Despite the ubiquity of *HspB1* in mouse tissue, particularly in musculature, these mice were viable, fertile, and lacked any apparent musculoskeletal or visual abnormalities. HspB1 was notably expressed in the spinal cord but expression was limited in the brain, and no neural phenotypes were reported. The lack of phenotypes was thought to be due to synergistic action as the levels of other Hsps, such as Hsp70, remained constant. However, upon heat stress, isolated mouse skin ear fibroblasts (MSFs) showed reduced viability (Huang et al., 2007). A second Hsp27 KO mouse model generated by Crowe *et al.* with deletion of all three exons of HspB1 demonstrated decreased wound healing and increased inflammatory markers in isolated mouse embryonic fibroblasts. This suggests that HspB1 may physiologically restrain the immune response and promote progression of the cell cycle (Crowe et al., 2013). A third HspB1 KO generated by homologous recombination resulted in male mice that exhibited diminished weight, reduced plasma lipids, and myofibrillar abnormalities in muscle organization (Kammoun et al., 2016).

Hsp27 KO in fruit flies (Hao et al., 2007) and transient reduction through antisense phosphorodiamidate morpholino oligonucleotides in zebrafish (Tucker et al., 2009) further suggest a lack of drastic developmental or neural phenotypes. Flies did have a reduced stress response to starvation and decreased mean lifespan. The lack of stark phenotypes in KO models may result from compensatory mechanisms from other sHsps that otherwise could not similarly compensate gain-of-function mutations observed in CMT2F (Kammoun et al., 2016).

4.2. Chaperone function

While Hsps operate as molecular chaperones under thermal and cellular stresses arising from ischemic, oxidative, and toxic etiologies, Hsps also can operate to promote these functions in unstressed conditions (Dierick et al., 2005). Given its intimate role as a chaperone, Hsp27 has been examined in many CMT2F studies in both stressed and unstressed states. Initially, the P182L Hsp27 mutation was found to irregularly cluster in the soma instead of spreading into neurites when overexpressed in primary cortical neurons (Ackerley et al., 2006). Minor levels of aggregation were also observed in the presence of wild-type (WT) Hsp27, but were drastically increased by co-transfection with P182L. This suggested that the P182L mutant could exert a dominant effect by drastically increasing the degree of a normal aggregative process. Moreover, unlike the majority of WT Hsp27, P182L mutant demonstrated predominantly insolubility in a Triton X-100 detergent solution, a property of other proteins mutated in neurodegenerative diseases, further suggesting that this mutation causes insoluble aggregates (Ackerley et al., 2006). However, this work did not clarify if this pathology resulted primarily from the formation of Hsp27 aggregates in the soma or the loss of WT Hsp27 in neurites.

The work by Ackerley *et al.* encouraged further exploration of aggregation of Hsp27 using other cellular models. Increased aggregation of Hsp27 was further observed in HeLa cells expressing the G84R, S135F, R136W, and P182L mutants compared to WT, as well as two mutants in Hsp22 (James et al., 2008), suggesting that many CMT2F mutations can disrupt Hsp27's chaperone function. The greatest levels of aggregation were measured with the P182L mutant, suggesting that mutations in the C-terminus may especially predispose Hsp27 to clumping in the cytoplasm (James et al., 2008). Recently, Echaniz-Laguna *et al.* have also shown that the S187L mutant, but not P7S, G53D, nor Q128R, displayed clumping, further suggesting that C-terminal mutations may selectively cause this phenotype. This group also presented evidence that these aggregates are cleared via proteasomes and not autophagosomes (Echaniz-Laguna et al., 2017). Increased aggregation was also observed with another similar mutation, P182S, and may be due to its increased propensity to form large oligomers, which have low thermal stability and easily form aggregates (Chalova et al., 2014). While these large oligomers can contain WT Hsp27, this effect was not observed for T180I, another C-terminal mutation, suggesting aggregation may be a mutation-specific phenomenon and not generalizable to all C-terminal mutations (Chalova et al., 2014). Furthermore, another study using patient-derived fibroblasts and stably transfected neuroblastoma cells failed to demonstrate aggregation occurring with the P182L mutant (Geuens et al., 2017). Overall, while multiple studies have reported aggregation induced by different mutants, these effects are still unclear.

Further studies examining the effects of different mutations also present less clear results. Three CMT2F mutations in the α-crystallin domain—R127W, S135F, and R136W—were interestingly found to *increase* Hsp27's chaperone function, associated with an increased monomeric state of Hsp27. This increase in function theoretically might have been able to explain the biological gain-of-function thought to underlie the typical autosomal dominant inheritance of CMT2F (Almeida-Souza et al., 2010). However, three other mutations—S156Y, T151I, and P182L—had no overall effect on chaperone activity, suggesting that increased chaperone activity is not necessary for CMT2F disease progression. Moreover, these mutants had varied effects on thermotolerance—increasing, decreasing, or not altering responses. This suggested that either specific mutants caused different pathologies that still resulted in a similar clinical phenotype, or direct measures of Hsp27 chaperone function may not be especially relevant in disease pathogenesis. Almeida-Souza *et al.* observed that the R127W and S135F mutants were increasingly present in monomeric form. The authors proposed that the mutant residues blocked otherwise stabilizing internal hydrogen bonds that facilitated protein dimerization. These two mutants, along with R136W, bound more to client proteins, further suggesting the mutants are hyperactivated and may have increased chaperone function in the monomeric form (Almeida-Souza et al.,

2010).

Additional results from other studies have made interpreting the effects of Hsp27 mutants on chaperone activity difficult. Other mutations (P7S, G53D, Q128R, and S187L) studied by Echaniz-Laguna *et al.* did not have an altered monomeric:dimeric ratio nor altered chaperone activity (Echaniz-Laguna et al., 2017). Given that Q128R occurs between R127 and S135F, these results suggest that it may be difficult to extrapolate effects on chaperone activity from the general location of mutations in the protein. Further complicating matters, the R127L and a truncating C-terminal mutation were shown to impair heat tolerance both immediately and 48 hours following heat stress in fibroblasts isolated from patients; the effect of the truncating mutation was much greater (Ylikallio et al., 2015). As the WT and truncated Hsp27 showed similar nuclear translocation and induction of heat shock protein genes, this suggests that immediate decreases in viability following heat shock could be due to structural deficits in Hsp27 induced by the mutation, preventing Hsp27 from binding and protecting denatured proteins. Both mutant conditions demonstrated an increased sensitivity to protein misfolding, as measured by increased apoptotic round cells, and the effect appeared earlier in the C-terminal truncated cells than the R127L (Ylikallio et al., 2015). However, Ylikallio *et al.* did not observe aggregates in the C-terminal truncation mutant as Ackerley *et al.*, observed in P182L (Ackerley et al., 2006; Ylikallio et al., 2015). Ylikallio *et al.* also did not observe an increased monomeric fraction of R127L as Almeida-Souza *et al.* did for R127W, suggesting that either the specific point mutation confers different phenotypic effects or overexpression in neuroblastoma lines may produce different results than patient-derived fibroblasts (Almeida-Souza et al., 2010; Ylikallio et al., 2015). Each of these models possesses a strength of either a neuronal background or endogenous expression levels. Induced pluripotent stem cells (iPSCs) largely carry both of these advantages and should be highly considered for future studies given this discrepancy.

Substantial work has also been undertaken to descriptively analyze the biochemical effects of mutations located in each portion of the protein. G84R and L99M mutants produce larger and less stable homooligomers and possess reduced chaperone activity, potentially by decreasing accessibility of the N-terminus and affecting dimer stability, respectively (Nefedova et al., 2013a). Additionally, three N-terminal mutations have also been shown to produce earlier heat-induced aggregation, increased susceptibility to chymotrypsin-induced lysis, and reduced chaperone activity. These mutants also decreased phosphorylation preventing Hsp27 oligomer dissociation and were observed to form larger homooligomers (Muranova et al., 2015). The R140G and K141Q mutations in the α-crystallin domain both decrease thermal stability; however, these mutants have varying effects on protein interactions, trypsinolysis, homo- and heterooligomer size, and chaperone activity, the latter which was decreased in R140G but not altered in K141Q (Nefedova et al., 2013b). The more drastic effects of R140G compared to K141Q is comparable to those observed for S135F and R136W and may be due to the fact that R140G mutation more severely disrupts the dimer interface than a mutation at the adjacent residue (Almeida-Souza et al., 2010; Nefedova et al., 2013b). Focusing on the C-terminus, Chalova *et al.*, analyzed parameters of chaperone function in four mutations and concluded that each confers a distinct biochemical mechanism of disruption. R188W possessed a decreased chaperone function likely due to altering polarity of the C-terminal extension while not altering quaternary structure. Conversely, the T164A mutation decreased quaternary structure and thermal stability without affecting chaperone function. While T180I and P182S occurred in a similar location in the C-terminal domain, T180I increased thermal stability while P182S decreased thermal stability and dramatically decreased chaperone activity (Chalova et al., 2014).

4.3. Neurofilaments

One potential mechanism for decreased axonal transport may be through neurofilament (NF) dysregulation. NFs in the peripheral nervous system are typically heteropolymers made up of NF light (NF-L), medium, heavy, and peripherin subunits (Yuan et al., 2012). NF-L chain levels have been shown to be highly associated in blood and cerebrospinal fluid with progression of neurodegenerative diseases in animal models and are becoming promising biomarkers in clinical settings. It is thought that their accumulation directly contributes to neuronal cell death (Alves and Bonanni, 2017; Bacioglu et al., 2016; Byrne et al., 2017; Yuan et al., 2012). Mutations in NF-L, which is abundant in neurons, are known to cause CMT2E, often with an early and severe phenotype (Dequen et al., 2010; Jordanova et al., 2003; Mersiyanova et al., 2000). In their seminal paper, Evgrafov *et al.* co-expressed NF-L with either WT or S135F mutant Hsp27 in adrenal carcinoma cells, and observed that the S135F mutant induced NF-L aggregates instead of a filamentous staining pattern and S135F overexpressed in N2a cells reduced cell viability (Evgrafov et al., 2004). Reductions in cell viability have since been supported by evidence from other groups (Amornvit et al., 2017; Heilman et al., 2017) although my not be generalizable in all cell lines (Schwartz et al., 2018). Ackerley *et al.* similarly showed that the P182L mutant disrupts the NF network in primary cortical neurons (Ackerley et al., 2006).

Further work has sought to build upon these initial findings. WT Hsp27 was found to directly bind to WT and CMT2E-mutant NF-L and reduce mutant NF-L-induced aggregation and mutant NF-L-mediated motor neuron death. This suggested that mutations of Hsp27 underlying CMT2F dysfunction may have a related mechanism to CMT2E (Zhai et al., 2007). Similar to mutant NF-L expression, expression of S135F mutant disrupted the NF network, causing NF-L aggregation and reducing viability of motor neurons. To determine if these effects on NF-L are necessary for CMT2F pathogenesis, Zhai *et al.* microinjected S135F cDNA into NFL KO mice and observed that isolated motor neurons had decreased growth. However, when WT mice were microinjected with S135F cDNA, there was a substantially greater decrease in motor neuron growth. Thus, while eliminating NF-L did not completely rescue cells from S135F-mediated toxicity, it hints at the possibility for common mechanism for CMT2E and CMT2F.

Further studies have largely focused on investigating NF regulation in CMT2F. Using neuroblastoma cell lines stably expressing CMT2F mutations, Holmgren *et al.* found CMT2F mutants reduced NF anterograde transport. This was partially explained by increased Cdk5 activity, which increased NF phosphorylation and thereby increased binding among NFs in all mutants tested but R127W; this caused decreased NF binding with kinesin (Holmgren et al., 2013). Increased phosphorylation of NFs has also been observed in S135F mouse tissue and SH-SY5Y cells stably transfected with P7S, G53N, Q128R, but not S187L mutants (Echaniz-Laguna et al., 2017; Lee et al., 2015). While R136W mutant mice generated by Srivastava *et al.* demonstrated an increased NF density in axons, other studies have not focused on using electron microscopy to study NF function (Srivastava et al., 2012).

