The expanding genetic landscape of hereditary motor neuropathies

REVIEW ARTICLE

The expanding genetic landscape of hereditary motor neuropathies

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Abstract

Hereditary motor neuropathies are clinically and genetically diverse disorders characterized by length-dependent axonal degeneration of lower motor neurons. Although currently as many as 26 causal genes are known, there is considerable missing heritability compared to other inherited neuropathies such as Charcot-Marie-Tooth disease. Intriguingly, this genetic landscape spans a discrete number of key biological processes within the peripheral nerve. Also, in terms of underlying pathophysiology, hereditary motor neuropathies show striking overlap with several other neuromuscular and neurological disorders. In this review, we provide a current overview of the genetic spectrum of hereditary motor neuropathies highlighting recent reports of novel genes and mutations or recent discoveries in the underlying disease mechanisms. In addition, we link hereditary motor neuropathies with various related disorders by addressing the main affected pathways of disease divided into five major processes: axonal transport, tRNA aminoacylation, RNA metabolism and DNA integrity, ion channels and transporters and endoplasmic reticulum.

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Abbreviations: ALS = amyotrophic lateral sclerosis; CMT = Charcot-Marie-Tooth disease; CMT2 = axonal Charcot-Marie-Tooth disease; ER = endoplasmic reticulum; HMN = hereditary motor neuropathies; HSP = hereditary spastic paraplegia; SMA = spinal muscular atrophy

Introduction

Hereditary motor neuropathies (HMN) are a type of neuromuscular disorder characterized by length-dependent lower motor neurons dysfunction. Classically, a patient with HMN presents with peroneal muscular atrophy and weakness without involvement of sensory dysfunction, relatively slow progressive wasting of the foot extensor muscles and intrinsic foots muscles occurs, with involvement of the muscles in the proximal lower limbs and distal upper limbs later on. Often atypical or 'complex'present with predominance in the upper limbs or by involvement of upper motor neurons, and vocal cord and/or diaphragm paralysis, among other features. Unlike Charcot-Marie-Tooth (CMT) disease, the most common group of inherited neuropathies and a sensorimotor neuropathy, HMN feature predominantly motor deficits and only mild, if any, sensory impairments. HMN are highly heterogeneous in terms of clinical presentation, as well as their age of onset and speed of progression. This review provides an overview of the current genetic landscape associated with distal HMN divided into five major processes: axonal transport, tRNA aminoacylation, RNA metabolism and DNA integrity, ion channels and transporters, and endoplasmic reticulum (Fig. 1). It should be noted, however, that such classification —although practical and insightful—is always arbitrary to some extent and that certain genes could fit into multiple clusters, such as the small heat shock proteins whose pathomechanism has been linked to both axonal transport, RNA metabolism, as well as processes of protein quality control. We will highlight recently reported genes and mutations, and the (presumed) underlying pathomechanisms that are associated with the specific genetic defects.

Axonal transport

The highly complex process of axonal transport allows movement of proteins, RNA and organelles though the axon. Impairment of this process can result in protein aggregation, reduced axonal outgrowth, impaired repair and regeneration and reduced exocytosis and endocytosis of large and small molecules, all of which affect the overall neuronal homeostasis (Beijer *et al.*, 2019*b*). A significant number of genes associated with HMN and related neuromuscular disorders have an underlying pathomechanism that impacts axonal transport (Table 1).

