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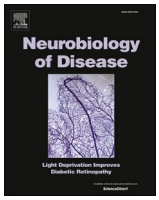

Review

Charcot-Marie-tooth disease type 2A: An update on pathogenesis and therapeutic perspectives

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ABSTRACT

Mutations in the gene encoding MFN2 have been identified as associated with Charcot–Marie–Tooth disease type 2A (CMT2A), a neurological disorder characterized by a broad clinical phenotype involving the entire nervous system. MFN2, a dynamin-like GTPase protein located on the outer mitochondrial membrane, is well-known for its involvement in mitochondrial fusion. Numerous studies have demonstrated its participation in a network crucial for various other mitochondrial functions, including mitophagy, axonal transport, and its controversial role in endoplasmic reticulum (ER)-mitochondria contacts. Considerable progress has been made in the last three decades in elucidating the disease pathogenesis, aided by the generation of animal and cellular models that have been instrumental in studying disease physiology. A review of the literature reveals that, up to now, no definitive pharmacological treatment for any CMT2A variant has been established; nonetheless, recent years have witnessed substantial progress. Many treatment approaches, especially concerning molecular therapy, such as histone deacetylase inhibitors, peptide therapy to increase mitochondrial fusion, the new therapeutic strategies based on MF1/MF2 balance, and SARM1 inhibitors, are currently in preclinical testing. The literature on gene silencing and gene replacement therapies is still limited, except for a recent study by Rizzo et al.(Rizzo et al., 2023), which recently first achieved encouraging results in in vitro and in vivo models of the disease. The near-future goal for these promising therapies is to progress to the stage of clinical translation.

1. Introduction

Charcot-Marie-Tooth disease (CMT) includes a wide spectrum of primary inherited sensory-motor neuropathies, also defined as hereditary sensory and motor neuropathies (HSMN). Characterized by the degeneration of the peripheral nerves' axonal or myelin structures, diseases within this spectrum exhibit pathological and molecular features impacting the motor and sensory neurons (Laurá et al., 2019; Reilly et al., 2011). The overall prevalence of the disease has been estimated at 1/2000-2500, making it the most common inherited neuromuscular disorder (Braathen, 2012). Its transmission is predominantly autosomal dominant, although some subtypes of autosomal recessive axonal and demyelinating CMT have been described. From a clinical perspective, CMT closely overlap with distal hereditary motor neuropathy (dHMN), a pure length-dependent motor nerve syndrome with no sensory involvement. CMTs can be classified based on their neurophysiological properties and inheritance patterns. Demyelinating CMT type 1 is distinguished by a decrease in motor nerve conduction velocity (MNCV), whereas axonal CMT type 2 exhibits normal motor nerve conduction velocity (Laurá et al., 2019). The most common form of CMT is type 2A, caused by mutations in the mitochondrial GTPase mitofusin 2 (OMIM 609260), accounting for around 30-40% of axonal CMT cases and representing 4-7% of all CMTs with a confirmed genetic diagnosis (Braathen, 2012; Fridman et al., 2015; Murphy et al., 2012). CMT2A patients present with a "classic phenotype" characterized by mild weakness and sensory loss during the first two decades of life, with slow progression thereafter. Some of patients experience pure motor neuropathies, while many others have significant loss of proprioception in addition to weakness. This suggests that in some cases, there is involvement of large-diameter sensory axons or their perikaryons, while

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in other cases they are spared. The distinction between motor and sensory involvement is not specific to the mutation, as the same mutations may cause both motor and sensorimotor phenotypes (Feely et al., 2011; Pipis et al., 2021). CMT2A is associated with mutations in the nuclear-encoded mitochondrial gene *mitofusin 2 (MFN2),* which is translated into the 757-amino acid long protein Mitofusin2 (MFN2) (Stuppia et al., 2015). The human *MFN2* gene is located at chromosome 1p (GRCh18 *1:11,980,181-12,015,211;* NM_014874.4) and has 19 exons. Two distinct variants of the transcript encoding the identical protein have been discovered: variant 1 (NM_014874.4) 4685 base pairs long and variant 2 (NM_001127660) 4540 base pairs long, which is distinct from the first for the untranslated region in the 5' UTR. The gene has ubiquitous expression in all human tissues; it is more abundant in heart, skeletal muscle, adipose tissue and liver (Casasnovas Pons et al., 2010). Although MFN2 is ubiquitously expressed, the predominant consequence of *MFN2* mutations is axonal neuropathy (Detmer and Chan, 2007). The significance of maintaining appropriate mitochondrial dynamics in motor axons has been discussed in previous studies (Detmer et al., 2008). This phenomenon can be attributed to the specific composition of peripheral nerves, where mitochondria must travel along the axon for extended distances, and the existence of a mitochondrial network is crucial for the maintenance of peripheral nerve axons (Kijima et al., 2005).

More than 100 *MFN2* mutations have been detected in CMT2A patients, predominantly in the form of missense mutations. However, a smaller proportion consists of nonsense variants or deletions.

Although few recessive and semi-dominant forms have been described (Nicholson et al., 2008; Piscosquito et al., 2015; Polke et al., 2011; Tomaselli et al., 2016), CMT2A is generally associated with heterozygous *MFN2* mutations, many of which are de novo dominant mutations (Cartoni and Martinou, 2009; Chen et al., 2003; Detmer and Chan, 2007; Feely et al., 2011; Pipis et al., 2021; Züchner et al., 2004). *MFN2* mutations seem to induce disease through a "dominant-negative" mechanism, meaning that expression of this variant results in prolific and stable mitochondrial tethering that also blocks mitochondrial fusion by endogenous wild-type mitofusins (Amiott et al., 2008; Baloh et al., 2007; Feely et al., 2011; Loiseau et al., 2007; Misko et al., 2010). These considerations emphasize the importance of maintaining proper functionality of the native protein.

As documented in other case series, the majority of mutations are localized within the highly conserved amino-terminal GTP binding domain and coiled-coil domains (heptad repeat HR1-2) that are mainly involved in the regulation of mitochondria-related processes, such as mitochondrial fusion, mitochondrial transport along axons, and mitophagy (Fig. 1) (Pipis et al., 2021). This indicates the need for further investigation into how the specific location of the domain mutation affects the characteristics of mitochondrial metabolism. Most of them (p.R94Q, p.R104W, p.T236M, p.S249C and p.R280H) are located in the GTPase domain of MFN2 and exhibit a diminished capacity to hydrolyze GTP to GDP, which is the final step in mitochondrial fusion. Consequently, this leads to the formation of shortened and depolarized "clumped mitochondria" that remain tethered together but are unable to undergo fusion due to the absence of GTPase activity [35]. The impact of loss-of-function mutations in the HR1 domain on the formation of perinuclear mitochondrial clumping aggregates with impaired GTP catalytic activity remains uncertain. An alternative hypothesis is that these mutations in the HR1 domain promote an inactive conformation of mitofusin, rendering it unable to complete the fusion process. As explained by Franco et al. (Franco et al., 2022)prolonged exposure to the mitofusin agonist MiM 111 effectively reverses the mitochondrial fusion dysfunction observed in the HR1 mutant (M376A) by promoting an open conformation. This finding suggests a potential therapeutic approach for addressing this variant. In a recent study conducted by Abati et al. (Abati et al., 2022), a novel heterozygous missense variant (p.K357E) was identified. This variant is located outside the functional domain, specifically within a helix bundle formed by helices from the N-terminal and C-terminal regions of the MFN2 protein, within the highly conserved R3 region. This helix bundle plays a crucial role in the proper expression of the GTPase domain. Additionally, the structural integrity of this helix bundle enables close interaction between the membranes of neighboring mitochondria, thereby facilitating precise mitochondrial fusion (Figs. 2 and 7).

1.1. The protein Mitofusin 2 and its interplay with Mitofusin 1 protein

Mammals express two Mitofusin (Mfn) paralogs, Mfn1 and Mfn2. In humans, MFN1 (UniProt ID: Q8IWA4, 741 amino acid residues [aa]) and MFN2 (UniProt ID: O95140, 757 aa) protein sequences share a 63% identity, 81% similarity and display a certain grade of functional redundancy in mice (Chen et al., 2003; Koshiba et al., 2004a). Both MFN1/2 are dynamin-like GTPases, embedded in the outer mitochondrial membrane (OMM) via two adjacent transmembrane regions (Chen et al., 2003; Li et al., 2019; Song et al., 2009). The relative mRNA or protein expression levels of MFN1 and MFN2 vary between tissues: MFN1 predominates in heart, liver, pancreas, adrenal glands and testis, whereas MFN2 is more abundant in heart, skeletal muscle, adrenal glands and brown adipose tissue and brain (Chandhok et al., 2018; Santel et al., 2003). In nerve cells, MFN1 exhibits lower basal expression, rendering these cells more vulnerable to harm caused by MFN2 mutations, particularly affecting the lower motor and dorsal root ganglia (DRG) neurons along with their extensive axons (Chen et al., 2007; Eura et al., 2003; Misko et al., 2010). The co-existence of two isoforms of MFN in mammalian cells raises the question as to whether they play similar, separate or dependent biological roles.

At a molecular level, the best-characterized function of MFN1 and MFN2 is to mediate the fusion of the OMM after GTP hydrolysis (Li et al., 2019). Before mitochondrial fusion, MFN1 and MFN2 interact with each other on the OMMs of juxtaposed mitochondria and form three distinct molecular complexes that are capable of promoting mitochondrial fusion–MFN1 homotypic oligomers, MFN2 homotypic oligomers, and MFN1-MFN2 heterotypic oligomers (Detmer and Chan, 2007; Samanas et al., 2020), a process named mitochondrial tethering (Franco et al., 2016; Zhou et al., 2021).

1.2. Mechanisms of MFN2 dysfunction

1.2.1. Mitochondrial fusion

Though the exact molecular mechanisms by which MFNs facilitate this process are still not fully understood, groundbreaking studies have demonstrated that MFNs, present on the OMM of two neighboring mitochondria, physically interact in a trans manner. This interaction occurs through the formation of antiparallel dimers between their HR2 domains (Koshiba et al., 2004b). Interestingly, a recent paper suggested the presence of two distinguishable and evolving conformational states in mammalian MFNs (Franco et al., 2016). According to this model, the MFNs in the resting state are tethering-non-permissive due to

Fig. 1. Schematic representation of MFN2 protein structure. The scheme represents the linear structure of MFN2. Note the large N-terminal GTPase domain, followed by the HR1- domain, the PR domain, the two TM domains, and the C-terminal HR2 domain. The numbers above indicate the initial and the terminal amino acids of the corresponding domains.

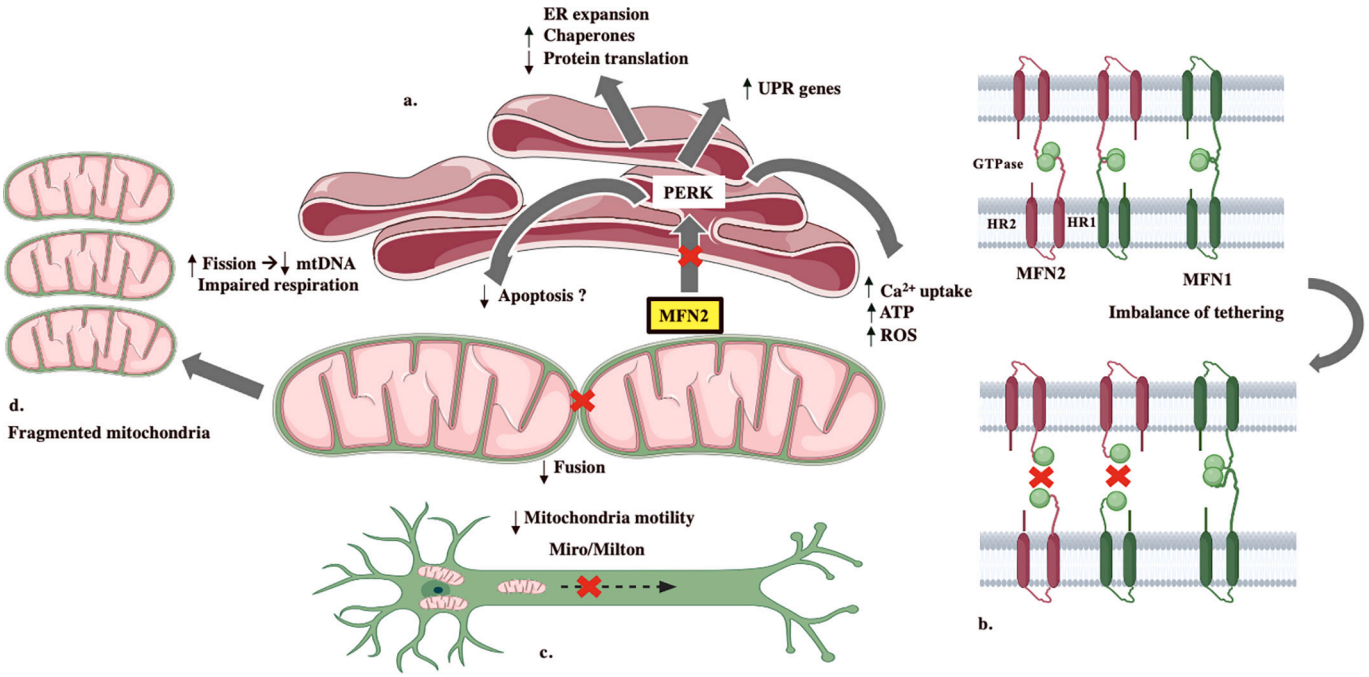

Fig. 2. Schematic representation of pathogenesis of CMT2A. a. Mfn2 ablation triggers the activation of UPR proteins present in ER membranes. Specifically, MFN2 has been suggested as an upstream regulator of PERK, which, under normal conditions, keeps the kinase in an inactive state. Upon activation, the UPR functions by expanding the ER, increasing chaperone levels, and inhibiting protein translation. Mitochondria exhibit elevated Ca2+ absorption and ATP generation to meet the heightened energy requirements. It remains uncertain whether the deletion of Mfn2 leads to an augmentation or reduction of ER stress-induced apoptosis. b. Scheme of the OMM-fusion activity of MFNs. Tethering is mediated by the interaction between HR2 domains belonging to MFNs on opposite OMM. Recent data suggest that dimerization of the GTPase domains, as well as a power stroke due to GTP hydrolysis, may be important for fusion. c. MFN2 has been proposed to be essential for the transport of mitochondria along axons, being involved in their attachment to microtubules through interaction with the two main motor proteins Miro and Milton. d. Excessive fragmentation can generate mitochondria that lack mtDNA, leading to impaired respiration.