These results raise many questions on the mechanism by which CMT2F mutations alter regulatory kinases and in which cell types this occurs. The results suggest that C-terminal mutations may lack this effect, and further experiments should be performed with other C-terminal mutants as have been done on chaperone function. Cdk5 knockdown provided a substantial but incomplete reduction of NF phosphorylation, suggesting mutants could induce other distinct regulatory kinases and pathways (Holmgren et al., 2013). However, contrary to earlier studies, Holmgren *et al.* did not observe NF aggregates nor changes in NF staining patterns in cells transfected with S135F or P182L mutants, Echaniz-Laguna *et al.* did not observe alterations in NF architecture in SH-SY5Y cells using the NF-H (NF heavy) as a marker, and Kalmar *et al.* did not observe changes in NF-L staining using transduced mouse primary motor neurons (Echaniz-Laguna et al., 2017; Holmgren et al., 2013; Kalmar et al., 2017). It is still unclear whether this pathology occurs *in vivo*, and if different mutants affect NF structure and transport to different degrees, as in chaperone function.

4.4. Microtubules

Another interesting and especially promising target for treatment in CMT2F is microtubules (MTs) and their associated dysfunction. MTs serve many important functions in axons such as regulating axonal morphology, conserving neuronal polarity, transporting of cargo, and modulating intracellular signaling through organizing hubs of kinases and other regulatory enzymes. They often exist in relatively dynamic states of assembly and disassembly that can be regulated by a variety of mechanisms including associating proteins or chemical modifications to tubulin, although MTs in neurons are generally considered to be relatively stable (Dubey et al., 2015; Gentil and Cooper, 2012).

Evaluation of WT and mutant Hsp27 binding properties as chaperones led to the discovery of tubulin as a potential binding partner with increased binding in mutants (Almeida-Souza et al., 2011; Almeida-Souza et al., 2010). While primary motor neurons transduced with viral constructs of mutants (P39L, R140G, and S135F) did not demonstrate changes in MT staining as evidenced by TUBB3 (beta III tubulin) (Kalmar et al., 2017), work performed *in vitro* and using dorsal root ganglia (DRGs) isolated from mice demonstrated that three of five CMT2F mutants tested (R127W, S135F, and R136W, but not T151I nor P182L) increasingly bound to both α-tubulin and assembled MTs. This increased binding of Hsp27 to MTs increased the stability of MTs in destabilizing conditions by reducing MT dynamic events (Almeida-Souza et al., 2011). Further exploration of this binding interaction demonstrated a significantly greater co-localization of mutant Hsp27 and the tubulin marker TUBB3. However, this difference was slight and there was still high co-localization of WT Hsp27 and no apparent difference in pattern of the MT network. Nevertheless, MTs in mutant cells showed marginally decreased migratory ability, suggesting a more stabilized state. In confirmation, thorough evaluation of kinetics of individual MTs revealed that MTs in mutant Hsp27 cells spent more time in the stationary phase; even though some MTs exhibited bouts of *faster* depolymerization, these occurred less often. It remains unclear whether the increased stability over time or more rapid depolymerization could potentially induce pathological effects.

These findings generated great interest in MTs in CMT2F. Many other neurodegenerative diseases are associated with MT dysfunction, however typically, but not always, with a less stable state (Brunden et al., 2017; Brunden et al., 2014; Dubey et al., 2015; Pareyson et al., 2015; Prior et al., 2017). In addition, peripheral neuropathy is widely known as a common dose-limiting side effect of many MT-targeting chemotherapeutic agents (Carlson and Ocean, 2011; Lee and Swain, 2006). Patient biopsy data from a clinical study also reveals an increased density of MTs in atrophic axons, potentially due to decreased NFs (Benedetti et al., 2010). In a seminal study by d'Ydewalle *et al.*, S135F and P182L mutant mice were found to have decreased acetylation of α-tubulin in sciatic and sural nerves, suggesting a contrarily less stabilized (and increased dynamic) state. Furthermore, the authors also observed decreased MT-mediated trafficking of mitochondria, which became a candidate to underlie the pathological features presenting exclusively in peripheral neurons, given their high requirements for mitochondria (d'Ydewalle et al., 2011). The decreased acetylation of α-tubulin was confirmed in median/ulnar and sciatic nerves from S135F transgenic mice generated by a different group, generating great excitement as a potential biological mechanism of degeneration (Lee et al., 2015).

However, like the variable in results observed in chaperone function and NF structure, decreased acetylation of α-tubulin has not been observed in many cell lines and may be strictly limited to peripheral tissue. Importantly, no change in acetylation of α-tubulin was observed for R127W, S135F, R136W, and P182L mutants—if anything, the R136W and P182L mutants demonstrate increased acetylation (Almeida-Souza et al., 2011). These results are similar to others that failed to show a lack of acetylation of α-tubulin in cell culture (Schwartz et al., 2018). Even more perplexing, these results suggest a more dynamic MT state, which falls in line with alterations observed in most neurological diseases, but opposes the results of Almeida-Souza et al. It is possible that an initially stabilizing environment is present, and the over-recruitment of histone deacetylase (HDAC) inhibitors could overcompensate, causing deacetylation in the PNS. However, this possibility would presumably result in an increase in acetylation of α-tubulin in the system of Almeida-Souza *et al.*, which was not observed, making reconciling these results somewhat difficult. As Almeida-Souza *et al.* mention, the increased speed of polymerization they observed could signify that the increased stabilization observed may only be a transient effect and not reflective of the true overall MT behavior (Almeida-Souza et al., 2011). It is also possible that these results are exclusive to the cellular environment of peripheral neurons.

4.5. Mitochondria

In addition to providing energy in the form of ATP, mitochondria have many important functions in neurons including responding to rapidly changing cellular conditions, buffering intracellular calcium, and triggering apoptosis (Prior et al., 2017; Sheng, 2014). Neurons require an especially high energy demand for synaptic functions including synaptic assembly, generation of action potentials, and regulation of synaptic transmission, all of which can occur far from the nucleus. As such, neurons face especially difficult challenges in transporting sufficient mitochondria to axon terminals for energy generation and removing them when they become damaged (Sheng, 2014). Defects in mitochondrial functioning, including fusion and fission, respiratory chain function, and mitophagy have been associated with a wide variety of neurodegenerative disorders (Burté et al., 2015).

Many studies point to a role of mitochondria in the pathology of CMT2F, as well as other types of CMT2. Multiple groups have discovered patients with mutations of both *MFN2* and *GDAP1*. As both of these genes influence mitochondrial dynamics, mitochondrial function may be a common mechanistic of CMT2 (Cassereau et al., 2011; Vital et al., 2012). Moreover, specific mutations that typically lead to mild disease alone present with severe symptomatology when inherited together, suggesting a synergistic or additive effect of mutation burden (Vital et al., 2012). Impaired mitochondrial trafficking has been implicated in CMT2A (Saporta et al., 2015), CMT2E (Brownlees et al., 2002; Saporta et al., 2015), and CMT2F (d'Ydewalle et al., 2011; Kalmar et al., 2017; Kim et al., 2016), while other mitochondrial defects and proteins involved in mitochondrial function are impaired in many other forms of CMT (Pareyson et al., 2015).

In relation to decreases in α-tubulin acetylation, d'Ydewalle *et al.* determined that mitochondrial transport decreased in S135F mouse DRGs (d'Ydewalle et al., 2011). Since MTs serve to transport mitochondria along axons, the group tried to rescue mitochondrial transport deficits by increasing MT acetylation using HDAC inhibitors. HDACs hydrolyze acetyl modifications selectively added by histone acetyltransferases (HATs) to lysine residues on histones and other molecules. HDAC6 ironically does not act on histones but is known to regulate acetylation of α-tubulin and other heat shock proteins (Shen et al., 2016). Indeed, HDAC6 inhibition using trichostatin A (TSA) and tubastatin increased mitochondrial movement, neuromuscular junction number and innervation, and rescued altered behavioral performance and compound muscle and sensory nerve action potentials (CMAPs and SNAPs) (d'Ydewalle et al., 2011). Yet, other studies have not been able to completely confirm these findings in CMT2F models. Kalmar *et al.*, observed no difference in percentage of moving mitochondria using three mutations (P39L, R140G, and S135F) in transduced mouse motor neurons (Kalmar et al., 2017). This group did, however, observe a decreased velocity of retrograde transport of mitochondria with no alterations in anterograde transport velocity or retrograde transport of other cargo, and increased mitochondrial pausing in all mutant conditions tested. The relative proportion of mitochondrial movement in the anterograde direction was increased only in the S135F mutant.

Similarly, the early results of Ackerley *et al*. also did not observe an effect on anterograde transport of mitochondria in primary cortical neurons transfected with P182L (Ackerley et al., 2006).

Nevertheless, given the stark findings of d'Ydewalle *et al.* on mitochondrial transport, HDAC6 inhibitors stand as a promising candidate treatment for CMT2F. Different groups have identified multiple small molecules with hydroxamic acid moieties with similar HDAC6 inhibition selectively as TSA. Inhibitors tested by one group were found to possess more potent increases in acetylation of α-tubulin at low concentrations in N2a cells than TSA, and at higher concentrations increased mitochondrial transport in CMT2F mutant mouse DRG neurons similar to the degree observed in mice treated with TSA or WT mice (Shen et al., 2016). Another recent screening study identified two molecules that also demonstrated potent and selective HDAC6 inhibition and increased mitochondrial axonal trafficking. They were verified as biologically relevant by increasing innervation at neuromuscular junctions and improving motor and sensory nerve conduction in mice (Benoy et al., 2017). Mitochondrial movement most drastically increased in a retrograde fashion, but also appeared to increase in anterograde motion as well, raising questions as to directional deficits in disease pathology and how HDAC6 inhibitor treatment may affect mitochondrial movement. Moreover, while these inhibitors seemed to rescue some motor and sensory function, compared to previously reported degree of deficits (and without a direct positive control from WT mice), they may not fully alleviate symptoms (Benoy et al., 2017). Two HDAC inhibitors developed by a third group were also shown to rescue defects in α-tubulin acetylation and mitochondrial trafficking observed in S135F and P182L cultured motor neurons differentiated from iPSCs derived from patient fibroblasts (Kim et al., 2016). While not a major conclusion of the paper, interestingly, two clones of P182L cells presented with markedly different mitochondrial properties—one clone, unlike the other, did not demonstrate a decreased absolute velocity of mitochondrial movement, but interestingly seemed to have an even more drastically diminished percentage of moving mitochondria (although the authors did not make this statistical comparison) (Kim et al., 2016). If these iPSC models are taken to be strong models of cellular pathology, this suggests that variability in phenotype may even exist within the same individual, potentially allowing a role for environmental influence. For this reason, more studies should be performed using iPSC models.

Further study also needs to evaluate other potential therapeutics and determine if long-term dosage can effectively reduce pathology in model systems. In addition, due to nucleotide substitution, hydroxyamates as a class are well known mutagens and safety profiling must be carefully performed to evaluate the potential carcinogenicity of potential treatments, especially since CMT is not a terminal diagnosis (Shen and Kozikowski, 2016). Studies on sensory nerve endings will also be useful, especially given the variable presentation of sensory symptoms in CMT2F. Further research may consider also utilizing HATs to directly induce acetylation.