HSPB1

HSPB1 (Hsp27) is a member of the class of small heat shock proteins and functions as a molecular chaperone. Small heat shock proteins act as a first line of defence against misfolded proteins and keep them in a folding-competent state until larger chaperones, like Hsp70, arrive at the scene to perform the active refolding (Haslbeck *et al.*, 2019). This holdase function allows small heat shock proteins to protect and stabilize dynamic molecular structures such as neurofilaments, microtubules and actin filaments, linking them to axonal transport (Beijer *et al.*, 2019*b*). Mutations in *HSPB1* seem to have a different impact on these processes depending on the protein domain that is affected by the mutation. For instance, mutations in the highly conserved α-crystallin domain decrease the oligomer size and give rise to aberrant protein-protein interactions with, for instance, tubulin, whereas mutations in the C-terminal domain cause an increase in oligomer size and affect different protein-protein interactions (Ackerley *et al.*, 2006; Zhai *et al.*, 2007; Almeida-Souza *et al.*, 2010, 2011; Chalova *et al.*, 2014; Geuens *et al.*, 2017; Kalmar *et al.*, 2017; Alderson *et al.*, 2019*a*, *b*). The only pathway for which it has been demonstrated that all HSPB1 missense mutations have a dominant-negative impact, irrespective of their location, is autophagy (Haidar *et al.*, 2019). The first disease association for *HSPB1* was established in 2004 when heterozygous missense mutations were detected in several axonal CMT (CMT2) families (Evgrafov *et al.*, 2004). Until today, most reported mutations remain missense mutations, although several stop-gained and frameshift mutations have been reported (Capponi *et al.*, 2011; Echaniz-Laguna *et al.*, 2017*b*; Vendredy *et al.*, 2020). Many specific *HSPB1* mutations cause both CMT2 and HMN, without a clear distinction in the previously described molecular deficits, as such the distinction of these two phenotypes in relation to *HSPB1* mutations remains unclear (Adriaenssens *et al.*, 2017; Vendredy *et al.*, 2020). Further expansion on the phenotype was reported in recent studies, where patients presented with hyperreflexia and pyramidal tract involvement, suggesting upper motor neuron deficits (Capponi *et al.*, 2011; Stancanelli *et al.*, 2015; Amornvit *et al.*, 2017). One novel report has identified the new *D129E* mutation in a family with motor neuropathy and a distal myopathy, linking *HSPB1* mutations with yet another clinical phenotype (Lewis-Smith *et al.*, 2016).

HSPB3

Mutations in small heat shock protein B3 (*HSPB3*/Hsp17) have been associated with HMN on two occasions. The initial report was based on the identification of the *R7S* mutation in two siblings (Kolb *et*

al., 2010). The second reported mutation was the *W118H*, identified in a multigenerational HMN family (Nam *et al.*, 2018). With its function as a molecular chaperone and family members *HSPB1* and *HSPB8* already known for their association with the inherited peripheral neuropathies spectrum of disease, *HSPB3* poses an interesting candidate gene. However, functional evidence for these specific *HSPB3* mutations is currently missing. In addition, the initial *R7S* mutation has since its report gained a (likely) benign status in the ClinVar database, presumably based on its high frequency in public sequencing databases, such as gnomAD (Lek *et al.*, 2016; Landrum *et al.*, 2018). As such the overall association between *HSPB3* mutations and the HMN phenotype remains unclear.

HSPB8

HSPB8 (Hsp22) belongs to the same small heat shock protein family as *HSPB1* and *HSPB3*, with similar chaperone activity. HSPB8 is part of the complex required for chaperone-assisted selective autophagy (CASA) and in this HSPB8 was shown to direct misfolded proteins to either Hsp70 for refolding or to SQSTM1/p62 for autophagosomal degradation (Kwok *et al.*, 2011). Mutations in *HSPB8* were first associated with HMN, although a year later the association with CMT2L also became evident (Irobi *et al.*, 2004*b*; Tang *et al.*, 2005). In addition, *HSPB8* mutations were identified in patients with a progressive myopathy associated with myofibrillar network disruption and rimmed vacuolar pathology (Ghaoui *et al.*, 2016; Al-Tahan *et al.*, 2019; Nicolau *et al.*, 2020). Novel mutations in *HSPB8* were rarely identified, highlighting their low prevalence, although a more recent report suggests that for distal HMN they could make up a significant part of genetic diagnoses (Echaniz-Laguna *et al.*, 2017*a*).