intramolecular, antiparallel interactions between HR1 and HR2, and strict adherence of the globular GTPase domain to the OMM. However, in the tethering-permissive state, the destabilization of the intramolecular HR1-HR2 interaction permits the HR2 domain to extend into the cytosol, where it can come into contact with and bind HR2 domains of MFNs in the opposing membrane, mediating tethering.

Interestingly, it has been demonstrated that a TAT-peptide-mediated strategy is effective in destabilizing the interaction between HR1 and HR2, which is not normally permissive. This strategy corrects the fusion defects that are observed with certain MFN2 mutants associated with CMT2A (see below) (Franco et al., 2016).

1.2.2. The controversial role in endoplasmic reticulum-mitochondria contacts and endoplasmic reticulum stress

While its undisputed function in mitochondrial fusion, MFNs have also been proposed as a crucial controller of the juxtaposition between endoplasmic reticulum (ER) and mitochondria. Interestingly, a fraction of MFN2 is also located at ER membrane, in particular at specialized sites of contacts with mitochondria called mitochondria-associated membranes (MAM).

However, the precise role of MFNs in this interaction between organelles continues to be a topic of intense debate. It is our partial opinion, however, that although the significant role of MFN2 in the modulation of ER-mitochondria tethering is universally acknowledged, the discovery of either an increased (Filadi et al., 2015; Cosson et al., 2012) or, at the very least, a mostly present ER-mitochondria connection in MFN2-KO cells (de Brito and Scorrano, 2008) (Naon et al., 2016) inevitably implies that this protein is not truly essential in the formation and maintenance of this inter-organelle tethering. Further investigations will be required to more precisely assess the influence of MFN2 in this crucial intracellular pathway. Finally, the ER is a key storage repository of intracellular Ca^{2+}, and Ca^{2+} that is released from the ER can be taken up by mitochondria. However, the proximity between mitochondria and ER, and thus mitochondria-ER contact sites (MERCs), are critical for this process.

Another hypothetical role performed by MFN2, which is connected to its participation in the association between the ER and mitochondria, relates to the response of ER stress. The ER stress response constitutes a process triggered by a variety of conditions that disturb protein folding within the organelle, e.g., protein synthesis impairment, Ca2+ imbalance, and redox capacity defects. During evolution, cells have developed a complex signal transduction mechanism, the unfolded protein response (UPR), that aims at clearing unfolded proteins and restoring ER homeostasis. In particular, Zorzano and colleagues(Muñoz et al., 2013) reported the induction of UPR mediators in MFN2-deficient MEFs under basal or ER stress conditions. MFN2 has been proposed as an upstream modulator of PERK protein kinase RNA (PKR)-like ER kinase, a specialized protein located in ER membranes which is responsible for recognizing unfolded proteins accumulation and activate specific signaling pathways. Once activated, UPR works by expanding the ER, upregulating chaperones and inhibiting protein translation. It is still unclear whether Mfn2 ablation induces an increase or decrease of ER stress-induced apoptosis (Ngoh et al., 2012).

1.2.3. Mitophagy

Furthermore, MFN2 performs a vital function in mitophagy, which is the extensively comprehended process for eliminating mitochondria, together with the mitochondrial serine-threonine kinase PTEN induced putative kinase 1 (PINK1) and the cytosolic ubiquitin ligase PARK2/Parkin. This process entails the degradation of cellular components, including mitochondria, by enclosing them in autophagosomes that fusion with lysosomes (Karbowski and Youle, 2011). Autophagy fulfils a crucial function in cellular health by decomposing protein aggregates and regenerating organelles, thereby ensuring the provision of vital

molecules during periods of nutrient scarcity (Webster et al., 2014). Mitophagy is regulated intricately through post-translational modifications such as ubiquitination and deacetylation of lysine residues. Various proteins, including Sirtuine1 (Sirt1), a cellular energy sensor, PINK1, Parkin, an E3 ubiquitin ligase, and MFN2, govern the dynamics of mitophagy. In cases where there is consistent damage to mitochondria, PINK1 phosphorylates MFN2, which in turn recruits Parkin. Parkin then ubiquitinates MFN2 and other proteins present in the outer mitochondrial membrane. This process leads to the recruitment of the adaptor protein p62/Sequestome 1, which binds to LC3-II on the autophagosome, ultimately resulting in the degradation of mitochondria (Chen and Dorn, 2013; Gegg et al., 2010; Tanaka et al., 2010; Webster et al., 2014). In addition, Jiajia Li et al. (Li et al., 2022) have untangled these intricate relationships and gained a deeper understanding of how PINK1-mitofusin interactions coordinate mitochondrial fate. The study has elucidated the tight dependence of this metabolic pathway on the site-specific phosphorylation of MFN2 (T111, S378, and S442) by PINK1. They observed that MFN2, when non-phosphorylated, exhibited fusogenic behavior. However, when phosphorylation was mimicked at specific sites such as T111, S378, or S442, it resulted in the recruitment of Parkin to mitochondria and initiated mitophagy. The impact of phosphorylation varied depending on the specific site, with MFN2 S378 playing a major role in disabling mitochondrial fusion, while having minimal effect on Parkin recruitment. On the other hand, mimicking phosphorylation at T111 or S442 had a similar effect to S378 in suppressing mitochondrial fusion, but phosphorylation at S442 in particular facilitated the translocation of Parkin to mitochondria.

1.2.4. Axonal transport

Mitochondrial trafficking is a critical process that guarantees the accurate localization of mitochondria within the cellular cytoplasm (Milone and Benarroch, 2012). For short distances, movement occurs along actin microfilaments and requires myosin motors, while for longer distances, movement occurs along microtubules and requires kinesins or dynein motors (Milone and Benarroch, 2012). Members of the kinesin-1 family of ATPases, such as KIF5 and KIF1, are responsible for rapid anterograde transport of mitochondria (Chen et al., 2009; Kang et al., 2008; Koutsopoulos et al., 2010). MFN2 is involved in this process by interacting with Miro 1 and Miro 2, outer mitochondrial membrane proteins that are engaged in the specific attachment of mitochondria to KIF5 in neurons. These Miro proteins also function as calcium and energy sensors, allowing mitochondria to be appropriately positioned in response to alterations in metabolic status and synaptic activity (Miller and Sheetz, 2004; Saotome et al., 2008). The survival and efficient nerve conduction in the axon heavily rely on substantial ATP levels, particularly at crucial locations such as the nodes of Ranvier of long neuronal axons. Nonetheless, disruptions in both mitochondrial transport and energy production could present a dual impediment. This combination might restrain the axon's capacity to meet the elevated energy requirements, ultimately leading to axonal dysfunction.

1.2.5. ROS production and inflammation

Recently, Juan Tur et al. (Tur et al., 2020)elucidated the role of MFN2 in the generation of reactive oxygen species (ROS) and inflammation in macrophages. The expression of Mfn2 was induced by pro-inflammatory stimuli such as lipopolysaccharide (LPS). By utilizing myeloid-conditional knockout (KO) mouse models for Mfn2 and Mfn1, it was observed that Mfn2, but not Mfn1, is necessary for the adaptation of mitochondrial respiration to stressful conditions and the generation of reactive oxygen species (ROS) following pro-inflammatory activation. The deficiency of Mfn2 specifically impairs the synthesis of pro-inflammatory cytokines and nitric oxide. Moreover, the absence of Mfn2, but not Mfn1, is associated with dysfunctions in autophagy, apoptosis, phagocytosis, and antigen processing. Mice with the Mfn2floxed;CreLysM fail to exhibit protection against Listeria, *Mycobacterium tuberculosis*, or LPS endotoxemia. These findings highlight an unexpected role of Mfn2 in ROS production and inflammation in macrophages.

1.3. Pathogenesis of Charcot-Marie-Tooth type 2A

MFN2 is a critical protein that sustains cellular bioenergetics through coordination of the mitochondrial functional network. This intricate system comprises highly conserved mechanisms such as mitochondrial fusion and fission, trafficking, interorganellar communication between mitochondria and the ER, and mitophagy function negatively, and this disbalance has been suggested as a potential mechanism for neurodegeneration.

Several studies have shown that mutations in the MFN2 gene disrupt mitochondrial fusion and subsequently alter mitochondrial shape, resulting in a wide range of spherical or oval mitochondrial sizes. Furthermore, in MFN2-mutant cells, mitochondria tend to aggregate in clusters (Rizzo et al., 2016; Saporta et al., 2015; Van Lent et al., 2021). Cells that lack mitochondrial fusion have a severe defect in respiratory capacity. Essentially, an excessive level of mitochondrial fragmentation leads to a depletion of mitochondrial DNA (mtDNA) (Chen et al., 2005). Since mtDNA encodes several proteins of the respiratory chain, mtDNA depletion leads to impairment of oxidative phosphorylation. The synaptic dysfunction thus induced is typically followed by dendritic and axonal degeneration, leading to neurodegeneration, especially in neurons that exhibit low levels of MFN1. These low levels are insufficient to compensate for the deficit of MFN2. MFN2 is also located at the ER, where it acts as a mediator of ER-mitochondria cross-talk at MAM level. Several studies have highlighted the role of MAM in neuronal function, with important implications in various neurodegenerative disorders (Krols et al., 2019; Bernard-Marissal et al., 2015; Hedskog et al., 2013). MFN2 has been suggested to modulate both positively and negatively the interplay between the two organelles. Marissal and colleagues, in their study (Bernard-Marissal et al., 2019) quantified ER–mitochondria contacts in neurons overexpressing either WT or mutated MFN2. The presence of a mutant protein results in a decrease in the interactions between the endoplasmic reticulum and mitochondria in fibroblasts derived from patients with CMT2A, primary neurons, and also in vivo, specifically in motoneurons of a mouse model of CMT2A. Those changes were concomitant with ER stress and calcium handling defects.

Another pathogenic mechanism associated with MFN2 mutations is the alteration of the mitophagy process, where MFN2 plays a central role. Mitophagy is essential in neurons due to their heightened susceptibility to the buildup of impaired cellular constituents (Nixon and Yang, 2012). The susceptibility of neurons to autophagic/lysosomal dysfunction is unsurprising when considering that neurons need to persist throughout the lifespan of the organism and lack the ability for mitosis, which aids in the disposal of accumulating waste materials by dividing cells. Rizzo and colleagues, in their study on induced pluripotent stem cell (IPSC)-derived CMT2A motor neurons (MNs), observed a significant reduction in mitochondria in human motor neurons expressing mutant MFN2. Additionally, they observed an increase in mitophagy within these motor neurons, suggesting that this could potentially be the main underlying mechanism responsible for this phenomenon (Rizzo et al., 2016).