The observed decreased trafficking of mitochondria has led other studies to focus on mitochondrial alterations beyond MT-mediated transport in axons. The fact that transport of other signaling proteins has not been altered by CMT2F mutations suggest that deficiencies of mitochondrial transport may be primarily due to mitochondrial defects rather than generalized defects of transport machinery (Kalmar et al., 2017). There is some evidence that mitochondrial content may be increased in mutant cells. R136W mice axons demonstrated an increased overall mitochondria content by electron microscopy (Srivastava et al., 2012), potentially due to increased individual mitochondrial size, as observed in S135F-transfected cells in another study (Schwartz et al., 2018). However, these results stand in opposition to d'Ydewalle et al., who observed decreased total mitochondria in DRGs from S135F mice at 10 months but not 2 months, and demonstrated increases in mitochondrial number by HDAC6 inhibition using kymographs and spatio-temporal maps (d'Ydewalle et al., 2011). The same group later showed slight increases in mitochondrial content in neurites with HDAC6 inhibition based on kymograph quantification (Benoy et al., 2017). It is possible that this discrepancy in results could result from the different imaging modalities used.

Other studies have focused on determining effects on mitochondrial function. Kalmar *et al*. observed mitochondria with decreased membrane potential in the neurites of S135F mouse motor neurons. Using S135F mouse motor neurons, they observed decreased mitochondrial Complex I (but not Complex II) activity, increased superoxide in response to antimycin A treatment, and increased glutathione release and nitrotyrosine expression, all of which suggest an increased susceptibility to oxidative stress (Kalmar et al., 2017). Many of these studies used other mutants which, in some cases, were significantly different from WT, and in other cases also showed similar trends to the S135F mutant. However, some notable exceptions still existed: P39L appeared to have similar levels of glutathione (if anything, increased in whole cells) and R140G similar levels of nitrotyrosine to WT, again suggesting that these mechanisms implicated may only pertain to certain mutants (Kalmar et al., 2017). The lack of a decrease in membrane potential in the neuronal cell bodies suggests that the mitochondrial defects occur in more distal processes and may be related to transport. Mitochondrial respiratory function has also been shown to be impaired by the S135F and P182L mutants using a Seahorse apparatus to measure oxygen consumption (Schwartz et al., 2018). It is unknown whether these structural and functional abnormalities in mitochondrial size and content, oxidative state, and respiratory function are related to trafficking defects. It is also unknown why decreased potential seems to be related to defects specifically in Complex I. However, decreased mitochondrial functioning could potentially lead to an increased basal level of oxidative stress over time, which may cause greatest effects in post-mitotic cells after extensive time, as observed in CMT.

4.6. Other molecular effects

Since CMT2F mutations are typically point mutations that produce a translated protein, investigators have typically focused on studying the biochemical properties and biological effects of the mutated protein rather than investigating potential defects in the molecular biology of the disease. However, one group recently discovered that the P182L mutation, but not WT nor R127W mutants, interacts with RNA-binding protein poly(C)binding protein 1 (PCBP1), another ubiquitously expressed protein that exists in peripheral nervous system tissue, and reduces its function of repressing translation. PCBP1 targets, which included many genes that when mutated are known causes of peripheral neuropathy, were expressed more highly in P182L mutants (Geuens et al., 2017). However, other mutations have not been studied for translational repression and the potential consequences of altered translational profiles pertaining to other neurodegenerative disease have not been mechanistically connected to CMT2F.

Other mutations have also been shown to increase Hsp27 binding to other proteins. S135F mutant has increased association with ceramide synthase 1, reducing ceramide synthase 1 and ceramide levels at the mitochondria (Schwartz et al., 2018). Mutant Hsp27 (S135F, R136W, and R127W) has also been suggested to increase Hsp27 binding to F-actin, leading to increased dissociation of Drebrin from F-actin (Mata et al., 2015).

Other biological responses have received limited study and warrant more attention. Motor neurons differentiated and cultured from patient-derived iPSCs and mouse motor neurons transduced with mutant lentiviral constructs do not appear to have a stark difference in morphology or length and express similar neuronal markers as control motor neurons (244, 260). However, SH-SY5Y cells transfected with mutants (P39L, R140G, and S135F) demonstrate increased lactate dehydrogenase release, suggesting increased cytolysis (Kalmar et al., 2017). R136W mice sciatic nerves contain cytoplasmic invaginations similar to secondary lysosomes (Srivastava et al., 2012). As mentioned

earlier, axonal transport has been shown to be affected in a wide array of neuropathies beyond simply mitochondrial trafficking (Gentil and Cooper, 2012; Prior et al., 2017). P182L was shown to co-localize and alter the localization of p150, a protein important in regulating neuronal retrograde transport, potentially sequestering p150 and preventing efficient retrograde transport (Ackerley et al., 2006). However, a later study did not find altered transport of p75, suggesting that generalized retrograde transport may not be altered (Kalmar et al., 2017) and necessitating further study to thoroughly evaluate transport deficits. Alterations in autophagy, a mechanism of delivering cell aggregates and debris to lysosomes for recycling, are another common mechanism thought to potentially underlie many types of CMT (Haidar and Timmerman, 2017). Schwartz et al. demonstrate a slightly increased level of autophagy in CMT2F (Schwartz et al., 2018), which parallels observations of increased autophagy in other types of CMT2. However, recently Haidar et al. observed that cells expressing R127W, S135F and P182L mutants as well as Hsp27 KO cells demonstrated a decrease in autophagic flux and patient-derived motor neurons. This may be due to an increased binding interaction of some of the mutants to the autophagosomal proteins (Haidar et al., 2019).

4.7. Biological models and pathophysiological effects

Different groups have attempted to study the pathophysiology of CMT2F using mouse models, many of which recapitulate the human disease to a strikingly similar degree. Physical deformities and abnormal behavior have been noted in multiple mouse models. S135F and P182L transgenic mice generated by d'Ydewalle *et al.* demonstrated noticeable phenotypes, although the latter presents more like dHMN II than CMT2F with a lack of sensory symptoms (d'Ydewalle et al., 2011). At 8 months of age, these mice have clawed hindpaws similar to the *pes cavus* that presents clinically. These mice present with clasping of hindpaws and decreased rotarod performance at 6 months of age and decreased grip strength at 4 and 7 months, in the P182L and S135F mutants, respectively; forepaw strength decreases a few months later, similar to clinical presentation. The 8-month old mice tested demonstrate an abnormal gait with a decreased stride length and an abnormal hindpaw, but not forepaw, angle and foot print area, again mirroring the distal lower limb alterations typically seen initially in humans (d'Ydewalle et al., 2011). Interestingly, grip strength appeared to be markedly worse in the P182L mice, suggesting that the P182L mutant might present with a stronger motor phenotype than S135F.

However, CMT2F mouse models generated by other groups have notable differences. Behavior deficits reported by Lee *et al.* occur slightly earlier at 5 versus 6 months of age, but appear to be much more severe than the mouse models of d'Ydewalle *et al.*, which show a more gradual decline in many motor phenotypes (d'Ydewalle et al., 2011; Lee et al., 2015). Further complicating these findings, Lee *et al.* report no sensory phenotype in S135F mice, suggesting their model presents with a strict motor loss, similar to the P182L, but not the S135F mice of d'Ydewalle *et al.* In further contrast, the R136W mouse model did not demonstrate any functional or behavioral deficits (Srivastava et al., 2012). A fourth transgenic model attempting to express physiological levels of R127W and P182L mutant proteins to alleviate concerns of artifacts due to overexpression revealed a lack of pathology and behavioral deficits. While the authors attributed this to insufficient expression of Hsp27 under the ROSA26 locus, it may also indicate heterogeneity in the presentation of CMT2F mutations in animal models as well (Bouhy et al., 2016). The authors postulate that the other mouse models with Hsp27 overexpression could potentially promote formation heter- and homooligomers that would likely carry distinct functional consequences, potential mislocalization of Hsp27, and may lead to artificial phenotypes.

Despite the stark differences in phenotype, these mouse models display expected trends in neural physiology based on behavioral data. CMAPs were reduced in the models of d'Ydewalle *et al.* and Lee *et al.*, and paralleling behavioral results, sensory nerve action potentials were decreased in the S135F but not P182L mice of d'Ydewalle *et al.* (d'Ydewalle et al., 2011; Lee et al., 2015). In the R136W mouse, decreases in sciatic motor amplitude were present at 1 year (but not 6 months), and no change in conduction velocity was observed (Srivastava et al., 2012). Similar to previously mentioned clinical data (Gaeta et al., 2012), fatty infiltration of the gastrocnemius muscle was described by Lee *et al.*, while d'Ydewalle *et al.* noted atrophic muscle fibers with abnormal organization (d'Ydewalle et al., 2011; Lee et al., 2015). While Lee *et al.* report demyelination in sciatic nerve tissue of 10 month mice, d'Ydewalle et al., 2011 show axonal loss but a lack of demyelination in sciatic nerve tissue from 10 month old mice. Interestingly, R136W mice have an increased myelin thickness, foldings, and increased number of Schmidt-Lanterman incisures observed at 6 months of age (Srivastava et al., 2012). d'Ydewalle also noted both the S135F and P182L mice have a reduced number of axons in the distal but not proximal sciatic nerve, decreased acetylcholine receptor clusters per axon terminal, and increased percentage of denervated neuromuscular junctions (d'Ydewalle et al., 2011). Despite some clear trends, such a wide variety of phenotypes should prompt additional CMT2F mouse models, both using the same as well as different mutations, to better understand the variability of CMT2F.

5. sHsps in neurodegenerative disease

sHsps have been suggested to play a large role in many instances of neuropathy. As motor neurons have a greater threshold for heat shock induction and Hsp expression generally decreases with increased age, a reduced Hsp stress response could play a pathological role in CMT disease with presentation later in life and exclusively in peripheral neurons. A mutation in the promotor of Hsp27 that causes decreased Hsp27 expression in neurons is associated with Amyotrophic Lateral Sclerosis (ALS) (Dierick et al., 2007). Hsp27 has been shown to accumulate in brains of patients with Alzheimer's and frontotemporal lobe dementia (Zhang et al., 2014). Hsp27 may also be distinctly linked to myopathy, although further work must focus to delineate this pathology from CMT (Adriaenssens et al., 2017).

Upregulation of Hsp27 has shown potential in alleviating many neurodegenerative diseases, although beneficial effects observed between different models are not always consistent. Hsp27 overexpression improved impaired long-term potentiation and spatial memory in a mouse model of Alzheimer's Disease(Tóth et al., 2013). Similarly, Hsp27 overexpression protects again protein aggregation and apoptotic effects from WT and mutant α-synuclein in cell models of Dementia with Lewy bodies and Parkinson's Disease, respectively (Outeiro et al., 2006; Zourlidou et al., 2004). Hsp27 overexpression reduces reactive oxygen species (ROS) levels and protects neuronal cells from cell death in a cellular model of Huntington's Disease (Wyttenbach et al., 2002). However, while there are hints *in vivo* viral vector expression of Hsp27 may reduce huntingtin protein aggregation (Perrin et al., 2007), overall brain ROS and phenotype in transgenic mice are not improved (Zourlidou et al., 2007). Furthermore, overexpression of Hsp27 did not protect superoxide dismutase 1 KO mice, a common model of ALS, from motor neuron degeneration(Krishnan et al., 2008).