Functional assessment of missense mutations in *HSPB8* associated with HMN and myopathy reported an increased affinity of mutant HSPB8 to bind to BAG3 in one study (Echaniz-Laguna *et al.*, 2017*a*), while two other studies reported a decreased affinity of mutant HSPB8 for BAG3 (Carra *et al.*, 2010; Shemetov and Gusev, 2011). It was shown *in vivo* that mutant HSPB8 forms aggregates, both in the sciatic nerve and the muscle tissue, and reduces autophagy of these HSPB8 aggregates (Bouhy *et al.*, 2018). The accumulation of toxic protein aggregates within the confined long peripheral axons could impair axonal transport, which could be one of the underlying pathomechanisms for these mutations.

DNAJB2

Similar to the heat shock proteins, *DNAJB2* (HSJ1) is a co-chaperone protein involved in ubiquitin-proteasome system-associated degradation and thus protection of neurons against cytotoxic protein aggregation (Westhoff *et al.*, 2005; Howarth *et al.*, 2007). Recessive mutations in *DNAJB2*, causing

abnormal splicing, were first associated with HMN in 2012 (Blumen *et al.*, 2012). Since then, *DNAJB2* missense, splice and frameshift mutations have been identified for CMT2T sometimes with associated parkinsonism (Gess *et al.*, 2014; Gonzaga-Jauregui *et al.*, 2015) and a specific 3.8 kb deletion of the first four exons of *DNAJB2* has been linked with spinal muscular atrophy (SMA) and parkinsonism (Sanchez *et al.*, 2016). The pathogenesis of recessive *DNAJB2* mutations neuropathies is likely the result from a loss-of-function mechanism, as demonstrated by reduced protein expression (Blumen *et al.*, 2012; Gess *et al.*, 2014). A cellular model demonstrated that while wildtype DNAJB2 is able to prevent protein aggregation, the *DNAJB2* splice mutations are not. Cells overexpressing the splice variant show increased protein aggregation and body inclusion formation similar to the other chaperones, such as HSPB1 and HSPB8, likely as an effect of an inability of the cell to properly degrade proteins that fail to refold properly (Blumen *et al.*, 2012).

DCTN1

DCTN1 encodes the dynactin protein, a prominent adaptor to the dynein molecular motor. Mutations in *DCTN1* are linked to Perry syndrome and HMN and have been associated with amyotrophic lateral sclerosis (ALS) (Puls *et al.*, 2003; Munch *et al.*, 2004; Farrer *et al.*, 2009; Tian *et al.*, 2020). The *G59S* mutant associated with HMN is located within the $p150^{glued}$ CAP-Gly domain, whereas the Perry syndrome-associated variants are located directly adjacent to the $p150^{glued}$ CAP-Gly GKNDG motif. The relevance of this difference in localization remains unknown as both Perry syndrome and HMN associated variants result in reduced microtubule binding and lead to dramatic redistribution of dynactin (Puls *et al.*, 2003; Levy *et al.*, 2006; Tian *et al.*, 2020). Since the original association of the *G59S* mutation with HMN, several reports have identified novel HMN and ALS associated missense mutations (Stockmann *et al.*, 2013; Liu *et al.*, 2017; Honda *et al.*, 2018; Tian *et al.*, 2020). Most recently, two novel *DCTN1* mutations were identified in association with HMN and ALS, *L210Afs*90* and *R1275C*, respectively (Tian *et al.*, 2020). The *L210Afs*90* is the first truncating *DCTN1* mutation to be identified. The *L210Afs*90* variant, however, would still fit with a supposed dominant-negative or gain-of-function, as the mutation results in the expression of a truncated DCTN1 protein (Tian *et al.*, 2020).