Indeed, deficient axonal mitochondrial transport is a common occurrence in neuronal models of CMT2A. In murine neuronal cells expressing mutant MFN2, axonal mitochondria demonstrate slower anterograde and retrograde movements compared to control cells (Bannerman et al., 2016; Detmer et al., 2008; Stavropoulos et al., 2021; Zhou et al., 2021). As demonstrated in the same study cited above the mitochondrial distribution in CMT2A cells was abnormal with prominent perinuclear localization and poor spreading in peripheral cellular regions (Zhou et al., 2021). These data were confirmed by the transduction of WT-MNs and CMT2A-MNs with viral particles expressing a fluorescent protein fused to a mitochondrial localization signal peptide. These findings, which were also observed in histological sections of

peripheral nerves from patients with CMT2A (Baloh et al., 2007; Barbullushi et al., 2019; Calvo et al., 2009; Koshiba et al., 2004a; Verhoeven et al., 2006), suggest the existence of a primary deficiency in mitochondrial axonal transport in vivo and its involvement in the pathogenesis of CMT2A in the human body. However, it is still a matter of debate whether or not such abnormalities are a cause or merely a consequence of a degenerating axon. Additional research is necessary to establish this elusive aspect.

1.4. Treatment of CMT2A disease

Much progress has been made in the last three decades in the identification of genes and mutations and in the elucidation of the disease pathogenesis, through the generation of animal and cellular models, that have been instrumental to study disease physiology. However, therapies are not yet available. Up to now, the mainstay of CMT2A treatment has relied mainly on supportive care, whose most important aspect is physical activity. Important aspects of physical therapy and rehabilitation for patients with CMT may involve gait training, therapeutic exercise, stretching, balance and postural stabilization, fall risk prevention strategies, aquatic therapy, energy conservation techniques, serial casting/night splinting, patient education, training on appropriate assistive devices, and prevention of secondary impairments. Protection of joint range of motion (ROM) to avoid the possibility of contractures and maximize functional use of all extremities should be stressed in the management of CMT (Abresch et al., 2012). It has been shown that various types of properly fitted ankle–foot orthoses (AFO) may significantly reduce the need for proximal compensations, increasing ROM and preventing painful contractions. Due to the relatively rare and varied presentation of CMT-related deformities, surgical treatment guidelines are lacking (Reilly et al., 2017). The objectives of surgical intervention typically involve realigning joints, rectifying bony deformities, and addressing muscle imbalance (Yagerman et al., 2012). Surgical determinations are commonly influenced by factors such as the patient's age, the extent of deformity severity, the necessity to forestall additional deterioration, and when pain and reduced function persist despite the aforementioned non-surgical interventions. Only a small number of studies have investigated the long-term outcomes of surgical procedures in patients with CMT(Ward et al., 2008; Leeuwesteijn et al., 2010). Consequently, providing specific guidance on the appropriate surgical procedures and optimal timing for individual patients remains challenging. In consideration of the frequent respiratory manifestations and given a multidisciplinary approach to the disease, consideration should be given to respiratory support and speech therapy. Moreover, there is a conspicuous lack of controlled trials focusing on the treatment of pain and fatigue, despite these symptoms being prominent in CMT (Herrmann, 2008).

A definitive cure for CMT remains elusive, and significant advancements in the field are still pending. This can be due to several issues: CMTs result from over 1500 mutations affecting at least 100 genes. Secondly, therapy necessitates targeting the peripheral nervous system (PNS), which, akin to the CNS, is safeguarded by the blood-nerve barrier. Thirdly, CMT rodents and cellular models not always reproduce the severity of disease, particularly for axonal CMT2A.

Transgenic or mutagenized mice currently represent the most faithful in vivo models of CMT2A (Detmer et al., 2008; Cartoni et al., 2010; Zhou et al., 2021; Stavropoulos et al., 2021; Bannerman et al., 2016; Hines et al., 2023). Among them, the most used so far was the MitoCharc1 mouse, carrying the R94Q amino acid substitution in the human *MFN2* gene under the neuron-specific enolase (*Eno*) promoter (Cartoni et al., 2010). A notable limitation of this model is the mild-to-late onset of pathological phenotypes, in contrast to the severe-early clinical presentation typically seen in CMT2A patients with the R94Q MFN2 mutation. In 2019, Zhou and colleagues developed the Tg(Thy1-MFN2R94Q)44Balo/J), characterized by hemizygous expression of the MFN2-R94Q transgene under the thymocyte differentiation antigen 1, allele 2 (Thy1.2) promoter integrated into the Y chromosome (Zhou et al., 2021. This model presents a more severe phenotype with precocious onset, sensorimotor symptoms, visual disturbances, diffuse axonal degenerations and mitochondrial abnormalities. Overall, the primary technical limitations of existing murine models is related to the level and pattern of expression of the MFN2 gene, since mutant MFN2 overexpression is often needed to achieve the phenotype. We reviewed thoroughly the characteristics and limitations of murine models of CMT2 in (Barbullushi et al., 2019).

Additionally, there is a requirement for natural history studies across numerous forms of CMT. These studies should focus on identifying biomarkers and developing informative outcome measures to effectively track the disease progression, which is frequently very slow (Juneja et al., 2019;Rossor et al., 2020). Despite these considerations, there has been a shift in the treatment paradigm in recent years. MFN2 agonists and histone deacetylase inhibitors have exhibited promising outcomes in experimental models (Picci et al., 2020; Rocha et al., 2018). Below, we review the progress made so far in preclinical research (Table 1). Within this context, uncovering the genetic and phenotypic range of CMT2A is increasingly crucial.

1.5. Peptide therapy to increase mitochondrial fusion

A novel therapeutic strategy strives to improve mitochondrial fusion rates by administering peptides that are specifically designed for this purpose (Fig. 3) (Franco et al., 2016; Rocha et al., 2018). As mentioned above, the initiation of mitochondrial fusion occurs due to intermolecular interactions between HR2 domains found on mitofusins situated across two opposing mitochondrial membranes. Additionally, the HR2 domain can undergo intramolecular binding with HR1-HR2. The state of mitofusins varies based on the accessibility of the HR2 domain, enabling them to transition between a fusion-restricted state (inactive) to a fusion-facilitating state (active) (Franco et al., 2016). Based on this model Franco and colleagues generated a cell-permeant minipeptide (MP1) that competes with the HR1-HR2 interaction to destabilize fusion-constrained mitofusin and promote the fusion-permissive conformation(Franco et al., 2016). They examined the functional import of intra-molecular Mfn2 HR1-HR2 binding by introducing into murine embryonic fibroblasts (MEFs) a recombinant Mfn2 HR1 fragment containing the sequences predicted to mediate intra-molecular HR1-HR2 binding (adeno-HR1) with destabilization of HR1-HR2 binding unfolds and extends HR2 into the cytosol, into a tethering-permissive state. In order to enhance its permeability and flexibility, the minipeptide was fused with the TAT sequence from the HIV transactivator protein, and glycine residues were substituted for leucine residues. The resulting molecule, TAT-MP1Gly, specifically restored the mitochondrial defect only in cultured neurons that expressed a wild-type MFN2 allele alongside the mutated variant (Franco et al., 2016). Taking into account that most CMT2A mutations are autosomal dominant (Cartoni and Martinou, 2009; Chen et al., 2003; Detmer and Chan, 2007; Feely et al., 2011; Pipis et al., 2021; Züchner et al., 2004), patients have one MFN2 mutant allele, one wild-type MFN2 allele and two normal MFN1 alleles, thus could be promising for future studies as an alternative to genetic therapeutic approaches.

Recently, Rocha and colleagues discovered a new class of small-molecules, referred to as mitofusin agonists, which have the ability to allosterically disrupt HR1-HR2 interactions, resulting in a conclusive shift toward the fusion-ready MFN2 conformation and an augmentation in fusion events. Mitofusin agonists have exhibited encouraging outcomes both in laboratory experiments and animal models. They effectively reversed the formation of immobile mitochondrial clusters in cultured primary murine neurons with R94Q or T105M mutations. Furthermore, they restored the compromised mitochondrial axonal transport in peripheral nerves of mice bearing the T105M mutation (Rocha et al., 2018). Similarly to the peptides that we described above, mitofusin agonists were not effective in *MFN1* or *MFN2 null* cells (Rocha

Table 1
Overview of preclinical studies for CMT2A treatment approaches.

Category	Compound	Delivery	Mechanism	Clinical trials	References
Drug therapy	MFN2 agonists (peptide therapy)	Intramuscular	Improve mitochondrial fusion rates (stabilize the fusion-permissive open conformation of MFN2) Restored the compromised mitochondrial axonal transport	N.A	Rocha et al., 2018 Franco et al., 2016, 2020
Drug therapy	6-phenylhexanamide derivative mitofusin activators	Intravenous	Improves mitochondrial motility	N.A	Dang et al., 2020
Drug therapy	HDAC6s inhibitors	Intraperitoneal	Reduces microtubles acetylation, rescued defective axonal transport	N.A	Picci et al., 2020 Shen et al., 2021
Molecular therapy	MFN1	Transgenic	To increase MFN1 expression	N.A	Zhou et al., 2021 Shahin et al., 2023 Stravopoulus et al. 2023
Molecular therapy	SARM1 inhibitors	Transgenic	To ablate SARM1 NADase activity at the basis of axonal degeneration	N.A	Geisler et al., 2019 Hughes et al., 2021 Sato-Yamada et al., 2022
Gene therapy	MFN2 gene replacement	Intrathecal (via adeno-associated virus 9 (AAV9))	introduction of an exogenous functional wild-type MFN2 gene copy	N.A	Rizzo et al., 2023

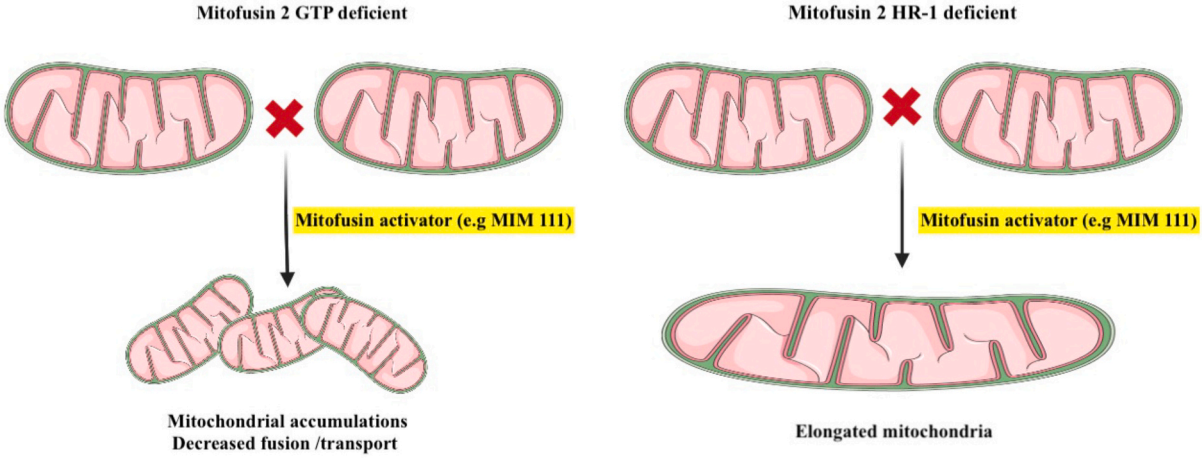

Fig. 3. Schematic representation of peptide-based therapy. Mutants of the GTPase mitofusin 2, when exposed to a mitofusin activator (MIM111), exhibit an inability to fuse two mitochondria, resulting in the formation of aggregated clusters of mitochondria, decreased fusion and transport. Conversely, HR1 mutants of mitofusin 2, when exposed to mitofusin activators (MIM111), demonstrate the ability to successfully accomplish mitochondrial fusion (Franco et al. 2020).

et al., 2018). Hence, mitofusin agonists don't restore the function of CMT2A MFN2 GTPase domain mutants. Instead, they stabilize the fusion-permissive open conformation of native, healthy MFN1 or MFN2, allowing them to counteract the dominant inhibition of mitochondrial fusion caused by these dysfunctional disease-associated proteins.