Mutations in Hsp22, another small heat shock protein also known as HspB8, can cause dHMN II (Irobi et al., 2004) and CMT2L (Tang et al., 2004; Tang et al., 2005b). Mutant Hsp22 is known to display greater binding to Hsp27, while S135F mutant Hsp27 has demonstrated greater binding to Hsp22, suggesting that increased binding of these proteins may contribute to observed neuropathy (Fontaine et al., 2006; Irobi et al., 2004). Furthermore, motor neurons transfected with mutant Hsp22 displayed substantial neuronal degeneration and reduced neurite growth. However, these phenotypes were absent in transfected cortical neurons and glial cells and only occurred in a small proportion of sensory neurons (Irobi et al., 2010). These results also suggest that dHMN may cause cellular defects predominantly in motor neurons

because they are more susceptible to damage, while impairment of sensory neurons may be below the threshold of producing clinical signs. If similar mechanisms occur in CMT2F, varying thresholds of impairment in different individuals may explain why some patients present with sensory loss with the same mutation while others do not.

Mutations in α-crystallin are known to cause cataracts and cardiomyopathy. Many of these mutations are known to cause protein aggregation similar to Hsp27 mutants, however, WT Hsp27 co-expression has been shown to reduce both levels of aggregation and levels of inclusion bodies caused by mutants, similar to Hsp27's effect on mutant NFs (Ito et al., 2003; Raju and Abraham, 2013; Zhai et al., 2007). Neuropathies associated with other small heat shock proteins are extensively reviewed by Boncoraglio *et al.*, Datskevich *et al.*, and Benndorf *et al.* (Benndorf et al., 2014; Boncoraglio et al., 2012; Datskevich et al., 2012).

6. Concluding themes

One common trend observed across various studies of CMT2F appears to be that different mutations produce different effects, even when studied by a single group. For example, R127W mutations may not affect NF interactions as S135F and P182L do(Holmgren et al., 2013), and T151I and P182L mutants were not found to bind and hyperstabilize MTs as S135F and R127W did (Almeida-Souza et al., 2011). Moreover, some phenotypes have shown opposing effects from different mutants, as cells transfected with R127W and S135F exhibit greater thermotolerance, those with P182L less thermotolerance, and those with T151I and S156Y not demonstrating significant change. In this same study, R136W was found to have greater chaperone activity while P182L was unchanged (Almeida-Souza et al., 2010). In a comparable disease state, CMT2D and distal spinal muscular atrophy type V are both caused by glycyl tRNA synthase mutations, further suggesting that variable sensory symptoms can exist in the same disease process (Antonellis et al., 2003). The slightly different phenotypes from clones isolated from iPSCs from a single individual discussed earlier provide even stronger evidence for variability in CMT2F (Kim et al., 2016).

As such, it will be of critical significance to focus efforts on generating models from iPSCs derived from patients as has been done recently (Kim et al., 2016; Saporta et al., 2015). Not only do such systems facilitate relatively easy study in human cells but they allow for the study and direct comparison of WT Hsp27 and mutant proteins at endogenous levels. iPSCs can also be propagated in cell culture indefinitely and are relatively easily expandable, especially in facilitating experiments that test potential therapeutics including HDAC6 inhibitors. This relative ease of expansion could further be applied to high-throughput testing of many different mutations to better understanding heterogeneity in presentation. As previously mentioned, isolating multiple clones from the same individual may uniquely allow for increased understanding of variability in phenotype and any potential environmental influences on pathology and presentation. More cost-effective sequencing and greater emphasis on analyzing sequencing data for a wide spectrum of diseases may also allow for a greater understanding of the prevalence of common CMT2F-inducing mutations in non-presenting individuals and better estimates of the penetrance of mutations. As the S135F mutation has consistently demonstrated some of the most striking pathologies in experimental reports, and is consistently reported as one of the most common mutations, it is possible that it has a higher penetrance than other mutations that may not always result in a phenotype.

Given the high variability of effects of different mutations and experimental systems, studies must be carefully designed and performed to ensure a complete understanding of CMT2F pathology. Research papers should carefully consider alternative conclusions and potential limitations of model systems used. This is especially critical in mouse models – while the first paper presenting a transgenic mouse model contained exciting findings that led to a search for therapeutic targets, a second mouse model did not replicate the same basic pathology, and later models suggested these findings may be spurious due to overexpression of Hsp27. Given the advent and relative ease of using CRISPR-Cas9 technology to generate mutants, generation of additional mouse models with physiological levels of mutant Hsp27 expression need to be assessed, and findings from mouse models with overexpressed protein must be carefully considered in appropriate context. Novel phenotypes should ideally be assayed using many different mutations, and negative data should be prominently displayed, given issues with reproducibility. Studies demonstrating differences in phenotypes for mutants should be especially careful to repeat experiments with sufficient power to show effects, especially in the face of high experimental variation.

Nevertheless, the great wealth of knowledge developed in the short time period since 2001, when CMT2F was discovered, and 2004, when Hsp27 was identified as a causative agent, provides confidence that research in CMT2F will continue to progress at a rapid pace. Investigators from increasingly diverse disciplines are becoming involved in studying CMT2F, and such a multiformity of perspectives can only aid in considering novel mechanisms and developing unconventional potential therapeutic strategies. It is likely that different forms of CMT2 share similar pathology and potential common mechanisms. CMT2E has been shown to present similarly to CMT2F with formation of aggregates, abnormal mitochondria, and resulting axonal transport defects (Ackerley et al., 2006; Tradewell et al., 2009), while Hsp27 has been shown to rescue defects in CMT2E, and removing NFL alleviated the CMT2F phenotype, suggesting these diseases may possess convergent mechanisms (Tradewell et al., 2009; Zhai et al., 2007). Multiple mitochondrial phenotypes including an increased average size have been observed in CMT2F, suggesting that mutations in mitofusin 2 that cause CMT2A may induce similar pathological mechanisms (Cartoni and Martinou, 2009). As an increasingly nuanced biochemical and cellular understanding of other forms of CMT2 develop, we can hope to extend our understanding CMT2F, and come closer to developing effective treatments.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interests

None

Author contributions

NUS wrote the manuscript.

Acknowledgements

I thank Dr. Lina M. Obeid for overall advice and feedback on this manuscript.

References

Ackerley, S., James, P.A., Kalli, A., French, S., Davies, K.E., Talbot, K., 2006. A mutation in the small heat-shock protein HSPB1 leading to distal hereditary motor neuronopathy disrupts neurofilament assembly and the axonal transport of specific cellular cargoes. Hum. Mol. Genet. 15, 347–354. https://doi.org/10.1093/hmg/ddi452.

Acunzo, J., Katsogiannou, M., Rocchi, P., 2012. Small heat shock proteins HSP27 (HspB1), αB-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. Int. J. Biochem. Cell Biol. 44, 1622–1631. https://doi.org/10.1016/j.biocel.2012.04.002.

Adriaenssens, E., Geuens, T., Baets, J., Echaniz-Laguna, A., Timmerman, V., 2017. Novel insights in the disease biology of mutant small heat shock proteins in neuromuscular diseases. Brain 1–9. https://doi.org/10.1093/brain/awx187.

Almeida-Souza, L., Goethals, S., de Winter, V., Dierick, I., Gallardo, R., Van Durme, J., Irobi, J., Gettemans, J., Rousseau, F., Schymkowitz, J., Timmerman, V., Janssens, S.,

2010. Increased monomerization of mutant HSPB1 leads to protein hyperactivity in Charcot-Marie-Tooth neuropathy. J. Biol. Chem. 285, 12778–12786. https://doi.org/10.1074/jbc.M109.082644.

Almeida-Souza, L., Asselbergh, B., d'Ydewalle, C., Moonens, K., Goethals, S., de Winter, V., Azmi, A., Irobi, J., Timmermans, J.-P., Gevaert, K., Remaut, H., Van Den Bosch, L., Timmerman, V., Janssens, S., 2011. Small heat-shock protein HSPB1 mutants stabilize microtubules in Charcot-Marie-Tooth neuropathy. J. Neurosci. 31, 15320–15328. https://doi.org/10.1523/JNEUROSCI.3266-11.2011.

Alves, G., Bonanni, L., 2017. Neurofilament light: a heavyweight diagnostic biomarker in neurodegenerative parkinsonism? Neurology 88, 922–923.

Amornvit, J., Yalvac, M.E., Chen, L., Sahenk, Z., 2017. A novel p.T139M mutation in HSPB1 highlighting the phenotypic spectrum in a family. Brain Behav. 7, 1–8. https://doi.org/10.1002/brb3.774.

Antonellis, A., Ellsworth, R.E., Sambuughin, N., Puls, I., Abel, A., Lee-Lin, S.-Q., Jordanova, A., Kremensky, I., Christodoulou, K., Middleton, L.T., Sivakumar, K., Ionasescu, V., Funalot, B., Vance, J.M., Goldfarb, L.G., Fischbeck, K.H., Green, E.D., 2003. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth Disease Type 2D and Distal Spinal Muscular Atrophy Type V. Am. J. Hum. Genet. 72, 1293–1299. https://doi.org/10.1086/375039.

Aquilina, J.A., Shrestha, S., Morris, A.M., Ecroyd, H., 2013. Structural and functional aspects of Hetero-oligomers formed by the small Heat Shock Proteins αB-Crystallin and Hsp27. J. Biol. Chem. 288, 13602–13609. https://doi.org/10.1074/jbc.M112.443812.

Asadi, S., 2019. Journal of brain and neurological disorder. J. Brain Res. 1.

Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D.R., Neumann, U., Kahle, P.J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J., Jucker, M., 2016. Neurofilament light chain in blood and CSF as Marker of Disease progression in mouse models and in Neurodegenerative Diseases. Neuron 91, 56–66. https://doi.org/10.1016/j.neuron.2016.05.018.

Bacquet, J., Stojkovic, T., Boyer, A., Martini, N., Audic, F., Chabrol, B., Salort-Campana, E., Delmont, E., Desvignes, J.P., Verschueren, A., Attarian, S., Chaussenot, A., Delague, V., Levy, N., Bonello-Palot, N., 2018. Molecular diagnosis of inherited peripheral neuropathies by targeted next-generation sequencing: molecular spectrum delineation. BMJ Open 8, e021632. https://doi.org/10.1136/bmjopen-2018-021632.

Baets, J., De Jonghe, P., Timmerman, V., 2014. Recent advances in Charcot – Marie – Tooth disease. Curr. Opin. Neurol. 27, 532–540. https://doi.org/10.1097/WCO.0000000000000131.

Bagnéris, C., Bateman, O., Naylor, C., Cronin, N., Boelens, W., Keep, N., Slingsby, C., 2009. Crystal structures of alpha-crystallin domain dimers of alphaB-crystallin and Hsp20. J. Mol. Biol. 392, 1242–1252. https://doi.org/10.1016/j.jmb.2009.07.069.

Bakthisaran, R., Tangirala, R., Rao, C.M., 2014. Small heat shock proteins: role in cellular functions and pathology. Biochim. Biophys. Acta 1854, 291–319. https://doi.org/10.1016/j.bbapap.2014.12.019.

Benedetti, S., Previtali, S.C., Coviello, S., Scarlato, M., Cerri, F., Di Pierri, E., Piantoni, L., Spiga, I., Fazio, R., Riva, N., Natali Sora, M.G., Dacci, P., Malaguti, M.C., Munerati, E., Edoardo Grimaldi, L.M., Marrosu Giovanna, M., De Pellegrin, M., Ferrari, M., Comi, G., Quattrini, A., Bolino, A., 2010. Analyzing Histopathological features of rare Charcot-Marie-Tooth Neuropathies to Unravel Their Pathogenesis. Arch. Neurol. 67, 1498–1505.

Benn, S.C., Perrelet, D., Kato, A.C., Scholz, J., Decosterd, I., Mannion, R.J., Bakowska, J.C., Woolf, C.J., 2002. Hsp27 Upregulation and Phosphorylation is required for injured sensory and motor neuron survival. Neuron 36, 45–56. https://doi.org/10.1016/S0896-6273(02)00941-8.