Considering the limited pharmacokinetic characteristics of the prototype peptidomimetic small molecule mitofusin activator developed by Rocha and colleagues, Dang et al. (Dang et al., 2020) have recently proposed a rational design of a series of 6-phenylhexanamide derivatives whose pharmacokinetic optimization yielded a 4-hydroxy cyclohexyl analog, with the potency, selectivity, and oral bioavailability of a preclinical candidate. The active trans-diastereomer, trans- 4-hydroxy cyclohexyl, demonstrated excellent cell permeability, microsomal stability, and in vivo pharmacokinetics, significantly enhancing pharmaceutical properties compared to its precursors. Upon systemic administration to a preclinical mouse model of CMT2A, this compound reversed the characteristic mitochondrial dismotility in sciatic nerve neurons, indicating in vivo target engagement in pathophysiologically relevant models and tissues. Preclinical profiling of 6-phenylhexanamide derivatives supports their potential as the inaugural disease-modifying treatments for CMT2A. Finally, Franco et al. (Franco et al., 2016) have recently proposed a new class of direct mitofusin activators that have shown excellent results regarding intermittent, or burst, mitofusin activation in CMT2A neuromuscular dysfunction. In this study, they demonstrated that pharmacological activation of endogenous normal mitofusins effectively mitigated the dominant inhibitory effects of CMT2A mutants in reprogrammed human patient motor neurons. This intervention resulted in the reversal of characteristic mitochondrial stasis and fragmentation, regardless of the underlying MFN2 mutation. In a murine model expressing the human MFN2 T105M variant, intermittent mitofusin activation using the small molecule MiM111 normalized the neuromuscular dysfunction associated with CMT2A. Additionally, it attenuated pre-existing axon and skeletal myocyte atrophy and facilitated axon regrowth by enhancing mitochondrial transport along peripheral axons. Moreover, MiM111 administration resulted in increased in vivo mitochondrial localization at neuromuscular junctional synapses. Neurons from MiM111-treated MFN2 T105M mice exhibited accelerated primary outgrowth and enhanced post-axotomy regrowth, indicative of heightened mitochondrial motility.

1.6. Histone-deacetylases inhibitors

Inhibitors of histone deacetylases (HDACs) are a promising class of novel therapeutics (Fig. 4). Epigenetic modifications of chromatin have profound effects on cell-specific gene expression. One type of

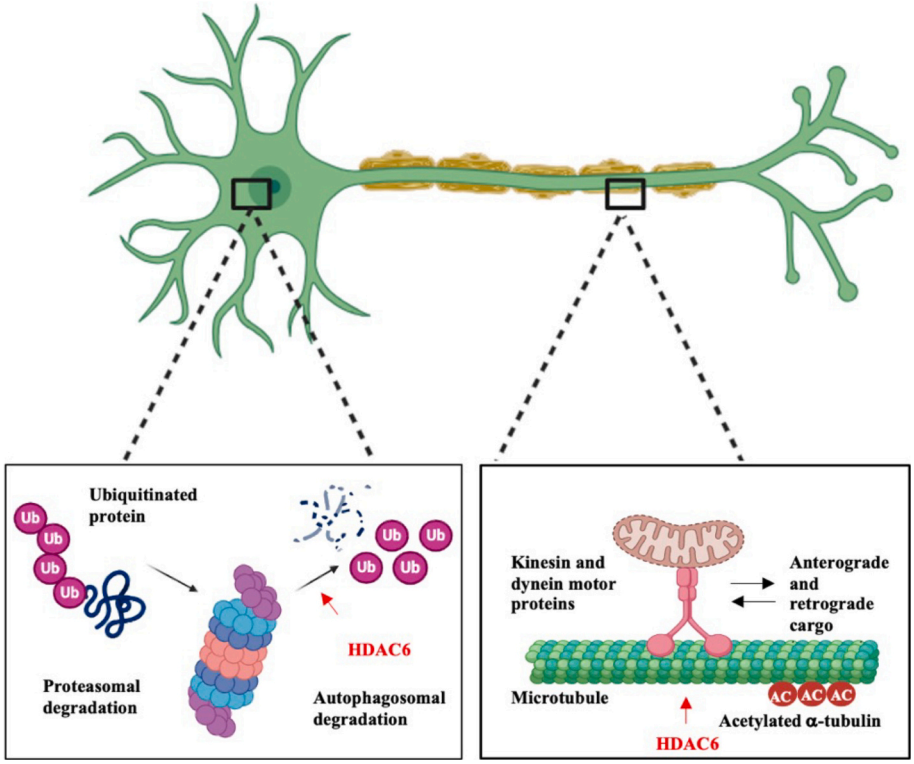

Fig. 4. Schematic representation of HDAC6s inhibitor therapy. (a) HDAC6 facilitates the transition from proteasomal-mediated to autophagy-mediated protein degradation. The proteasomal pathway serves as the primary route for protein degradation. However, if the proteasomal degradation pathway is impaired or overwhelmed, HDAC6 redirects ubiquitinated proteins to the perinuclear region, promoting the formation of aggresomes. (b) HDAC6 negatively regulates the anterograde and retrograde transport of various organelles, including mitochondria. This is primarily achieved through the deacetylation of α-tubulin, a key component of microtubule tracks. Deacetylation of α-tubulin reduces the affinity of motor proteins to microtubules, resulting in a slowdown of axonal transport. Consequently, HDAC6 plays a neurotoxic role by suppressing the proper transport of vital cargoes along axons. Based on these pathogenic mechanisms, the role of HDAC6 inhibitors is established.

modification is the acetylation and deacetylation of lysine residues within the amino terminal tails of histone proteins. This coordinated process is carried out by 2 classes of nuclear enzymes, histone acetyltransferases (HATs) and HDACs. HDACs promote chromatin condensation and gene silencing. Although histones are the most intensively investigated substrates, various nonhistone proteins may be also modified by HATs and HDACs, including transcription factors, cytoskeletal proteins, signal transduction mediators, and molecular chaperons (Ganai, 2016; Glozak et al., 2005). HDACs are a family of 11 zinc-dependent enzymes that catalyze the deacetylation of conserved lysine residues within histones as well as non-histone proteins impacting various cellular functions without affecting chromatin acetylation (Ganai, 2016; Glozak et al., 2005). Among these proteins, HDAC6 stands out due to its distinct characteristics. It comprises tandem catalytic domains (CD1 and CD2), a C-terminal zinc finger ubiquitin-binding domain (ZnF-UBP), and an N-terminal microtubule-binding domain (MBD). Notably, CD1 has exhibited minimal deacetylase activity on substrates containing internal lysine residues,whereas CD2 primarily facilitates the deacetylation process for numerous HDAC6 nonhistone substrates. These substrates include α-tubulin, heat shock protein 90, peroxiredoxin, cortactin, survivin, and β-catenin (Kutil et al., 2019; Miyake et al., 2016; Osko and Christianson, 2019).

Furthermore, the zinc finger domain exhibits a strong affinity for ubiquitin, contributing to the process of protein clearance and degradation through the aggresomal pathway. α-Tubulin was among the initial substrates recognized for cytoplasmic HDAC6. Subsequent extensive demonstrations have illustrated that pharmacological inhibition of HDAC6 elevates the levels of acetylated α-tubulin at lysine. This action effectively reinstates the compromised mitochondrial axonal transport observed in models simulating neurodegenerative disorders (Hammond et al., 2010). Recent evidence indicates that various genetic forms of CMT2 might arise from comparable pathogenic pathways, marked by disturbances in α-tubulin acetylation and impaired axonal transport (Prior et al., 2018). While the exploration of new HDAC6 inhibitors (HDAC6i) has gained interest in the past decade, the research has focused on a restricted set of drug candidates with only a limited number of druglike candidates that have been investigated and shown to possess efficacy in animal models. Significantly, in mutant heat shock protein β-1- induced CMT type 2F (CMT2F) models (d'Ydewalle et al., 2011)and mutant glycyltRNA synthetase-induced CMT type 2D (CMT2D) models (Benoy et al., 2018;Mo et al., 2018), the intra peritoneal (IP) injection of a selective HDAC6i Tubastatin A (TubA), restored the impaired levels of Ac-αtub in sciatic nerves, rescued defective axonal transport, and ameliorated the CMT phenotype. Subsequent to these findings, it was observed that HDAC6i also exhibit the potential to ameliorate disrupted mitochondrial fusion and mitochondrial transport (Guedes-Dias et al., 2015; Kalinski et al., 2019). Therefore, this strategy was repurposed also for testing in CMT2A models, Shen and colleagues recently published about the effect of pharmacological HDAC6 inhibition with the compound termed SW-100 on MitoCharc1 mice, which was designed by simplification of the tricyclic-capped HDAC6i, TubA (Picci et al., 2020). SW-100 is a phenylhydroxamate-based HDAC6i bearing a tetrahydroquinoline (THQ) capping group, In the MFN2R94Q-induced mouse model of CMT2A, SW-100 (20 mg/kg, administered twice a day IP) was observed to reverse α-tubulin acetylation defects in distal nerve regions and improve motor and sensory function (Picci et al., 2020). However, they discovered that the half-life of SW-100 was relatively brief in liver microsomes and hepatocytes. Consequently, this led to low drug concentrations in the brain and plasma, necessitating a repeated daily dose for animal efficacy studies. This limitation restricted

its further preclinical development. Structure–activity relationship studies led to the identification of a new THQ-based HDAC6i, named SW-101 that has shown a significantly enhanced drug exposure in plasma and the brain in comparison with SW-100. They discovered that administering SW-101 once daily at a dose of 20 mg/kg increased the levels of acetylated α-tubulin in the distal sciatic nerve, counteract progressive motor dysfunction and improved both motor and sensory symptoms in Mitocharc1 mice (Shen et al., 2021).

1.7. Strategies based on MFN1 overexpression

As explained above, before mitochondrial fusion, MFN1 and MFN2 engage with each other on the Outer Mitochondrial Membranes (OMMs) of adjacent mitochondria, creating both heterotypic and homotypic complexes, in a process known as mitochondrial tethering (Franco et al., 2016; Zhou et al., 2021). Among these interactions, the MFN1-MFN2 heterotypic interaction is notably preferred (Samanas et al., 2020). In experiments conducted with MEFs, it was noted that $MFN1^{WT}$ complements various MFN2 mutants in mitochondrial fusion more effectively than $MFN2^{WT}$ itself (Detmer and Chan, 2007). This method also demonstrated promising outcomes in murine models (Fig. 5).

In 2019, Zhou and colleagues provided what we believe is the first in vivo proof of concept that restoring MFN1:MFN2 balance by augmenting levels of MFN1 in the nervous system is a viable therapeutic approach for CMT2A (Zhou et al., 2019). They overexpressed a Flag-tagged human MFN1 under the mouse prion protein (PrP) promoter – which drives expression within the CNS – in a transgenic mouse model expressing the R94Q mutation under the *Thy1.2* promoter, generating the double transgenic (*$MFN2^{R94Q}$:MFN1*) mice (Zhou et al., 2021). Elevating the level of MFN1 in the nervous system was well tolerated and resulted in complete or near-complete restoration of deficiencies in locomotor activity, sensorimotor coordination, and vision loss in MFN2R94Q mice.

It is unknown why MFN1 levels are very low in the adult nervous system, but the fact that *Prp-MFN1* transgenic mice are phenotypically normal indicates that MFN1 overexpression at levels equivalent to MFN2 is well tolerated.

Simultaneously, they demonstrated that at the molecular level, the augmentation of MFN1 rescued mitochondrial aggregation and axon degeneration. Additionally, the authors analyzed the transcriptomic signatures of MFN2R94Q, (MFN2R94Q:MFN1), and nontransgenic mice. This analysis revealed that the affected mice exhibited down-regulation in oxidative phosphorylation and respiration electron transport pathways, which were subsequently restored by the overexpression of MFN1.

In a subsequent study involving the same transgenic animals, Shahin and colleagues evaluated the impact of increasing MFN1 levels on retinal degeneration associated with CMT2A (Shahin et al., 2023). The results indicated that the imbalance in the MFN1/MFN2 ratio, caused by the R94Q mutation and the subsequent impairment in mitochondrial fusion-fission pathways, led to retinal degeneration in MFN2R94Q mice through P62/LC3B-mediated mitophagy and autophagy. Conversely, the augmentation of MFN1 levels restored vision and maintained retinal morphology by re-establishing a well-balanced homeostasis in mitochondrial fusion-fission (MFN1/MFN2 ratio) (Shahin et al., 2023; Zhou et al., 2021). Stavropoulos and co-researchers subsequently applied a similar approach in a cellular model using SH-SY5Y cells, an immortalized human neuroblastoma line, that expressed the MFN2K357T mutation through the identical Thy1.2 expression cassette utilized by Zhou et al. (Stavropoulos et al., 2023).