Benndorf, R., Martin, J.L., Kosakovsky Pond, S.L., Wertheim, J.O., 2014. Neuropathy- and myopathy-associated mutations in human small heat shock proteins: characteristics and evolutionary history of the mutation sites. Mutat. Res. Rev. Mutat. Res. 761, 15–30. https://doi.org/10.1016/j.mrrev.2014.02.004.

Benoy, V., Vanden Berghe, P., Jarpe, M., Van Damme, P., Robberecht, W., Van Den Bosch, L., 2017. Development of improved HDAC6 Inhibitors as pharmacological therapy for Axonal Charcot–Marie–Tooth Disease. Neurotherapeutics 14, 417–428. https://doi.org/10.1007/s13311-016-0501-z.

Boncoraglio, A., Minoia, M., Carra, S., 2012. The family of mammalian small heat shock proteins (HSPBs): implications in protein deposit diseases and motor neuropathies. Int. J. Biochem. Cell Biol. 44, 1657–1669. https://doi.org/10.1016/j.biocel.2012.03.011.

Bouhy, D., Geuens, T., De Winter, V., Almeida-Souza, L., Katona, I., Weis, J., Hochepied, T., Goossens, S., Haigh, J.J., Janssens, S., Timmerman, V., 2016. Characterization of New Transgenic mouse models for Two Charcot-Marie-Tooth-Causing HspB1 mutations using the Rosa26 Locus. J. Neuromuscul. Dis. 3, 183–200. https://doi.org/10.3233/JND-150144.

Braathen, G.J., Sand, J.C., Lobato, A., Høyer, H., Russell, M.B., 2011. Genetic epidemiology of Charcot-Marie-Tooth in the general population. Eur. J. Neurol. 18, 39–48. https://doi.org/10.1111/j.1468-1331.2010.03037.x.

Brownlees, J., Ackerley, S., Grierson, A.J., Jacobsen, N.J.O., Shea, K., Anderton, B.H., Leigh, P.N., Shaw, C.E., Miller, C.C.J., 2002. Charcot-Marie-Tooth disease neurofilament mutations disrupt neurofilament assembly and axonal transport. Hum. Mol. Genet. 11, 2837–2844. https://doi.org/10.1093/hmg/11.23.2837.

Brunden, K.R., Trojanowski, J.Q., Smith, A.B., Lee, V.M.Y., Ballatore, C., 2014. Microtubule-stabilizing agents as potential therapeutics for neurodegenerative disease. Bioorg. Med. Chem. 22, 5040–5049. https://doi.org/10.1016/j.bmc.2013.12.046.

Brunden, K.R., Lee, V.M.Y., Smith, A.B., Trojanowski, J.Q., Ballatore, C., 2017. Altered microtubule dynamics in neurodegenerative disease: therapeutic potential of microtubule-stabilizing drugs. Neurobiol. Dis. 105, 328–335. https://doi.org/10.1016/j.nbd.2016.12.021.

Burté, F., Carelli, V., Chinnery, P.F., Yu-Wai-Man, P., 2015. Disturbed mitochondrial dynamics and neurodegenerative disorders. Nat. Rev. Neurol. 11, 11–24.

Byrne, L.M., Rodrigues, F.B., Blennow, K., Durr, A., Leavitt, B.R., Roos, R.A.C., Scahill, R.I., Tabrizi, S.J., Zetterberg, H., Langbehn, D., Wild, E.J., 2017. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol. 16, 601–609. https://doi.org/10.1016/S1474-4422(17)30124-2.

Callaghan, B.C., Price, R.S., Feldman, E.L., Arbor, A., 2015. Diagnostic and Therapeutic advances: distal Symmetric Polyneuropathy. JAMA 314, 2172–2181. https://doi.org/10.1001/jama.2015.13611.Diagnostic.

Capponi, S., Geroldi, A., Fossa, P., Grandis, M., Ciotti, P., Gulli, R., Schenone, A., Mandich, P., Bellone, E., 2011. HSPB1 and HSPB8 in inherited neuropathies: study of an Italian cohort of dHMN and CMT2 patients. J. Peripher. Nerv. Syst. 16, 287–294. https://doi.org/10.1111/j.1529-8027.2011.00361.x.

Carlson, K., Ocean, A.J., 2011. Peripheral neuropathy with microtubule-targeting agents: occurrence and management approach. Clin. Breast Cancer 11, 73–81. https://doi.org/10.1016/j.clbc.2011.03.006.

Carra, S., Crippa, V., Rusmini, P., Boncoraglio, A., Minoia, M., Giorgetti, E., Kampinga, H.H., Poletti, A., 2012. Alteration of protein folding and degradation in motor neuron diseases: implications and protective functions of small heat shock proteins. Prog. Neurobiol. 97, 83–100. https://doi.org/10.1016/j.pneurobio.2011.09.009.

Cartoni, R., Martinou, J.-C., 2009. Role of mitofusin 2 mutations in the physiopathology of Charcot-Marie-Tooth disease type 2A. Exp. Neurol. 218, 268–273. https://doi.org/10.1016/j.expneurol.2009.05.003.

Cassereau, J., Casasnovas, C., Guegeun, N., Malinge, M.C., Guillet, V., Reynier, P., Bonneau, D., Amati-Bonneau, P., Banchs, I., Volpini, V., Procaccio, V., Chevrollier, A., 2011. Simultaneous MFN2 and GDAP1 mutations cause major mitochondrial defects in a patient with CMT. Neurology 76, 1524–1526.

Chalova, A.S., Sudnitsyna, M.V., Strelkov, S.V., Gusev, N.B., 2014. Characterization of human small heat shock proten hspb1 that carries c-terminal domain mutations associated with hereditary motor neuron diseases. Biochim. Biophys. Acta 1884, 2116–2126. https://doi.org/10.1016/j.bbapap.2014.09.005.

Charcot, J.M., Marie, P., 1886. Sur une forme particulière d'atrophie musculaire progressive souvent familiale débutant par les pieds et les jambes et atteignant plus tard les. Rev. Méd. 6, 96–138.

Choi, B., Koo, S.K., Park, M., Rhee, H., Yang, S., Choi, K., Jung, S., Kim, H.S., Hyun, Y.S., Nakhro, K., Lee, H.J., Woo, H., Chung, K.W., 2012. Exome sequencing is an efficient tool for genetic screening of Charcot–Marie–Tooth Disease. Hum. Mutat. 33, 1610–1615. https://doi.org/10.1002/humu.22143.

Chung, K.W., Kim, S.-B., Cho, S.Y., Hwang, S.J., Park, S.W., Kang, S.H., Kim, J., Yoo, J.H., Choi, B.-O., 2008. Distal hereditary motor neuropathy in Korean patients with a small heat shock protein 27 mutation. Exp. Mol. Med. 40, 304–312. https://doi.org/10.3858/emm.2008.40.3.304.

Cox, D., Ecroyd, H., 2017. The small heat shock proteins α B-crystallin (HSPB5) and Hsp27 (HSPB1) inhibit the intracellular aggregation of α-synuclein. Cell Stress Chaperones 22, 589–600. https://doi.org/10.1007/s12192-017-0785-x.

Crowe, J., Aubareda, A., McNamee, K., Przybycien, P.M., Lu, X., Williams, R.O., Bou-Gharios, G., Saklatvala, J., Dean, J.L.E., 2013. Heat shock protein B1-deficient mice display impaired wound healing. PLoS One 8, e77383. https://doi.org/10.1371/journal.pone.0077383.

d'Ydewalle, C., Krishnan, J., Chiheb, D.M., Van Damme, P., Irobi, J., Kozikowski, A.P., Vanden Berghe, P., Timmerman, V., Robberecht, W., Van Den Bosch, L., 2011. HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. Nat. Med. 17, 968–974. https://doi.org/10.1038/nm.2396.

Datskevich, P.N., Nefedova, V.V., Sudnitsyna, M.V., Gusev, N.B., 2012. Mutations of small heat shock proteins and human congenital diseases. Biochemistry 77, 1500–5014. https://doi.org/10.1134/S0006297912130081.

Delbecq, S.P., Jehle, S., Klevit, R., 2012. Binding determinants of the small heat shock protein, αB-crystallin: recognition of the "IxI" motif. EMBO J. 31, 4587–4594. https://doi.org/10.1038/emboj.2012.318.

Dequen, F., Filali, M., Larivière, R.C., Perrot, R., Hisanaga, S.I., Julien, J.P., 2010. Reversal of neuropathy phenotypes in conditional mouse model of Charcot-Marie-Tooth disease type 2E. Hum. Mol. Genet. 19, 2616–2629. https://doi.org/10.1093/hmg/ddq149.

Dierick, I., Irobi, J., De Jonghe, P., Timmerman, V., 2005. Small heat shock proteins in inherited peripheral neuropathies. Ann. Med. 37, 413–422. https://doi.org/10.1080/07853890500296410.

Dierick, I., Irobi, J., Janssens, S., Theuns, J., Lemmens, R., Jacobs, A., Corsmit, E., Hersmus, N., Van Den Bosch, L., Robberecht, W., De Jonghe, P., Van Broeckhoven, C., Timmerman, V., 2007. Genetic variant in theHSPB1 promoter region impairs the HSP27 stress response. Hum. Mutat. 28https://doi.org/10.1002/humu.9503. (830–830).

Dierick, I., Baets, J., Irobi, J., Jacobs, A., De Vriendt, E., Deconinck, T., Merlini, L., Van Den Bergh, P., Rasic, V.M., Robberecht, W., Fischer, D., Morales, R.J., Mitrovic, Z., Seeman, P., Mazanec, R., Kochanski, A., Jordanova, A., Auer-Grumbach, M., Helderman-Van Den Enden, A.T.J.M., Wokke, J.H.J., Nelis, E., De Jonghe, P., Timmerman, V., 2008. Relative contribution of mutations in genes for autosomal dominant distal hereditary motor neuropathies: a genotype-phenotype correlation study. Brain 131, 1217–1227. https://doi.org/10.1093/brain/awn029.

DiVincenzo, C., Elzinga, C.D., Medeiros, A.C., Karbassi, I., Jones, J.R., Evans, M.C., Braastad, C.D., Bishop, C.M., Jaremko, M., Wang, Z., Liaquat, K., Hoffman, C.A., York, M.D., Batish, S.D., Lupski, J.R., Higgins, J.J., 2014. The allelic spectrum of Charcot-Marie-Tooth disease in over 17,000 individuals with neuropathy. Mol. Genet. Gen. Med. 2, 522–529. https://doi.org/10.1002/mgg3.106.

Dubey, J., Ratnakaran, N., Koushika, S.P., 2015. Neurodegeneration and microtubule

dynamics: death by a thousand cuts. Front. Cell. Neurosci. 9, 1–15. https://doi.org/10.3389/fncel.2015.00343.
Dyck, P.J., Lambert, E.H., 1968. Lower motor and primary sensory neuron Diseases with Peroneal Muscular Atrophy (I). Arch. Neurol. 18, 603–618. https://doi.org/10.1001/archneur.1968.00470360025002.
Echaniz-Laguna, A., Geuens, T., Petiot, P., Pereon, Y., Adriaenssens, E., Haidar, M., Capponi, S., Maisonobe, T., Fournier, E., Dubourg, O., Degos, B., Salachas, F., Lenglet, T., Eymard, B., Delmont, E., Pouget, J., Morales, R.J., Goizet, C., Latour, P., Timmerman, V., Stojkovic, T., 2017. Axonal Neuropathies due to mutations in small Heat Shock Proteins: clinical, Genetic, and functional insights into novel mutations. Hum. Gen. Var. 38, 556–568. https://doi.org/10.1002/humu.23189.
Ekins, S., Litterman, N.K., Arnold, R.J.G., Burgess, R.W., Freundlich, J.S., Gray, S.J., Higgins, J.J., Langley, B., Willis, D.E., Notterpek, L., Pleasure, D., Sereda, M.W., Moore, A., 2015. A brief review of recent Charcot-Marie-Tooth research and priorities. F1000Research 4, 53. https://doi.org/10.12688/f1000research.6160.1.
England, J.D., Gronseth, G.S., Franklin, G., Carter, G.T., Kinsella, L.J., Cohen, J.A., Asbury, A.K., Szigeti, K., Lupski, J.R., Latov, N., Lewis, R.A., Low, P.A., Fisher, M.A., Herrmann, D., Howard, J.F., Lauria, G., Miller, R.G., Polydefkis, M., Sumner, A.J., 2009. Evaluation of distal symmetric polyneuropathy: the role of laboratory and genetic testing (an evidence-based review). Muscle Nerve 39, 116–125. https://doi.org/10.1002/mus.21226.
Evgrafov, O.V., Mersiyanova, I., Irobi, J., Van Den Bosch, L., Dierick, I., Leung, C.L., Schagina, O., Verpoorten, N., Van Impe, K., Fedotov, V., Dadali, E., Auer-Grumbach, M., Windpassinger, C., Wagner, K., Mitrovic, Z., Hilton-Jones, D., Talbot, K., Martin, J.-J., Vasserman, N., Tverskaya, S., Polyakov, A., Liem, R.K.H., Gettemans, J., Robberecht, W., De Jonghe, P., Timmerman, V., 2004. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat. Genet. 36, 602–606. https://doi.org/10.1038/ng1354.
Fontaine, J.M., Sun, X., Hoppe, A.D., Simon, S., Vicart, P., Welsh, M.J., Benndorf, R., 2006. Abnormal small heat shock protein interactions involving neuropathy-associated HSP22 (HSPB8) mutants. FASEB J. 20, 2168–2170. https://doi.org/10.1096/fj.06-5911fje.
Gaeta, M., Mileto, A., Mazzeo, A., Donato, R., Ascenti, G., Blandino, A., 2012. MRI findings, patterns of disease distribution, and muscle fat fraction calculation in five patients with Charcot-Marie-Tooth type 2 F disease. Skelet. Radiol. 41, 515–524. https://doi.org/10.1007/s00256-011-1199-y.
Gentil, B.J., Cooper, L., 2012. Molecular basis of axonal dysfunction and traffic impairments in CMT. Brain Res. Bull. 88, 444–453. https://doi.org/10.1016/j.brainresbull.2012.05.003.
Geuens, T., De Winter, V., Rajan, N., Achsel, T., Mateiu, L., Almeida-Souza, L., Asselbergh, B., Bouhy, D., Auer-Grumbach, M., Bagni, C., Timmerman, V., 2017. Mutant HSPB1 causes loss of translational repression by binding to PCBP1, an RNA binding protein with a possible role in neurodegenerative disease. Acta Neuropathol. Commun. 5, 1–15. https://doi.org/10.1186/s40478-016-0407-3.
Ghodasara, M.K., Sankhla, C.S., Lovhale, N.S., Borse, S.T., 2018. Charcot Marie Tooth disease 2F and a novel mutation from India. Neurol. India 66, 528–529. https://doi.org/10.4103/0028-3886.227317.
Gilliatt, R.W., Thomas, P.K., 1957. Extreme slowing of nerve conduction in peroneal muscular atrophy. Rheumatology 4, 104–106. https://doi.org/10.1093/rheumatology/4.3.104.
Gonzaga-Jauregui, C., Harel, T., Gambin, T., Kousi, M., Griffin, L.B., Francescatto, L., Ozes, B., Karaca, E., Jhangiani, S.N., Bainbridge, M.N., Lawson, K.S., Pehlivan, D., Okamoto, Y., Withers, M., Mancias, P., Slavotinek, A., Reitnauer, P.J., Goksungur, M.T., Shy, M., Crawford, T.O., Koenig, M., Willer, J., Flores, B.N., Pediaditrakis, I., Us, O., Wiszniewski, W., Parman, Y., Antonellis, A., Muzny, D.M., Katsanis, N., Battaloglu, E., Boerwinkle, E., Gibbs, R.A., Lupski, J.R., 2015. Exome sequence analysis suggests that genetic burden contributes to Phenotypic variability and complex Neuropathy. Cell Rep. 12, 1169–1183. https://doi.org/10.1016/j.celrep.2015.07.023.
Gutmann, L., Shy, M., 2015. Update on Charcot–Marie–Tooth disease. Curr. Opin. Neurol. 28, 462–467. https://doi.org/10.1097/WCO.0000000000000237.
Haidar, M., Timmerman, V., 2017. Autophagy as an emerging common Pathomechanism in Inherited Peripheral Neuropathies. Front. Mol. Neurosci. 10, 1–17. https://doi.org/10.3389/fnmol.2017.00143.
Haidar, M., Asselbergh, B., Adriaenssens, E., De Winter, V., Timmermans, J.P., Auer-Grumbach, M., Juneja, M., Timmerman, V., 2019. Neuropathy-causing mutations in HSPB1 impair autophagy by disturbing the formation of SQSTM1/p62 bodies. Autophagy 15, 1–18. https://doi.org/10.1080/15548627.2019.1569930.
Hao, X., Zhang, S., Timakov, B., Zhang, P., 2007. The Hsp27 gene is not required for Drosophila development but its activity is associated with starvation resistance. Cell Stress Chaperones 12, 364–372. https://doi.org/10.1379/CSC-308.1.
Harding, A., Thomas, P., 1980. The clinical features of hereditary motor and sensory neuropathy types I and II. Brain J. Neurol. 17, 259–280.
Haslbeck, M., Vierling, E., 2015. A first line of stress Defense : small Heat Shock Proteins and their function in Protein Homeostasis. J. Mol. Biol. 427, 1537–1548. https://doi.org/10.1016/j.jmb.2015.02.002.
Heilman, P.L., Song, S.W., Miranda, C.J., Meyer, K., Srivastava, A.K., Knapp, A., Wier, C.G., Kaspar, B.K., Kolb, S.J., 2017. HSPB1 mutations causing hereditary neuropathy in humans disrupt non-cell autonomous protection of motor neurons. Exp. Neurol. 297, 101–109. https://doi.org/10.1016/j.expneurol.2017.08.002.
Hickey, E., Brandon, S.E., Potter, R., Stein, G., Stein, J., Weber, L.A., 1986. Sequence and organization of genes encoding the human 27 kDa heat shock protein. Nucleic Acids Res. 14, 4127–4145. https://doi.org/10.1093/nar/11.7.1997.
Holmgren, A., Bouhy, D., De Winter, V., Asselbergh, B., Timmermans, J.-P., Irobi, J., Timmerman, V., 2013. Charcot-Marie-Tooth causing HSPB1 mutations increase Cdk5-mediated phosphorylation of neurofilaments. Acta Neuropathol. 126, 93–108. https://doi.org/10.1007/s00401-013-1133-6.
Houlden, H., De Vrie, F.W., Blake, J., Wood, N., Reilly, M.M., 2008. Mutations in the HSP27 (HSPB1) gene cause dominant, recessive, and sporadic distal HMN/CMT type 2. Neurology 71, 1660–1668.
Huang, L., Min, J., Masters, S., Mivechi, N.F., Moskophidis, D., 2007. Insights into function and regulation of small Heat Shock Protein 25 (HSPB1) in a mouse model with targeted Gene Disruption. Genesis 45, 487–501. https://doi.org/10.1002/dvg.
Ikeda, Y., Abe, A., Ishida, C., Takahashi, K., Hayasaka, K., Yamada, M., 2009. A clinical phenotype of distal hereditary motor neuronopathy type II with a novel HSPB1 mutation. J. Neurol. Sci. 277, 9–12. https://doi.org/10.1016/j.jns.2008.09.031.
Irobi, J., Van Impe, K., Seeman, P., Jordanova, A., Dierick, I., Verpoorten, N., Michalik, A., De Vriendt, E., Jacobs, A., Van Gerwen, V., Vennekens, K., Mazanec, R., Tournev, I., Hilton-Jones, D., Talbot, K., Kremensky, I., Van Den Bosch, L., Robberecht, W., Vandekerckhove, J., Van Broeckhoven, C., Gettemans, J., De Jonghe, P., Timmerman, V., 2004. Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. Nat. Genet. 36, 597–601. https://doi.org/10.1038/ng1328.
Irobi, J., Almeida-Souza, L., Asselbergh, B., de Winter, V., Goethals, S., Dierick, I., Krishnan, J., Timmermans, J.P., Robberecht, W., de Jonghe, P., van den Bosch, L., Janssens, S., Timmerman, V., 2010. Mutant HSPB8 causes motor neuron-specific neurite degeneration. Hum. Mol. Genet. 19, 3254–3265. https://doi.org/10.1093/hmg/ddq234.
Ismailov, S.M., Fedotov, V.P., Dadali, E.L., Polyakov, A.V., Van Broeckhoven, C., Ivanov, V.I., De Jonghe, P., Timmerman, V., Evgrafov, O.V., 2001. A new locus for autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2F) maps to chromosome 7q11-q21. Eur. J. Hum. Genet. 9, 646–650.
Ito, H., Kamei, K., Iwamoto, I., Inaguma, Y., Tsuzuki, M., Kishikawa, M., Shimada, A., Hosokawa, M., Kato, K., 2003. Hsp27 suppresses the formation of inclusion bodies induced by expression of R120G a B-crystallin, a cause of desmin- related myopathy. Cell. Mol. Life Sci. 60, 1217–1223. https://doi.org/10.1007/s00018-003-3024-9.
James, P.A., Rankin, J., Talbot, K., 2008. Asymmetrical late onset motor neuropathy associated with a novel mutation in the small heat shock protein HSPB1 (HSP27). J. Neurol. Neurosurg. Psychiatry 79, 461–463. https://doi.org/10.1136/jnnp.2007.125179.
Jordanova, A., De Jonghe, P., Boerkoel, C.F., Takashima, H., De Vriendt, E., Ceuterick, C., Martin, J.J., Butler, I.J., Mancias, P., Papasozomenos, S.C., Terespolsky, D., Potocki, L., Brown, C.W., Shy, M., Rita, D.A., Tournev, I., Kremensky, I., Lupski, J.R., Timmerman, V., 2003. Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease. Brain 126, 590–597. https://doi.org/10.1093/brain/awg059.
Kalmar, B., Innes, A., Wanisch, K., Kolaszynska, A.K., Pandraud, A., Kelly, G., Abramov, A.Y., Reilly, M.M., Schiavo, G., Greensmith, L., 2017. Mitochondrial deficits and abnormal mitochondrial retrograde axonal transport play a role in the pathogenesis of mutant Hsp27-induced Charcot Marie Tooth Disease. Hum. Mol. Genet. 26, 3313–3326. https://doi.org/10.1093/hmg/ddx216.
Kammoun, M., Picard, B., Astruc, T., Gagaoua, M., Aubert, D., Bonnet, M., Blanquet, V., Cassar-Malek, I., 2016. The invalidation of HspB1 gene in mouse alters the ultrastructural phenotype of muscles. PLoS One 11, 1–19. https://doi.org/10.1371/journal.pone.0158644.
Kijima, K., Numakura, C., Goto, T., Takahashi, T., Otagiri, T., Umetsu, K., Hayasaka, K., 2005. Small heat shock protein 27 mutation in a Japanese patient with distal hereditary motor neuropathy. J. Hum. Genet. 50, 473–476.
Kim, H.J., Lee, J., Bin, Hong Y., Kim, Y.J., Lee, J.H., Nam, S.H., Choi, B.-O., Chung, K.W., 2014. Ser135Phe mutation in HSPB1 (HSP27) from Charcot–Marie–Tooth disease type 2F families. Genes Gen. 37, 295–303. https://doi.org/10.1007/s13258-014-0259-9.
Kim, J.-Y., Woo, S.-Y., Bin, Hong Y., Choi, H., Kim, J., Choi, H., Mook-Jung, I., Ha, N., Kyung, J., Koo, S.K., Jung, S.-C., Choi, B.-O., 2016. HDAC6 Inhibitors rescued the defective Axonal Mitochondrial Movement in Motor neurons derived from the induced Pluripotent stem cells of peripheral Neuropathy Patients with HSPB1 mutation. Stem Cells Int. 2016, 1–14. https://doi.org/10.1155/2016/9475981.
Kostenko, S., Moens, U., 2009. Heat shock protein 27 phosphorylation : kinases, phosphatases, functions and pathology. Cell. Mol. Life Sci. 66, 3289–3307. https://doi.org/10.1007/s00018-009-0086-3.
Kriehuber, T., Rattei, T., Weinmaier, T., Bepperling, A., Haslbeck, M., Buchner, J., 2010. Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J. 24, 3633–3642. https://doi.org/10.1096/fj.10-156992.
Krishnan, J., Vannuvel, K., Andries, M., Waelkens, E., Robberecht, W., Van Den Bosch, L., 2008. Over-expression of Hsp27 does not influence disease in the mutant SOD1(G93A) mouse model of amyotrophic lateral sclerosis. J. Neurochem. 106, 2170–2183. https://doi.org/10.1111/j.1471-4159.2008.05545.x.
Lee, J.J., Swain, S.M., 2006. Peripheral neuropathy induced by microtubule-stabilizing agents. J. Clin. Oncol. 24, 1633–1642. https://doi.org/10.1200/JCO.2005.04.0543.
Lee, J., Jung, S.-C., Joo, J., Choi, Y.-R., Moon, H.W., Kwak, G., Yeo, H.K., Lee, J.-S., Ahn, H.-J., Jung, N., Hwang, S., Rheey, J., Woo, S.-Y., Kim, J.Y., Hong, Y., Bin, Choi B.-O., 2015. Overexpression of mutant HSP27 causes axonal neuropathy in mice. J. Biomed. Sci. 22. https://doi.org/10.1186/s12929-015-0154-y.
Lewis-Smith, D.J., Duff, J., Pyle, A., Griffin, H., Polvikoski, T., Birchall, D., Horvath, R., Chinnery, P.F., 2016. Novel HSPB1 mutation causes both motor neuronopathy and distal myopathy. Neurol. Genet. 2, e110. https://doi.org/10.1212/NXG.0000000000000110.
Lin, K.-P., Soong, B.-W., Yang, C.-C., Huang, L.-W., Chang, M.-H., Lee, I.-H., Antonellis, A., Antonellis, A., Lee, Y.-C., 2011. The mutational spectrum in a cohort of Charcot-Marie-Tooth disease type 2 among the Han Chinese in Taiwan. PLoS One 6, e29393. https://doi.org/10.1371/journal.pone.0029393.
Luigetti, M., Fabrizi, G.M., Madia, F., Ferrarini, M., Conte, A., Del Grande, A., Tasca, G., Tonali, P.A., Sabatelli, M., 2010. A novel HSPB1 mutation in an Italian patient with