Then, they transfected these cells with various plasmids containing *$MFN2^{K357T}$*, *$MFN2^{WT}$* or *$MFN1^{WT}$*. Cells that overexpressed $MFN2^{WT}$ exhibited mitochondria widely distributed from the cell body to the axon-like processes. Conversely, overexpression of $MFN1^{WT}$ led to highly interconnected mitochondria across both the cell body and the axon-like processes, accompanied by the presence of mitochondrial aggregates. Cells that overexpressed $MFN2^{K357T}$ showed prominent mitochondrial aggregates around the nucleus and an absence of detectable mitochondria in the axon-like processes. These defects induced by $MFN2^{K357T}$ were more effectively resolved when co-overexpressing $MFN1^{WT}$ rather than $MFN2^{WT,}$ showcasing the greater capacity of $MFN1^{WT}$ to alleviate the CMT2A disease phenotype in vitro. The hypothesis that has been formulated is that, although the interaction between $MFN1^{WT}$ and $MFN2^{K357T}$ might not suffice to stimulate mitochondrial fusion in cells co-transfected with both, the interaction between $MFN1^{WT}$ and $MFN2^{WT}$, as well as between $MFN1^{WT}$ and itself, can facilitate fusion when the expression of $MFN1^{WT}$ is heightened. This effectively counteracts the mitochondrial fusion impairments induced by $MFN2^{K357T}$ in a manner dose-dependent manner. This mechanism, observed similarly in MFN2 activators presents an advantage as it can be

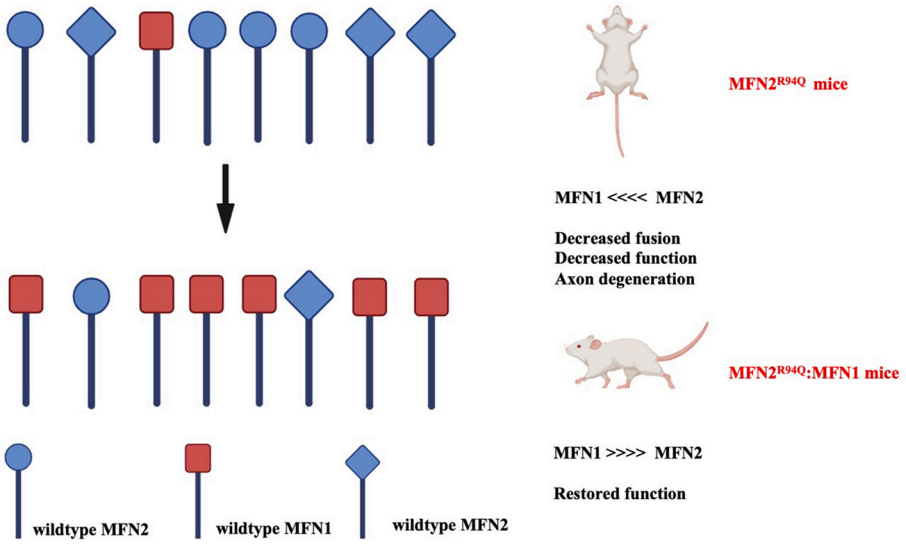

Fig. 5. Schematic representation of MFN1 overexpression-based therapy. Enhanced expression of MFN1 in the nervous system successfully rescued MFN2R94Q mutant mice phenotypes, indicating that the relative levels of MFN isoforms play a crucial role in conferring selectivity to the nervous system in CMT2A. This study provides in vivo evidence supporting the concept that increasing MFN1 levels could be a promising therapeutic approach for the treatment of this disease (Zhou et al. 2019).

applied to various MFN2 mutation types, as long as CMT2A patients still possess one $MFN2^{WT}$ allele or two $MFN1^{WT}$ alleles. Beyond the aforementioned discussion, it's important to note that MFN2, in contrast to MFN1, serves as a mediator in ER-mitochondrial tethering, a process that facilitates mitochondrial Ca2+ uptake, a process that is. also altered in CMT2A. Before identifying the most appropriate MFN candidate for CMT2A gene therapy, it's essential to evaluate their therapeutic effectiveness in additional molecular functions in which MFN2 is involved and that are impacted by MFN2 mutations.

1.8. SARM1 pathways

Axonal degeneration, a characteristic hallmark of various neurological disorders, such as CMT2A, is perceived as an inherent program of subcellular self-destruction that is genetically encoded. Within this process, the SARM1, a unique member of the Myd88 family of adaptor proteins, has been recognized as the central mediator of axonal degeneration in pathological conditions (Fig. 6) (Gerdts et al., 2015;Osterloh et al., 2012). The activation of SARM1 initiates axonal degeneration, even in the absence of identifiable damage (Gerdts et al., 2016). A decrease in mitochondrial membrane potential presumably triggers a decline in the survival factor within the axon, specifically nicotinamide mononucleotide adenylyl-transferase 2 (NMNAT2), which subsequently activates SARM1, culminating in axonal degeneration (Summers et al., 2020). Geisler and colleagues (Geisler et al., 2019) developed SARM1 dominant-negatives that potently block axonal degeneration in cellular models of axotomy and neuropathy. To evaluate in vivo efficacy, adeno-associated virus (AAV) was employed to express the most effective SARM1 dominant-negative construct, alongside nerve transection serving as a severe axonal degeneration model. In vehicle-treated mice, axonal degeneration occurred swiftly; however, in mice expressing the SARM1 dominant-negative construct, axons remained intact for over 10 days post-transection. This preservation of axons mirrored the protection observed in SARM1-null mice. Another interesting study in the field of SARM1 inhibitors is that of Hughes and colleagues (Hughes et al., 2021) developed a biochemical screen to find small molecule inhibitors of the SARM1 NADase, identified and further optimized a class of isoquinolines effective in the biochemical SARM1 assay, and generated DSRM-3716, a potent small molecule inhibitor that showed double-digit nM IC50 against recombinant SARM1. Considering that SARM1 activity can be tracked by measuring cADPR levels, they monitored changes in cADPR levels and found that DSRM-3716 prevented increases in axonal cADPR induced by transection or rotenone exposure in a dose-dependent manner. Additionally, they demonstrated how DSR-3716 can prevent axonal degeneration even when axons have already transitioned into a metastable state in which the regression of axon blebs, which characterizes it, has been confirmed. The potential for axonal protection and the prospect of even restoring an intermediate pool of damaged axons to a healthy state through SARM1 inhibition can hold significant therapeutic implications as a therapeutic target for CMT2A. Another intriguing animal model, which is relevant to the potential therapeutic efficacy of SARM1 inhibition, is the recently developed model by Sato-Yamada et al. (Sato-Yamada et al., 2022) They investigated the involvement of SARM1 in a rat model carrying a dominant CMT2A mutation (Mfn2H361Y) characterized by all characteristic features of the corresponding human disease. Through the generation of Sarm1-knockout (Sarm1−/−) and Mfn2H361Y Sarm1 double-mutant rats, they observed that the deletion of Sarm1 rescued various phenotypes related to axonal, synaptic, muscle, and functional impairments. This finding demonstrates that SARM1 plays a significant role in the neuropathology observed in this model. Remarkably, despite the presence of mutant MFN2 protein in these double-mutant rats, the loss of SARM1 also notably mitigated several mitochondrial defects, including abnormalities in the number, size, and cristae density of synaptic mitochondria.

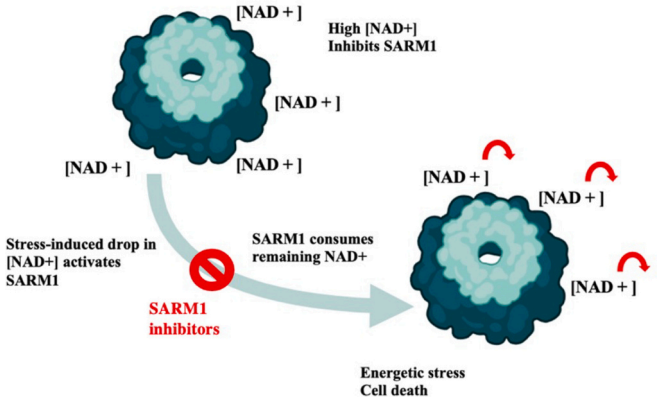

Fig. 6. Schematic representation of SARM1 inhibitors therapy. In homeostasis, the cellular concentration of NAD+ is sufficiently high to inhibit SARM1. However, when cellular NAD+ levels decrease due to reduced NAD+ synthesis (e.g., inhibition of NMNAT1/2) or increased NAD+ consumption, the inhibitory NAD+ molecules dissociate from SARM1, resulting in subsequent NADase activity. This leads to an almost complete depletion of the cellular NAD+ pool and induces energetic stress, ultimately resulting in cell death.

2. Perspectives on gene therapy: RNA interference and gene replacement therapy targeting MFN2

Despite gene therapies being widely approved as treatments for neuromuscular diseases like SMA, there is a lack of literature regarding gene therapy studies for CMT2A. In recent years, advancements in treating genetic diseases have been significant developments. Various investigations into gene therapy-based approaches hold promise to revolutionize therapeutic possibilities for rare diseases, these approaches involve replacing missing or defective genes using viral vectors (Kagiava et al., 2019; Schiza et al., 2019).Despite gene therapies being widely approved for neuromuscular diseases like SMA (Mendell et al., 2017), literature concerning this specific pathology remains lacking. Rizzo et al. have recently proposed a study (Rizzo et al., 2023)related to a combined RNA interference and gene replacement therapy targeting *MFN2*. They implemented this approach in motor neurons differentiated from induced pluripotent stem cells (iPSCs) derived from CMT2A patients, displaying the accurate suppression of native MFN2 and the introduction of an exogenous functional wild-type gene copy. This methodology notably ameliorates the CMT2A motor neuron phenotype in vitro, stabilizing the abnormal distribution of axonal mitochondria and rectifying irregular mitophagic processes. The accurate molecular correction of MFN2 was further validated in vivo using the MitoCharc1 CMT2A transgenic mouse model following the administration of constructs through cerebrospinal fluid delivery in newborn mice via adeno-associated virus 9 (AAV9). Demonstrating efficacy both in vitro and in vivo, their findings pave the way for future preclinical and clinical trials.

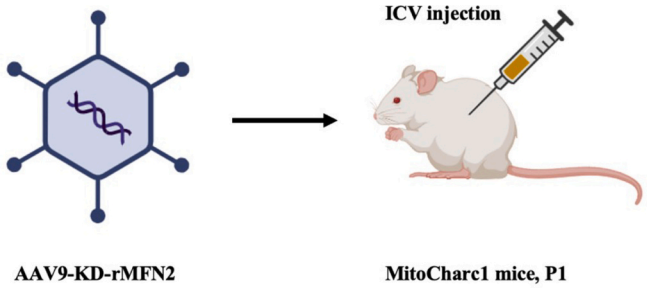

Fig. 7. Schematic representation of gene therapy based on MFN2 gene replacement. Newborn (P1) MitoCharc1mice CMT2A are injected intracerebroventricularly (ICV) with AAV9-KD-rMFN2. (Rizzo et al., 2023).

3. Conclusion

We are entering a groundbreaking phase in CMT2A research marked by the elucidation of pathophysiological mechanisms and the advancement of innovative treatments. Understanding the underlying causes and mechanisms driving axonal degeneration is fundamental to finding a cure. A comprehensive understanding of the temporal progression of axonal degeneration will pave the way for implementing molecular therapies like SARM1 inhibitors (Summers et al., 2020; Geisler et al., 2019), capable of preventing degeneration in both in vivo and in vitro disease models.

Recent exploration of the multifaceted roles of the MFN2 protein has revealed its involvement not only in established mechanisms like mitochondrial fusion, axonal mitochondrial transport, and mitophagy but also its role in interorganellar communication between mitochondria and the ER. Additionally, its contentious role in ER-stress induced apoptosis underscores the criticality of preserving the central role of native MFN2 in gene silencing and MFN2 agonists therapies. These discoveries have also uncovered potential new therapeutic targets.

Our review underscores the need for a personalized medicine approach tailored to the diverse mechanisms underlying MFN2-related diseases while targeting the common pathways of axonal degeneration.

Despite the transformative impact of gene therapy on other neuromuscular diseases, its potential benefits have not yet been effectively realized in CMT2A therapy. The pioneering study by Rizzo et al.(Rizzo et al., 2023), focusing on gene silencing and gene replacement therapies, suggests employing a combined RNAi and gene therapy strategy as a potential therapeutic avenue for addressing the broad spectrum of human diseases linked to MFN2 mutations.

As promising treatments advance toward clinical application, the identification of optimal outcome measures, novel biomarkers, and suitable trial designs becomes imperative. These steps are crucial to facilitate the successful testing and validation of these novel treatments for patients affected by CMT2A.

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Consent for publication

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Availability of data and material

Not applicable.

Authors' contributions

EA and CA collected the data and drafted the manuscript. AA, FR, SC and GC revised the manuscript adding important intellectual contributions. CA drafted the figures.

CRediT authorship contribution statement

Claudia Alberti: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Federica Rizzo:** Writing – review & editing, Funding acquisition, Conceptualization. **Alessia Anastasia:** Writing – review & editing, Investigation, Conceptualization. **Giacomo Comi:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Stefania Corti:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Elena Abati:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

Abati, E., Manini, A., Velardo, D., Del Bo, R., Napoli, L., Rizzo, F., Moggio, M., Bresolin, N., Bellone, E., Bassi, M.T., D'Angelo, M.G., Comi, G. Pietro, Corti, S., 2022. Clinical and genetic features of a cohort of patients with MFN2-related neuropathy. Sci. Rep. 12, 6181. https://doi.org/10.1038/s41598-022-10220-0.