CMT2/dHMN phenotype. J. Neurol. Sci. 298, 114–117. https://doi.org/10.1016/j.jns.2010.09.008.
Lupski, J.R., Montes de Oca-luna, R., Slaugenhaupt, S., Pentao, L., Guzzetta, V., Trask, B.J., Saucedo-Carclenas, O., Barker, D.F., Killian, J.M., Garcia, C.A., Chakravarti, A., Patel, P.I., 1991. DNA duplication associated with Charcot-Marie-Tooth Disease Type 1A. Cell 66, 219–232.
Lupski, J.R., Reid, J.G., Gonzaga-Jauregui, C., Rio Deiros, D., Chen, D.C., Nazareth, L., Bainbridge, M., Dinh, H., Jing, C., Wheeler, D.A., Mcguire, A.L., Zhang, F., Satnkiewicz, P., Halperin, J.L., Yang, C., Gehman, C., Guo, D., Irikat, R.K., Tom, W., Fantin, N.J., Muzny, D.M., Muzny, D.M., Gibbs, R.A., 2010. Whole-Genome sequencing in a patient with Charcot–Marie–Tooth Neuropathy. N. Engl. J. Med. 362, 1181–1191.
MacMillan, J.C., Harper, P.S., 1994. The Charcot-Marie-Tooth syndrome: clinical aspects from a population study in South Wales, UK. Clin. Genet. 45, 128–134. https://doi.org/10.1111/j.1399-0004.1994.tb04009.x.
Maeda, K., Idehara, R., Hashiguchi, A., Takashima, H., 2014. A Family with Distal Hereditary Motor Neuropathy and a K141Q mutation of small Heat Shock Protein HSPB1. Intern. Med. 53, 1655–1658. https://doi.org/10.2169/internalmedicine.53.2843.
Mandich, P., Grandis, M., Varese, A., Geroldi, A., Acquaviva, M., Ciotti, P., Gulli, R., Doria-lamba, L., Fabrizi, G.M., Giribaldi, G., Pizzuti, A., Schenone, A., 2010. Severe Neuropathy after Diphtheria- Tetanus-Pertussis Vaccination in a Child Carrying a Novel Frame-Shift mutation in the small Heat-Shock Protein 27 Gene. J. Child Neurol. 25, 107–109.
Mata, M., Sun, X., Fink, D., 2015. Regulation of Actin interacting Protein Drebrin by mutations in HSPB1. FASEB J. 29.
Mathis, S., Goizet, C., Tazir, M., Magdelaine, C., Lia, A., Magy, L., Vallat, J., 2015. Charcot – Marie – Tooth diseases : an update and some new proposals for the classi fi cation. J. Med. Genet. 52, 681–690. https://doi.org/10.1136/jmedgenet-2015-103272.
Mersiyanova, I.V., Perepelov, A.V., Polyakov, A.V., Sitnikov, V.F., Dadali, E.L., Oparin, R.B., Petrin, A.N., Evgrafov, O.V., 2000. A new variant of Charcot-Marie-Tooth Disease Type 2 Is Probably the Result of a Mutation in the Neurofilament-Light Gene. Am. J. Hum. Genet. 67, 37–46. https://doi.org/10.1086/302962.
Mogk, A., Bukau, B., 2017. Role of sHsps in organizing cytosolic protein aggregation and disaggregation. Cell Stress Chaperones 22, 493–502. https://doi.org/10.1007/s12192-017-0762-4.
Muranova, L.K., Weeks, S.D., Strelkov, S.V., Gusev, N.B., 2015. Characterization of mutants of human small heat shock protein HspB1 carrying replacements in the N-terminal domain and associated with hereditary motor neuron diseases. PLoS One 10, 1–24. https://doi.org/10.1371/journal.pone.0126248.
Nefedova, V.V., Sudnitsyna, M.V., Strelkov, S.V., Gusev, N.B., 2013a. Structure and properties of G84R and L99M mutants of human small heat shock protein HspB1 correlating with motor neuropathy. Arch. Biochem. Biophys. 538, 16–24. https://doi.org/10.1016/j.abb.2013.07.028.
Nefedova, V.V., Datskevich, P.N., Sudnitsyna, M.V., Strelkov, S.V., Gusev, N.B., 2013b. Biochimie Physico-chemical properties of R140G and K141Q mutants of human small heat shock protein HspB1 associated with hereditary peripheral neuropathies. Biochimie 95, 1582–1592. https://doi.org/10.1016/j.biochi.2013.04.014.
Nicholson, G., Myers, S., 2006. Intermediate forms of Charcot-Marie-Tooth Neuropathy. Neuro Mol. Med. 8, 123–130. https://doi.org/10.1385/NMM.
Nishibayashi, M., Kokubun, N., Nakamura, A., Hirata, K., Yamamoto, M., Sobue, G., 2007. Distal hereditary motor neuropathy type II with mutation in heat shock protein 27 gene. A Case Rep. Clin. Neurol. 47, 50–52.
Oberstadt, M., Mitter, D., Classen, J., Baum, P., 2016. Late onset dHMN II caused by c.404C > G mutation in HSPB1 gene. J. Peripher. Nerv. Syst. 21, 111–113. https://doi.org/10.1111/jns.12165.
Outeiro, T.F., Klucken, J., Strathearn, K.E., Liu, F., Nguyen, P., Rochet, J.-C., Hyman, B.T., McLean, P.J., 2006. Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. Biochem. Biophys. Res. Commun. 351, 631–638. https://doi.org/10.1016/j.bbrc.2006.10.085.
Pareyson, D., Saveri, P., Sagnelli, A., Piscosquito, G., 2015. Mitochondrial dynamics and inherited peripheral nerve diseases. Neurosci. Lett. 596, 66–77. https://doi.org/10.1016/j.neulet.2015.04.001.
Pareyson, D., Saveri, P., Pisciotta, C., 2017. New developments in Charcot–Marie–Tooth neuropathy and related diseases. Curr. Opin. Neurol. 30, 471–480. https://doi.org/10.1097/WCO.0000000000000474.
Pearce, J.M., 2000. Howard Henry Tooth (1856-1925). J. Neurol. 247, 3–4.
Penno, A., Reilly, M.M., Houlden, H., Laurá, M., Rentsch, K., Niederkofler, V., Stoeckli, E.T., Nicholson, G., Eichler, F., Brown, R.H., von Eckardstein, A., Hornemann, T., 2010. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. J. Biol. Chem. 285, 11178–11187. https://doi.org/10.1074/jbc.M109.092973.
Perrin, V., Régulier, E., Abbas-Terki, T., Hassig, R., Brouillet, E., Aebischer, P., Luthi-Carter, R., Déglon, N., 2007. Neuroprotection by Hsp104 and Hsp27 in Lentiviral-based Rat Models of Huntington's Disease. Mol. Ther. J. Am. Soc. Gene Ther. 15, 677–686. https://doi.org/10.1038/mt.
Prior, R., Van Helleputte, L., Benoy, V., Van Den Bosch, L., 2017. Defective axonal transport: a common pathological mechanism in inherited and acquired peripheral neuropathies. Neurobiol. Dis. 105, 300–320. https://doi.org/10.1016/j.nbd.2017.02.009.
Raju, I., Abraham, E.C., 2013. Biochemical and biophysical research communications mutants of human a B-crystallin cause enhanced protein aggregation and apoptosis in mammalian cells : influence of co-expression of HspB1. Biochem. Biophys. Res. Commun. 430, 107–112. https://doi.org/10.1016/j.bbrc.2012.11.051.
Rossor, A.M., Davidson, G.L., Blake, J., Polke, J.M., Murphy, S.M., Houlden, H., Innes, A., Kalmar, B., Greensmith, L., Reilly, M.M., 2012a. A novel p.Glu175X premature stop mutation in the C-terminal end of HSP27 is a cause of CMT2. J. Peripher. Nerv. Syst. 17, 201–205. https://doi.org/10.1111/j.1529-8027.2012.00400.x.
Rossor, A.M., Kalmar, B., Greensmith, L., Reilly, M.M., 2012b. The distal hereditary motor neuropathies. J. Neurol. Neurosurg. Psychiatry 83, 6–14. https://doi.org/10.1136/jnnp-2011-300952.
Rossor, A.M., Tomaselli, P.J., Reilly, M.M., 2016. Recent advances in the genetic neuropathies. Curr. Opin. Neurol. 29, 537–548. https://doi.org/10.1097/WCO.0000000000000373.
Saporta, M.A., 2014. Charcot-Marie-Tooth Disease and Other Inherited Neuropathies. Continuum (N Y) 20, 1208–1225.
Saporta, M.A., Dang, V., Volfson, D., Zou, B., Xie, X.S., Adebola, A., Liem, R.K., Shy, M., Dimos, J.T., 2015. Axonal Charcot-Marie-Tooth disease patient-derived motor neurons demonstrate disease-specific phenotypes including abnormal electrophysiological properties. Exp. Neurol. 263, 190–199. https://doi.org/10.1016/j.expneurol.2014.10.005.
Schwartz, N.U., Linzer, R.W., Truman, J.-P., Gurevich, M., Hannun, Y.A., Senkal, C.E., Obeid, L.M., 2018. Decreased ceramide underlies mitochondrial dysfunction in Charcot-Marie-Tooth 2F. FASEB J. 32https://doi.org/10.1096/fj.201701067R. fj.201701067R.
Shen, S., Kozikowski, A.P., 2016. Why Hydroxamates may not be the best Histone Deacetylase Inhibitors - what some may Have Forgotten or Would Rather Forget? ChemMedChem 11, 15–21. https://doi.org/10.1002/cmdc.201500486.
Shen, S., Benoy, V., Bergman, J.A., Kalin, J.H., Frojuello, M., Vistoli, G., Haeck, W., Van Den Bosch, L., Kozikowski, A.P., 2016. Bicyclic-Capped Histone Deacetylase 6 Inhibitors with improved activity in a model of Axonal Charcot-Marie-Tooth Disease. ACS Chem. Neurosci. 7, 240–258. https://doi.org/10.1021/acschemneuro.5b00286.
Sheng, Z.H., 2014. Mitochondrial trafficking and anchoring in neurons: new insight and implications. J. Cell Biol. 204, 1087–1098. https://doi.org/10.1083/jcb.201312123.
Skre, H., 1974. Genetic and clinical aspects of Carcat-Marie-Tooth's disease. Clin. Genet. 6, 98–118.
Solla, P., Vannelli, A., Bolino, A., Marrosu, G., Coviello, S., Murru, M.R., Tranquilli, S., Corongiu, D., Benedetti, S., Marrosu, M.G., 2010. Heat shock protein 27 R127W mutation: evidence of a continuum between axonal Charcot-Marie-Tooth and distal hereditary motor neuropathy. J. Neurol. Neurosurg. Psychiatry 81, 958–962. https://doi.org/10.1136/jnnp.2009.181636.
Srivastava, A.K., Renusch, S.R., Naiman, N.E., Gu, S., Sneh, A., Arnold, W.D., Sahenk, Z., Kolb, S.J., 2012. Mutant HSPB1 overexpression in neurons is sufficient to cause age-related motor neuronopathy in mice. Neurobiol. Dis. 47, 163–173. https://doi.org/10.1016/j.nbd.2012.03.035.
Stancanelli, C., Fabrizi, G.M., Ferrarini, M., Cavallaro, T., Taioli, F., Di Leo, R., Russo, M., Gentile, L., Toscano, A., Vita, G., Mazzeo, A., 2015. Charcot-Marie-Tooth 2F: phenotypic presentation of the Arg136Leu HSP27 mutation in a multigenerational family. Neurol. Sci. 36, 1003–1006. https://doi.org/10.1007/s10072-014-2050-8.
Stromer, T., Ehrnsperger, M., Gaestel, M., Buchner, J., 2003. Analysis of the interaction of Small Heat Shock Proteins with Unfolding Proteins. J. Biol. Chem. 278, 18015–18021. https://doi.org/10.1074/jbc.M301640200.
Sun, Y., Macrae, T.H., 2005. Small heat shock proteins: molecular structure and chaperone function. Cell. Mol. Life Sci. 62, 2460–2476. https://doi.org/10.1007/s00018-005-5190-4.
Szigeti, K., Lupski, J.R., 2009. Charcot–Marie–Tooth disease. Eur. J. Hum. Genet. 17, 703–710. https://doi.org/10.1038/ejhg.2009.31.
Tanabe, H., Higuchi, Y., Yuan, J.H., Hashiguchi, A., Yoshimura, A., Ishihara, S., Nozuma, S., Okamoto, Y., Matsuura, E., Ishiura, H., Mitsui, J., Takashima, R., Kokubun, N., Maeda, K., Asano, Y., Sunami, Y., Kono, Y., Ishigaki, Y., Yanamoto, S., Fukae, J., Kida, H., Morita, M., Tsuji, S., Takashima, H., 2018. Clinical and genetic features of Charcot-Marie-Tooth disease 2F and hereditary motor neuropathy 2B in Japan. J. Peripher. Nerv. Syst. 23, 40–48. https://doi.org/10.1111/jns.12252.
Tang, B.-S., Luo, W., Xia, K., Xiao, J.-F., Jiang, H., Shen, L., Tang, J.-G., Zhao, G.-H., Cai, F., Pan, Q., Dai, H.-P., Yang, Q.-D., Xia, J.-H., Evgrafov, O.V., 2004. A new locus for autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2L) maps to chromosome 12q24. Hum. Genet. 114, 527–533. https://doi.org/10.1007/s00439-004-1102-1.
Tang, B., Liu, X., Zhao, G., Luo, W., Xia, K., Pan, Q., Cai, F., Hu, Z., Zhang, C., Chen, B., Zhang, F., Shen, L., Zhang, R., Jiang, H., 2005a. Mutation analysis of the small heat shock protein 27 gene in chinese patients with Charcot-Marie-Tooth disease. Arch. Neurol. 62, 1201–1207. https://doi.org/10.1001/archneur.62.8.1201.
Tang, B.S., Zhao Hua, G., Luo, W., Xia, K., Cai, F., Pan, Q., Zhang Xu, R., Zhang Feng, F., Liu Min, X., Chen, B., Zhang, C., Shen, L., Jiang, H., Long Gao, Z., Dai Ping, H., 2005b. Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. Hum. Genet. 116, 222–224. https://doi.org/10.1007/s00439-004-1218-3.
Teunissen, Laurien, Notermans, N., 2003. Disease Course of Charcot-Marie-Tooth Disease Type 2. Arch. Neurol. 60, 823–828.
Timmerman, V., Strickland, A.V., Zuchner, S., 2014. Genetics of Charcot-Marie-Tooth (CMT) disease within the frame of the human genome project success. Genes (Basel) 5, 13–32. https://doi.org/10.3390/genes5010013.
Tooth, H.H., 1886. The Peroneal Type of Progressive Muscular Atrophy : Thesis for the Degree of M.D. in the University of Cambridge.
Tóth, M.E., Szegedi, V., Varga, E., Juhász, G., Horváth, J., Borbély, E., Csibrány, B., Alföldi, R., Lénárt, N., Penke, B., Sántha, M., 2013. Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. Cell Stress Chaperones 18, 759–771. https://doi.org/10.1007/s12192-013-0428-9.
Tradewell, M.L., Durham, H.D., Mushynski, W.E., Gentil, B.J., 2009. Mitochondrial and Axonal Abnormalities Precede Disruption of the Neurofilament Network in a Model of Charcot-Marie-Tooth Disease Type 2E and Are Prevented by Heat Shock Proteins in a Mutant-Specific Fashion. J. Neuropathol. Exp. Neurol. 68, 642–652.