Abresch, R.T., Carter, G.T., Han, J.J., McDonald, C.M., 2012. Exercise in neuromuscular diseases. Phys. Med. Rehabil. Clin. N. Am. 23, 653–673. https://doi.org/10.1016/j.pmr.2012.06.001.

Amiott, E.A., Lott, P., Soto, J., Kang, P.B., McCaffery, J.M., DiMauro, S., Abel, E.D., Flanigan, K.M., Lawson, V.H., Shaw, J.M., 2008. Mitochondrial fusion and function in Charcot-Marie-tooth type 2A patient fibroblasts with mitofusin 2 mutations. Exp. Neurol. 211, 115–127. https://doi.org/10.1016/j.expneurol.2008.01.010.

Baloh, R.H., Schmidt, R.E., Pestronk, A., Milbrandt, J., 2007. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-tooth disease from Mitofusin 2 mutations. J. Neurosci. 27, 422–430. https://doi.org/10.1523/JNEUROSCI.4798-06.2007.

Bannerman, P., Burns, T., Xu, J., Miers, L., Pleasure, D., 2016. Mice hemizygous for a pathogenic Mitofusin-2 allele exhibit hind limb/foot gait deficits and phenotypic perturbations in nerve and muscle. PloS One 11, e0167573. https://doi.org/10.1371/JOURNAL.PONE.0167573.

Barbullushi, K., Abati, E., Rizzo, F., et al., 2019. Disease modeling and therapeutic strategies in CMT2A: state of the art. Mol. Neurobiol. 56, 6460–6471. https://doi.org/10.1007/s12035-019-1533-2.

Benoy, V., Van Helleputte, L., Prior, R., d'Ydewalle, C., Haeck, W., Geens, N., Scheveneels, W., Schevenels, B., Cader, M.Z., Talbot, K., Kozikowski, A.P., Vanden Berghe, P., Van Damme, P., Robberecht, W., Van Den Bosch, L., 2018. HDAC6 is a therapeutic target in mutant GARS-induced Charcot-Marie-tooth disease. Brain 141, 673–687. https://doi.org/10.1093/brain/awx375.

Bernard-Marissal, N., Médard, J.-J., Azzedine, H., Chrast, R., 2015. Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. Brain 138, 875–890. https://doi.org/10.1093/brain/awv008.

Bernard-Marissal, N., van Hameren, G., Juneja, M., Pellegrino, C., Louhivuori, L., Bartesaghi, L., Rochat, C., El Mansour, O., Médard, J.-J., Croisier, M., Maclachlan, C., Poirot, O., Uhlén, P., Timmerman, V., Tricaud, N., Schneider, B.L., Chrast, R., 2019. Altered interplay between endoplasmic reticulum and mitochondria in Charcot–Marie–tooth type 2A neuropathy. Proc. Natl. Acad. Sci. 116, 2328–2337. https://doi.org/10.1073/pnas.1810932116.

Braathen, G.J., 2012. Genetic epidemiology of Charcot-Marie-tooth disease. Acta Neurol. Scand. Suppl. iv–22. https://doi.org/10.1111/ane.12013.

Calvo, J., Funalot, B., Ouvrier, R.A., Lazaro, L., Toutain, A., De Mas, P., Bouche, P., Gilbert-Dussardier, B., Arne-Bes, M.C., Carrière, J.P., Journel, H., Minot-Myhie, M. C., Guillou, C., Ghorab, K., Magy, L., Sturtz, F., Vallat, J.M., Magdelaine, C., 2009. Genotype-phenotype correlations in Charcot-Marie-tooth disease type 2 caused by Mitofusin 2 mutations. Arch. Neurol. 66, 1511–1516. https://doi.org/10.1001/ARCHNEUROL.2009.284.

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.

Cartoni, R., Arnaud, E., Médard, J.-J., Poirot, O., Courvoisier, D.S., Chrast, R., Martinou, J.-C., 2010. Expression of mitofusin 2(R94Q) in a transgenic mouse leads to Charcot-Marie-tooth neuropathy type 2A. Brain 133, 1460–1469. https://doi.org/10.1093/brain/awq082.

Casasnovas Pons, C., Banchs, I., Cassereau, J., Gueguen, N., Chevrollier, A., Martínez-Matos, J.A., Bonneau, D., Volpini, V., 2010. Phenotypic spectrum of MFN2 mutations in the Spanish population. J. Med. Genet. 47, 249–256. https://doi.org/10.1136/JMG.2009.072488.

Chandhok, G., Lazarou, M., Neumann, B., 2018. Structure, function, and regulation of mitofusin-2 in health and disease. Biol. Rev. Camb. Philos. Soc. 93, 933–949. https://doi.org/10.1111/brv.12378.

Chen, Y., Dorn, G.W., 2013. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science 340, 471–475. https://doi.org/10.1126/SCIENCE.1231031.
Chen, H., Detmer, S.A., Ewald, A.J., Griffin, E.E., Fraser, S.E., Chan, D.C., 2003. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J. Cell Biol. 160, 189–200. https://doi.org/10.1083/jcb.200211046.
Chen, H., Chomyn, A., Chan, D.C., 2005. Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J. Biol. Chem. 280, 26185–26192. https://doi.org/10.1074/jbc.M503062200.
Chen, H., McCaffery, J.M., Chan, D.C., 2007. Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130, 548–562. https://doi.org/10.1016/J.CELL.2007.06.026.
Chen, Y.M., Gerwin, C., Sheng, Z.H., 2009. Dynein light chain LC8 regulates syntaphilin-mediated mitochondrial docking in axons. J. Neurosci. 29, 9429–9438. https://doi.org/10.1523/JNEUROSCI.1472-09.2009.
Cosson, P., Marchetti, A., Ravazzola, M., Orci, L., 2012. Mitofusin-2 independent juxtaposition of endoplasmic reticulum and mitochondria: an ultrastructural study. PloS One 7, e46293. https://doi.org/10.1371/journal.pone.0046293.
Dang, X., Zhang, L., Franco, A., Li, J., Rocha, A.G., Devanathan, S., Dolle, R.E., Bernstein, P.R., Dorn, G.W., 2020. Discovery of 6-Phenylhexanamide derivatives as potent Stereoselective Mitofusin activators for the treatment of mitochondrial diseases. J. Med. Chem. 63, 7033–7051. https://doi.org/10.1021/acs.jmedchem.0c00366.
de Brito, O.M., Scorrano, L., 2008. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456, 605–610. https://doi.org/10.1038/nature07534.
Detmer, S.A., Chan, D.C., 2007. Complementation between mouse Mfn1 and Mfn2 protects mitochondrial fusion defects caused by CMT2A disease mutations. J. Cell Biol. 176, 405–414. https://doi.org/10.1083/jcb.200611080.
Detmer, S.A., Velde, C. Vande, Cleveland, D.W., Chan, D.C., 2008. Hindlimb gait defects due to motor axon loss and reduced distal muscles in a transgenic mouse model of Charcot–Marie–tooth type 2A. Hum. Mol. Genet. 17, 367–375. https://doi.org/10.1093/hmg/ddm314.
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.
Eura, Y., Ishihara, N., Yokota, S., Mihara, K., 2003. Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. J. Biochem. 134, 333–344. https://doi.org/10.1093/JB/MVG150.
Feely, S.M.E., Laura, M., Siskind, C.E., Sottile, S., Davis, M., Gibbons, V.S., Reilly, M.M., Shy, M.E., 2011. MFN2 mutations cause severe phenotypes in most patients with CMT2A. Neurology 76, 1690–1696. https://doi.org/10.1212/WNL.0b013e31821a441e.
Filadi, R., Greotti, E., Turacchio, G., Luini, A., Pozzan, T., Pizzo, P., 2015. Mitofusin 2 ablation increases endoplasmic reticulum–mitochondria coupling. Proc. Natl. Acad. Sci. 112 https://doi.org/10.1073/pnas.1504880112.
Franco, A., Kitsis, R.N., Fleischer, J.A., Gavathiotis, E., Kornfeld, O.S., Gong, G., Biris, N., Benz, A., Qvit, N., Donnelly, S.K., Chen, Y., Mennerick, S., Hodgson, L., Mochly-Rosen, D., Dorn, G.W., 2016. Correcting mitochondrial fusion by manipulating mitofusin conformations. Nature 540, 74–79. https://doi.org/10.1038/nature20156.
Franco, A., Walton, C.E., Dang, X., 2022. Mitochondria clumping vs. mitochondria fusion in CMT2A diseases. Life 12, 2110. https://doi.org/10.3390/life12122110.
Fridman, V., Bundy, B., Reilly, M.M., Pareyson, D., Bacon, C., Burns, J., Day, J., Feely, S., Finkel, R.S., Grider, T., Kirk, C.A., Herrmann, D.N., Laurá, M., Li, J., Lloyd, T., Sumner, C.J., Muntoni, F., Piscosquito, G., Ramchandren, S., Shy, R., Siskind, C.E., Yum, S.W., Moroni, I., Pagliano, E., Zuchner, S., Scherer, S.S., Shy, M.E., Inherited Neuropathies Consortium, 2015. CMT subtypes and disease burden in patients enrolled in the inherited neuropathies consortium natural history study: a cross-sectional analysis. J. Neurol. Neurosurg. Psychiatry 86, 873–878. https://doi.org/10.1136/jnnp-2014-308826.
Ganai, S., 2016. Histone deacetylase inhibitors modulating non-epigenetic players: the novel molecular targets for therapeutic intervention. Curr. Drug Targets 17, 1. https://doi.org/10.2174/1389450117666160527143257.
Gegg, M.E., Cooper, J.M., Chau, K.Y., Rojo, M., Schapira, A.H.V., Taanman, J.W., 2010. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Hum. Mol. Genet. 19, 4861–4870. https://doi.org/10.1093/HMG/DDQ419.
Geisler, S., Huang, S.X., Strickland, A., Doan, R.A., Summers, D.W., Mao, X., Park, J., DiAntonio, A., Milbrandt, J., 2019. Gene therapy targeting SARM1 blocks pathological axon degeneration in mice. J. Exp. Med. 216, 294–303. https://doi.org/10.1084/jem.20181040.
Gerdts, J., Brace, E.J., Sasaki, Y., DiAntonio, A., Milbrandt, J., 2015. SARM1 activation triggers axon degeneration locally via NAD $^{+}$ destruction. Science 1979 (348), 453–457. https://doi.org/10.1126/science.1258366.
Gerdts, J., Summers, D.W., Milbrandt, J., DiAntonio, A., 2016. Axon self-destruction: new links among SARM1, MAPKs, and NAD+ metabolism. Neuron 89, 449–460. https://doi.org/10.1016/j.neuron.2015.12.023.
Glozak, M.A., Sengupta, N., Zhang, X., Seto, E., 2005. Acetylation and deacetylation of non-histone proteins. Gene 363, 15–23. https://doi.org/10.1016/J.GENE.2005.09.010.
Guedes-Dias, P., de Proença, J., Soares, T.R., Leitão-Rocha, A., Pinho, B.R., Duchen, M.R., Oliveira, J.M.A., 2015. HDAC6 inhibition induces mitochondrial fusion, autophagic flux and reduces diffuse mutant huntingtin in striatal neurons. Biochim. Biophys. Acta 1852, 2484–2493. https://doi.org/10.1016/J.BBADIS.2015.08.012.
Hammond, J.W., Huang, C.F., Kaech, S., Jacobson, C., Banker, G., Verhey, K.J., 2010. Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. Mol. Biol. Cell 21, 572–583. https://doi.org/10.1091/MBC.E09-01-0044/ASSET/IMAGES/LARGE/ZMK0041093500007.JPEG.
Hedskog, L., Pinho, C.M., Filadi, R., Rönnbäck, A., Hertwig, L., Wiehager, B., Larssen, P., Gellhaar, S., Sandebring, A., Westerlund, M., Graff, C., Winblad, B., Galter, D., Behbahani, H., Pizzo, P., Glaser, E., Ankarcrona, M., 2013. Modulation of the endoplasmic reticulum–mitochondria interface in Alzheimer's disease and related models. Proc. Natl. Acad. Sci. 110, 7916–7921. https://doi.org/10.1073/pnas.1300677110.
Herrmann, D.N., 2008. Experimental therapeutics in hereditary neuropathies: the past, the present, and the future. Neurotherapeutics 5, 507–515. https://doi.org/10.1016/J.NURT.2008.07.001.
Hines, T.J., Bailey, J., Liu, H., et al., 2023. A novel ENU-induced Mfn2 mutation causes motor deficits in mice without causing peripheral neuropathy. Biology (Basel) 12, 953. https://doi.org/10.3390/BIOLOGY12070953/S1.
Hughes, R.O., Bosanac, T., Mao, X., Engber, T.M., DiAntonio, A., Milbrandt, J., Devraj, R., Krauss, R., 2021. Small molecule SARM1 inhibitors recapitulate the SARM1−/− phenotype and allow recovery of a metastable Pool of axons fated to degenerate. Cell Rep. 34, 108588 https://doi.org/10.1016/j.celrep.2020.108588.
Juneja, M., Burns, J., Saporta, M.A., Timmerman, V., 2019. Challenges in modelling the Charcot-Marie-tooth neuropathies for therapy development. J. Neurol. Neurosurg. Psychiatry 90, 58–67. https://doi.org/10.1136/jnnp-2018-318834.
Kagiava, A., Richter, J., Tryfonos, C., Karaiskos, C., Heslegrave, A.J., Sargiannidou, I., Rossor, A.M., Zetterberg, H., Reilly, M.M., Christodoulou, C., Kleopa, K.A., 2019. Gene replacement therapy after neuropathy onset provides therapeutic benefit in a model of CMT1X. Hum. Mol. Genet. 28, 3528–3542. https://doi.org/10.1093/hmg/ddz199.
Kalinski, A.L., Kar, A.N., Craver, J., Tosolini, A.P., Sleigh, J.N., Lee, S.J., Hawthorne, A., Brito-Vargas, P., Miller-Randolph, S., Passino, R., Shi, L., Wong, V.S.C., Picci, C., Smith, D.S., Willis, D.E., Havton, L.A., Schiavo, G., Giger, R.J., Langley, B., Twiss, J. L., 2019. Deacetylation of Miro1 by HDAC6 blocks mitochondrial transport and mediates axon growth inhibition. J. Cell Biol. 218 https://doi.org/10.1083/JCB.201702187.
Kang, J.S., Tian, J.H., Pan, P.Y., Zald, P., Li, C., Deng, C., Sheng, Z.H., 2008. Docking of axonal mitochondria by Syntaphilin controls their mobility and affects short-term facilitation. Cell 132, 137–148. https://doi.org/10.1016/J.CELL.2007.11.024.
Karbowski, M., Youle, R.J., 2011. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. Curr. Opin. Cell Biol. 23, 476–482. https://doi.org/10.1016/J.CEB.2011.05.007.
Kijima, K., Numakura, C., Izumino, H., Umetsu, K., Nezu, A., Shiiki, T., Ogawa, M., Ishizaki, Y., Kitamura, T., Shozawa, Y., Hayasaka, K., 2005. Mitochondrial GTPase mitofusin 2 mutation in Charcot?Marie?Tooth neuropathy type 2A. Hum. Genet. 116, 23–27. https://doi.org/10.1007/s00439-004-1199-2.
Koshiba, T., Detmer, S.A., Kaiser, J.T., Chen, H., McCaffery, J.M., Chan, D.C., 2004a. Structural basis of mitochondrial tethering by mitofusin complexes. Science 305, 858–862. https://doi.org/10.1126/SCIENCE.1099793.
Koshiba, T., Detmer, S.A., Kaiser, J.T., Chen, H., McCaffery, J.M., Chan, D.C., 2004b. Structural basis of mitochondrial tethering by Mitofusin complexes. Science 1979 (305), 858–862. https://doi.org/10.1126/science.1099793.
Koutsopoulos, O.S., Laine, D., Osellame, L., Chudakov, D.M., Parton, R.G., Frazier, A.E., Ryan, M.T., 2010. Human Miltons associate with mitochondria and induce microtubule-dependent remodeling of mitochondrial networks. Biochimica et Biophysica Acta (BBA) - molecular. Cell Res. 1803, 564–574. https://doi.org/10.1016/J.BBAMCR.2010.03.006.
Krols, M., Asselbergh, B., De Rycke, R., De Winter, V., Seyer, A., Müller, F.-J., Kurth, I., Bultynck, G., Timmerman, V., Janssens, S., 2019. Sensory neuropathy-causing mutations in ATL3 affect ER–mitochondria contact sites and impair axonal mitochondrial distribution. Hum. Mol. Genet. 28, 615–627. https://doi.org/10.1093/hmg/ddy352.
Kutil, Z., Skultetyova, L., Rauh, D., Meleshin, M., Snajdr, I., Novakova, Z., Mikesova, J., Pavlicek, J., Hadzima, M., Baranova, P., Havlinova, B., Majer, P., Schutkowski, M., Barinka, C., 2019. The unraveling of substrate specificity of histone deacetylase 6 domains using acetylome peptide microarrays and peptide libraries. FASEB J. 33, 4035–4045. https://doi.org/10.1096/FJ.201801680R.
Laurá, M., Pipis, M., Rossor, A.M., Reilly, M.M., 2019. Charcot-marie-tooth disease and related disorders: an evolving landscape. Curr. Opin. Neurol. https://doi.org/10.1097/WCO.0000000000000735.
Leeuwesteijn, A.E.E.P.M., de Visser, E., Louwerens, J.W.K., 2010. Flexible cavovarus feet in Charcot-Marie-tooth disease treated with first ray proximal dorsiflexion osteotomy combined with soft tissue surgery: a short-term to mid-term outcome study. Foot Ankle Surg. 16, 142–147. https://doi.org/10.1016/j.fas.2009.10.002.
Li, Y.J., Cao, Y.L., Feng, J.X., Qi, Y., Meng, S., Yang, J.F., Zhong, Y.T., Kang, S., Chen, X., Lan, L., Luo, L., Yu, B., Chen, S., Chan, D.C., Hu, J., Gao, S., 2019. Structural insights of human mitofusin-2 into mitochondrial fusion and CMT2A onset. Nat. Commun. 10 https://doi.org/10.1038/S41467-019-12912-0.
Li, J., Dang, X., Franco, A., Dorn, G.W., 2022. Reciprocal regulation of Mitofusin 2-mediated Mitophagy and mitochondrial fusion by different PINK1 phosphorylation events. Front. Cell Dev. Biol. 10 https://doi.org/10.3389/fcell.2022.868465.
Loiseau, D., Chevrollier, A., Verny, C., Guillet, V., Gueguen, N., Pou De Crescenzo, M.-A., Ferré, M., Malinge, M.-C., Guichet, A., Nicolas, G., Amati-Bonneau, P., Malthièry, Y., Bonneau, D., Reynier, P., 2007. Mitochondrial coupling defect in Charcot-Marie-tooth type 2A disease. Ann. Neurol. 61, 315–323. https://doi.org/10.1002/ana.21086.

Mendell, J.R., Al-Zaidy, S., Shell, R., Arnold, W.D., Rodino-Klapac, L.R., Prior, T.W., Lowes, L., Alfano, L., Berry, K., Church, K., Kissel, J.T., Nagendran, S., L'Italien, J., Sproule, D.M., Wells, C., Cardenas, J.A., Heitzer, M.D., Kaspar, A., Corcoran, S., Braun, L., Likhite, S., Miranda, C., Meyer, K., Foust, K.D., Burghes, A.H.M., Kaspar, B. K., 2017. Single-dose gene-replacement therapy for spinal muscular atrophy. N. Engl. J. Med. 377, 1713–1722. https://doi.org/10.1056/NEJMoa1706198.

Miller, K.E., Sheetz, M.P., 2004. Axonal mitochondrial transport and potential are correlated. J. Cell Sci. 117, 2791–2804. https://doi.org/10.1242/JCS.01130.

Milone, M., Benarroch, E.E., 2012. Mitochondrial dynamics: general concepts and clinical implications. Neurology 78, 1612–1619. https://doi.org/10.1212/WNL.0B013E3182563C46.

Misko, A., Jiang, S., Wegorzewska, I., Milbrandt, J., Baloh, R.H., 2010. Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. J. Neurosci. 30, 4232–4240. https://doi.org/10.1523/JNEUROSCI.6248-09.2010.

Miyake, Y., Keusch, J.J., Wang, L., Saito, M., Hess, D., Wang, X., Melancon, B.J., Helquist, P., Gut, H., Matthias, P., 2016. Structural insights into HDAC6 tubulin deacetylation and its selective inhibition. Nature Chemical Biology 12 (9), 748–754. https://doi.org/10.1038/nchembio.2140.

Mo, Z., Zhao, X., Liu, H., Hu, Q., Chen, X.-Q., Pham, J., Wei, N., Liu, Z., Zhou, J., Burgess, R.W., Pfaff, S.L., Caskey, C.T., Wu, C., Bai, G., Yang, X.-L., 2018. Aberrant GlyRS-HDAC6 interaction linked to axonal transport deficits in Charcot-Marie-tooth neuropathy. Nat. Commun. 9, 1007. https://doi.org/10.1038/s41467-018-03461-z.

Muñoz, J.P., Ivanova, S., Sánchez-Wandelmer, J., Martínez-Cristóbal, P., Noguera, E., Sancho, A., Díaz-Ramos, A., Hernández-Alvarez, M.I., Sebastián, D., Mauvezin, C., Palacín, M., Zorzano, A., 2013. Mfn2 modulates the UPR and mitochondrial function via repression of PERK. EMBO J. 32, 2348–2361. https://doi.org/10.1038/emboj.2013.168.

Murphy, S.M., Laura, M., Fawcett, K., Pandraud, A., Liu, Y.-T., Davidson, G.L., Rossor, A. M., Polke, J.M., Castleman, V., Manji, H., Lunn, M.P.T., Bull, K., Ramdharry, G., Davis, M., Blake, J.C., Houlden, H., Reilly, M.M., 2012. Charcot-Marie-tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J. Neurol. Neurosurg. Psychiatry 83, 706–710. https://doi.org/10.1136/jnnp-2012-302451.

Naon, D., Zaninello, M., Giacomello, M., Varanita, T., Grespi, F., Lakshminaranayan, S., Serafini, A., Semenzato, M., Herkenne, S., Hernández-Alvarez, M.I., Zorzano, A., De Stefani, D., Dorn, G.W., Scorrano, L., 2016. Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum–mitochondria tether. Proc. Natl. Acad. Sci. 113, 11249–11254. https://doi.org/10.1073/pnas.1606786113.

Ngoh, G.A., Papanicolaou, K.N., Walsh, K., 2012. Loss of Mitofusin 2 promotes endoplasmic reticulum stress. J. Biol. Chem. 287, 20321–20332. https://doi.org/10.1074/jbc.M112.359174.

Nicholson, G.A., Magdelaine, C., Zhu, D., Grew, S., Ryan, M.M., Sturtz, F., Vallat, J.-M., Ouvrier, R.A., 2008. Severe early-onset axonal neuropathy with homozygous and compound heterozygous MFN2 mutations. Neurology 70, 1678–1681. https://doi.org/10.1212/01.wnl.0000311275.89032.22.

Nixon, R.A., Yang, D.S., 2012. Autophagy and neuronal cell death in neurological disorders. Cold Spring Harb. Perspect. Biol. 4 https://doi.org/10.1101/CSHPERSPECT.A008839.

Osko, J.D., Christianson, D.W., 2019. Structural basis of catalysis and inhibition of HDAC6 CD1, the enigmatic catalytic domain of histone deacetylase 6. Biochemistry 58, 4912–4924. https://doi.org/10.1021/ACS.BIOCHEM.9B00934/ASSET/IMAGES/LARGE/BI9B00934_0009.JPEG.

Osterloh, J.M., Yang, J., Rooney, T.M., Fox, A.N., Adalbert, R., Powell, E.H., Sheehan, A. E., Avery, M.A., Hackett, R., Logan, M.A., MacDonald, J.M., Ziegenfuss, J.S., Milde, S., Hou, Y.-J., Nathan, C., Ding, A., Brown, R.H., Conforti, L., Coleman, M., Tessier-Lavigne, M., Züchner, S., Freeman, M.R., 2012. dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. Science 1979 (337), 481–484. https://doi.org/10.1126/science.1223899.

Picci, C., Wong, V.S.C., Costa, C.J., McKinnon, M.C., Goldberg, D.C., Swift, M., Alam, N. M., Prusky, G.T., Shen, S., Kozikowski, A.P., Willis, D.E., Langley, B., 2020. HDAC6 inhibition promotes α-tubulin acetylation and ameliorates CMT2A peripheral neuropathy in mice. Exp. Neurol. 328, 113281 https://doi.org/10.1016/j.expneurol.2020.113281.

Pipis, M., Feely, S.M.E., Polke, J.M., Skorupinska, M., Perez, L., Shy, R.R., Laura, M., Morrow, J.M., Moroni, I., Pisciotta, C., Taroni, F., Vujovic, D., Lloyd, T.E., Acsadi, G., Yum, S.W., Lewis, R.A., Finkel, R.S., Herrmann, D.N., Day, J.W., Li, J., Saporta, M., Sadjadi, R., Walk, D., Burns, J., Muntoni, F., Ramchandren, S., Horvath, R., Johnson, N.E., Züchner, S., Pareyson, D., Scherer, S.S., Rossor, A.M., Shy, M.E., Reilly, M.M., Inherited Neuropathies Consortium - Rare Disease Clinical Research Network (INC-RDCRN), 2021. Natural history of Charcot-Marie-Tooth disease type 2A: a large international multicentre study. Brain. https://doi.org/10.1093/brain/awaa323.