Tucker, N.R., Ustyugov, A., Bryantsev, A.L., Konkel, M.E., Shelden, 2009. Hsp27 is persistently expressed in zebrafish skeletal and cardiac muscle tissues but dispensable for their morphogenesis. Cell Stress Chaperones 14, 521–533. https://doi.org/10.1007/s12192-009-0105-1.

Vidyasagar, A., Wilson, N.A., Djamali, A., 2012. Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair 5, 7. https://doi.org/10.1186/1755-1536-5-7.

Vital, A., Latour, P., Sole, G., Ferrer, X., Rouanet, M., Tison, F., Vital, C., Goizet, C., 2012. A French family with Charcot-Marie-Tooth disease related to simultaneous heterozygous MFN2 and GDAP1 mutations. Neuromuscul. Disord. 22, 735–741. https://doi.org/10.1016/j.nmd.2012.04.001.

Wagstaff, M.J.D., Collaco-Moraes, Y., Smith, J., de Belleroche, J.S., Coffin, R.S., Latchman, D.S., 1999. Protection of Neuronal Cells from Apoptosis by Hsp27 Delivered with a Herpes Simplex Virus-based Vector. J. Biol. Chem. 274, 5061–5069. https://doi.org/10.1074/jbc.274.8.5061.

Wilhelmus, M.M.M., Boelens, W.C., Otte-höller, I., Kamps, B., De Waal, R.M.W., Verbeek, M.M., 2006. Small heat shock proteins inhibit amyloid- β protein aggregation and cerebrovascular amyloid- β protein toxicity. Brain Res. 1089, 67–78. https://doi.org/10.1016/j.brainres.2006.03.058.

Wyttenbach, A., Sauvageot, O., Carmichael, J., Diaz-latoud, C., Arrigo, A., Rubinsztein, D.C., 2002. Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. 11, 1137–1151.

Yerbury, J.J., Gower, D., Vanags, L., Roberts, K., Lee, J.A., Ecroyd, H., 2013. The small heat shock proteins α B-crystallin and Hsp27 suppress SOD1 aggregation in vitro. Cell Stress Chaperones 18, 251–257. https://doi.org/10.1007/s12192-012-0371-1.

Ylikallio, E., Johari, M., Konovalova, S., Moilanen, J.S., Kiuru-enari, S., Auranen, M., Pajunen, L., Tyynismaa, H., 2014. Targeted next-generation sequencing reveals further genetic heterogeneity in axonal Charcot–Marie–Tooth neuropathy and a mutation in HSPB1. Eur. J. Hum. Genet. 22, 522–527. https://doi.org/10.1038/ejhg.2013.190.

Ylikallio, E., Konovalova, S., Dhungana, Y., Hilander, T., Junna, N., Partanen, J.V., 2015. Truncated HSPB1 causes axonal neuropathy and impairs tolerance to unfolded protein stress. BBA Clin. 3, 233–242. https://doi.org/10.1016/j.bbacli.2015.03.002.

Yuan, A., Rao, M.V., Veeranna, Nixon, R.A., 2012. Neurofilaments at a glance. J. Cell Sci. 125, 3257–3263. https://doi.org/10.1242/jcs.104729.

Zhai, J., Lin, H., Julien, J.-P., Schlaepfer, W.W., 2007. Disruption of neurofilament network with aggregation of light neurofilament protein: a common pathway leading to motor neuron degeneration due to Charcot-Marie-Tooth disease-linked mutations in NFL and HSPB1. Hum. Mol. Genet. 16, 3103–3116. https://doi.org/10.1093/hmg/ddm272.

Zhang, X., Shi, J., Tian, J., Robinson, A.C., Davidson, Y.S., Mann, D.M., 2014. Expression of one important chaperone protein, heat shock protein 27, in neurodegenerative diseases. Alzheimers Res. Ther. 6, 78. https://doi.org/10.1186/s13195-014-0078-x.

Zourlidou, A., Payne Smith, M.D., Latchman, D.S., 2004. HSP27 but not HSP70 has a potent protective effect against α-synuclein-induced cell death in mammalian neuronal cells. J. Neurochem. 88, 1439–1448. https://doi.org/10.1046/j.1471-4159.2003.02273.x.

Zourlidou, A., Gidalevitz, T., Kristiansen, M., Landles, C., Woodman, B., Wells, D.J., Latchman, D.S., de Belleroche, J., Tabrizi, S.J., Morimoto, R.I., Bates, G.P., 2007. Hsp27 overexpression in the R6/2 mouse model of Huntington's disease: chronic neurodegeneration does not induce Hsp27 activation. Hum. Mol. Genet. 16, 1078–1090. https://doi.org/10.1093/hmg/ddm057.