Piscosquito, G., Saveri, P., Magri, S., Ciano, C., Di Bella, D., Milani, M., Taroni, F., Pareyson, D., 2015. Mutational mechanisms in MFN2-related neuropathy: compound heterozygosity for recessive and semidominant mutations. J. Peripher. Nerv. Syst. 20, 380–386. https://doi.org/10.1111/jns.12145.

Polke, J.M., Laurá, M., Pareyson, D., Taroni, F., Milani, M., Bergamin, G., Gibbons, V.S., Houlden, H., Chamley, S.C., Blake, J., Devile, C., Sandford, R., Sweeney, M.G., Davis, M.B., Reilly, M.M., 2011. Recessive axonal Charcot-Marie-tooth disease due to compound heterozygous mitofusin 2 mutations. Neurology 77, 168–173. https://doi.org/10.1212/WNL.0b013e3182242d4d.

Prior, R., Van Helleputte, L., Klingl, Y.E., Van Den Bosch, L., 2018. HDAC6 as a potential therapeutic target for peripheral nerve disorders. Expert Opin. Ther. Targets 22, 993–1007. https://doi.org/10.1080/14728222.2018.1541235.

Reilly, M.M., Murphy, S.M., Laurá, M., 2011. Charcot-Marie-tooth disease. J. Peripher. Nerv. Syst. 16, 1–14. https://doi.org/10.1111/j.1529-8027.2011.00324.x.

Reilly, M.M., Pareyson, D., Burns, J., Laurá, M., Shy, M.E., Singh, D., Agren, P.H., Altmann, V., Baets, J., Briggs, P., Burns, J., Butcher, K., Gaiani, L., Genovese, F., Gibbons, P., Laurá, M., Louwerens, J.W., Manzur, A., Moroni, I., Martinelli, N., Pareyson, D., Pfeffer, G., Ramdharry, G., Reilly, M.M., Shy, M., Singh, D., van der Linden, M., Wenz, W., 2017. 221st ENMC international workshop. Neuromuscul. Disord. 27, 1138–1142. https://doi.org/10.1016/j.nmd.2017.09.005.

Rizzo, F., Ronchi, D., Salani, S., Nizzardo, M., Fortunato, F., Bordoni, A., Stuppia, G., Del Bo, R., Piga, D., Fato, R., Bresolin, N., Comi, G.P., Corti, S., 2016. Selective mitochondrial depletion, apoptosis resistance, and increased mitophagy in human Charcot-Marie-tooth 2A motor neurons. Hum. Mol. Genet. 25, 4266–4281. https://doi.org/10.1093/hmg/ddw258.

Rizzo, F., Bono, S., Ruepp, M.D., Salani, S., Ottoboni, L., Abati, E., Melzi, V., Cordiglieri, C., Pagliarani, S., De Gioia, R., Anastasia, A., Taiana, M., Garbellini, M., Lodato, S., Kunderfranco, P., Cazzato, D., Cartelli, D., Lonati, C., Bresolin, N., Comi, G., Nizzardo, M., Corti, S., 2023. Combined RNA interference and gene replacement therapy targeting MFN2 as proof of principle for the treatment of Charcot–Marie–tooth type 2A. Cell. Mol. Life Sci. 80, 373. https://doi.org/10.1007/s00018-023-05018-w.

Rocha, A.G., Franco, A., Krezel, A.M., Rumsey, J.M., Alberti, J.M., Knight, W.C., Biris, N., Zacharioudakis, E., Janetka, J.W., Baloh, R.H., Kitsis, R.N., Mochly-Rosen, D., Townsend, R.R., Gavathiotis, E., Dorn, G.W., 2018. MFN2 agonists reverse mitochondrial defects in preclinical models of Charcot-Marie-tooth disease type 2A. Science 360, 336–341. https://doi.org/10.1126/science.aao1785.

Rossor, A.M., Shy, M.E., Reilly, M.M., 2020. Are we prepared for clinical trials in Charcot-Marie-tooth disease? Brain Res. 1729, 146625 https://doi.org/10.1016/j.brainres.2019.146625.

Samanas, N.B., Engelhart, E.A., Hoppins, S., 2020. Defective nucleotide-dependent assembly and membrane fusion in Mfn2 CMT2A variants improved by Bax. Life Sci Alliance 3. https://doi.org/10.26508/LSA.201900527.

Santel, A., Frank, S., Gaume, B., Herrler, M., Youle, R.J., Fuller, M.T., 2003. Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. J. Cell Sci. 116, 2763–2774. https://doi.org/10.1242/jcs.00479.

Saotome, M., Safiulina, D., Szabadkai, G., Das, S., Fransson, Å., Aspenstrom, P., Rizzuto, R., Hajnóczky, G., 2008. Bidirectional Ca2+-dependent control of mitochondrial dynamics by the Miro GTPase. Proc. Natl. Acad. Sci. U. S. A. 105, 20728–20733. https://doi.org/10.1073/PNAS.0808953105.

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.

Sato-Yamada, Y., Strickland, A., Sasaki, Y., Bloom, J., DiAntonio, A., Milbrandt, J., 2022. A SARM1-mitochondrial feedback loop drives neuropathogenesis in a Charcot-Marie-tooth disease type 2A rat model. J. Clin. Investig. 132 https://doi.org/10.1172/JCI161566.

Schiza, N., Georgiou, E., Kagiava, A., Médard, J.-J., Richter, J., Tryfonos, C., Sargiannidou, I., Heslegrave, A.J., Rossor, A.M., Zetterberg, H., Reilly, M.M., Christodoulou, C., Chrast, R., Kleopa, K.A., 2019. Gene replacement therapy in a model of Charcot-Marie-tooth 4C neuropathy. Brain 142, 1227–1241. https://doi.org/10.1093/brain/awz064.

Shahin, S., Lu, B., Zhou, Y., Xu, H., Chetsawang, J., Baloh, R.H., Wang, S., 2023. MFN1 augmentation prevents retinal degeneration in a Charcot-Marie-Tooth type 2A mouse model. iScience 26, 106270. https://doi.org/10.1016/J.ISCI.2023.106270.

Shen, S., Picci, C., Ustinova, K., Benoy, V., Kutil, Z., Zhang, G., Tavares, M.T., Pavlíček, J., Zimprich, C.A., Robers, M.B., Van Den Bosch, L., Bařinka, C., Langley, B., Kozikowski, A.P., 2021. Tetrahydroquinoline-capped histone deacetylase 6 inhibitor SW-101 ameliorates pathological phenotypes in a Charcot-Marie-tooth type 2A mouse model. J. Med. Chem. 64, 4810–4840. https://doi.org/10.1021/ACS.JMEDCHEM.0C02210.

Song, Z., Ghochani, M., McCaffery, J.M., Frey, T.G., Chan, D.C., 2009. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. Mol. Biol. Cell 20, 3525–3532. https://doi.org/10.1091/MBC.E09-03-0252.

Stavropoulos, F., Sargiannidou, I., Potamiti, L., Kagiava, A., Panayiotidis, M.I., Bae, J.H., Yeom, S.C., Lee, J.Y., Kleopa, K.A., 2021. Aberrant mitochondrial dynamics and exacerbated response to neuroinflammation in a novel mouse model of cmt2a. Int. J. Mol. Sci. 22 https://doi.org/10.3390/IJMS222111569/S1.

Stavropoulos, F., Georgiou, E., Schiza, N., Bell, S., Baloh, R.H., Kleopa, K.A., Sargiannidou, I., 2023. Mitofusin 1 overexpression rescues the abnormal mitochondrial dynamics caused by the Mitofusin 2 K357T mutation in vitro. J. Peripher. Nerv. Syst. 28 https://doi.org/10.1111/JNS.12564.

Stuppia, G., Rizzo, F., Riboldi, G., Del Bo, R., Nizzardo, M., Simone, C., Comi, G.P., Bresolin, N., Corti, S., 2015. MFN2-related neuropathies: clinical features, molecular pathogenesis and therapeutic perspectives. J. Neurol. Sci. 356, 7–18. https://doi.org/10.1016/j.jns.2015.05.033.

Summers, D.W., Frey, E., Walker, L.J., Milbrandt, J., DiAntonio, A., 2020. DLK activation synergizes with mitochondrial dysfunction to downregulate axon survival factors and promote SARM1-dependent axon degeneration. Mol. Neurobiol. 57, 1146–1158. https://doi.org/10.1007/s12035-019-01796-2.

Tanaka, A., Cleland, M.M., Xu, S., Narendra, D.P., Suen, D.F., Karbowski, M., Youle, R.J., 2010. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J. Cell Biol. 191, 1367–1380. https://doi.org/10.1083/JCB.201007013.

Tomaselli, P.J., Rossor, A.M., Polke, J.M., Poh, R., Blake, J., Reilly, M.M., 2016. Semi-dominant mutations in MFN2-related neuropathy and implications for genetic counselling. J. Peripher. Nerv. Syst. 21, 52–54. https://doi.org/10.1111/jns.12155.

Tur, J., Pereira-Lopes, S., Vico, T., Marín, E.A., Muñoz, J.P., Hernández-Alvarez, M., Cardona, P.-J., Zorzano, A., Lloberas, J., Celada, A., 2020. Mitofusin 2 in macrophages links mitochondrial ROS production, cytokine release, phagocytosis, autophagy, and bactericidal activity. Cell Rep. 32, 108079 https://doi.org/10.1016/j.celrep.2020.108079.

Van Lent, J., Verstraelen, P., Asselbergh, B., Adriaenssens, E., Mateiu, L., Verbist, C., De Winter, V., Eggermont, K., Van Den Bosch, L., De Vos, W.H., Timmerman, V., 2021. Induced pluripotent stem cell-derived motor neurons of CMT type 2 patients reveal progressive mitochondrial dysfunction. Brain 144, 2471–2485. https://doi.org/10.1093/BRAIN/AWAB226.

Verhoeven, K., Claeys, K.G., Züchner, S., Schröder, J.M., Weis, J., Ceuterick, C., Jordanova, A., Nelis, E., De Vriendt, E., Van Hul, M., Seeman, P., Mazanec, R., Saifi, G.M., Szigeti, K., Mancias, P., Butler, I.J., Kochanski, A., Ryniewicz, B., De Bleecker, J., Van Den Bergh, P., Verellen, C., Van Coster, R., Goemans, N., Auer-Grumbach, M., Robberecht, W., Milic Rasic, V., Nevo, Y., Tournev, I., Guergueltcheva, V., Roelens, F., Vieregge, P., Vinci, P., Moreno, M.T., Christen, H.J., Shy, M.E., Lupski, J.R., Vance, J.M., De Jonghe, P., Timmerman, V., 2006. MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-tooth type 2. Brain 129, 2093–2102. https://doi.org/10.1093/brain/awl126.

Ward, C.M., Dolan, L.A., Bennett, D.L., Morcuende, J.A., Cooper, R.R., 2008. Long-term results of reconstruction for treatment of a flexible Cavovarus foot in Charcot-Marie-tooth disease. The Journal of Bone and Joint Surgery-American 90, 2631–2642. https://doi.org/10.2106/JBJS.G.01356.

Webster, B.R., Scott, I., Traba, J., Han, K., Sack, M.N., 2014. Regulation of autophagy and mitophagy by nutrient availability and acetylation. Biochim. Biophys. Acta 1841, 525–534. https://doi.org/10.1016/J.BBALIP.2014.02.001.

Yagerman, S.E., Cross, M.B., Green, D.W., Scher, D.M., 2012. Pediatric orthopedic conditions in Charcot–Marie–tooth disease. Curr. Opin. Pediatr. 24, 50–56. https://doi.org/10.1097/MOP.0b013e32834e9051.

Zhou, Y., Carmona, S., Muhammad, A.K.M.G., Bell, S., Landeros, J., Vazquez, M., Ho, R., Franco, A., Lu, B., Dorn, G.W., Wang, S., Lutz, C.M., Baloh, R.H., 2021. Restoring mitofusin balance prevents axonal degeneration in a Charcot-Marie-tooth type 2A model. J. Clin. Invest. 131 https://doi.org/10.1172/JCI147307.

Züchner, S., Mersiyanova, I.V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J., Parman, Y., Evgrafov, O., De Jonghe, P., Takahashi, Y., Tsuji, S., Pericak-Vance, M.A., Quattrone, A., Battologlu, E., Polyakov, A.V., Timmerman, V., Schröder, J.M., Vance, J.M., Battologlu, E., 2004. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-tooth neuropathy type 2A. Nat. Genet. 36, 449–451. https://doi.org/10.1038/ng1341.