SPTAN1

SPTAN1 encodes αII-spectin, a critical component of the cytoskeleton. Heterozygous *SPTAN1* missense and in-frame deletion mutations were initially associated with a spectrum of epilepsy phenotypes (Syrbe *et al.*, 2017). More recently, mutations in *SPTAN1* have been described in patients with complex neurodevelopmental phenotypes, HMN and hereditary spastic paraplegia (HSP), although HSP-associated mutations are the only reported recessively inherited *SPTAN1* mutations (Gartner *et al.*, 2018; Beijer *et al.*, 2019*a*; Leveille *et al.*, 2019). The heterotetramers formed with two αII-spectrin and two β-

spectrin subunits are essential for the axon submembrane network and the formation of membrane periodic structures, which allow the maintenance of the longitudinal architecture of polarized neurons, likely influencing actin stability and axonal transport (Hauser *et al.*, 2018; Unsain *et al.*, 2018; Lorenzo *et al.*, 2019). While the β-spectrin (βII–βIV) subunits may vary depending on the subcellular localization, αII-spectrin is the only α-spectrin subtype expressed in the nervous system (Liu and Rasband, 2019). αII-spectrin and the spectrin cytoskeleton have been credited with numerous different functions, including subcellular spacing of ion channels and modulating AIS position (Hauser *et al.*, 2018; Unsain *et al.*, 2018; Liu and Rasband, 2019). Currently, three nonsense mutations have been associated with HMN, and the loss of αII-spectrin protein in patient-derived lymphoblasts suggests a haploinsufficiency mechanism (Beijer *et al.*, 2019*a*). In contrast, the C-terminal in-frame variants associated with West syndrome are associated with spectrin aggregate formation, suggesting a dominant-negative or gain-of-function mechanism (Syrbe *et al.*, 2017; Wang *et al.*, 2018*b*). Despite this, the underlying pathomechanisms of *SPTAN1* mutations associated with the different phenotypes remain largely unknown (Beijer *et al.*, 2019*a*; Liu and Rasband, 2019).

SYT2

Transport of synaptic vesicles to the nerve terminals and the subsequent release of neurotransmitters is essential for proper synapse function. In this way the v-SNARE (vesicle soluble *N*-ethylmaleimide-sensitive factor protein receptors), are essential for the exocytosis process of the vesicles with the presynaptic membrane (Littleton *et al.*, 1993; Mackler *et al.*, 2002). *SYT2* encodes synaptotagmin-2, a synaptic vesicle protein and the major isoform of synaptotagmin at mammalian neuromuscular junctions (Littleton *et al.*, 1993; Mackler *et al.*, 2002). In 2014, next-generation sequencing (NGS) in one family with childhood-onset Lambert Eaton-like myasthenia and a second family with motor neuropathy revealed causal dominant missense mutations in *SYT2* (Herrmann *et al*., 2014). Functional investigation of the myasthenia-associated mutation in *Drosophila* revealed that in *SYT2*-null animals, the myasthenia *D307A* failed to rescue the lack of synchronous neurotransmitter release and enhanced asynchronous release and elevated spontaneous vesicle fusion rates (Herrmann *et al.*, 2014). It is thought that the synaptotagmin-II function as a fusion clamp preventing spontaneous exocytosis and possibly its role as calcium sensors for evoked neurotransmitter release, is lost in the *SYT2* mutants and thereby causing neuromuscular junction dysfunction (Littleton *et al.*, 1993; Pang *et al.*, 2006; Herrmann *et al.*, 2014).

PLEKHG5

A single homozygous missense mutation, *F647S*, in *PLEKHG5* was first identified in one Malian family with HMN (Maystadt *et al.*, 2007). Since then, compound heterozygous missense and homozygous truncating mutations have been identified in intermediate CMT (Azzedine *et al.*, 2013; Kim *et al.*, 2013).

The *F647S* and other missense mutants result in reduced protein expression and stability and reduced capability to activate NFκB signalling (Maystadt *et al.*, 2007; Kim *et al.*, 2013). *PLEKHG5*-null mice also display a lower motor neuron phenotype, similar to that of the patients (Azzedine *et al.*, 2013; Luningschror *et al.*, 2017). *PLEKHG5* encodes a guanine exchange factor (GEF) of the RhoGEF family of proteins, capable of activating the Rho family of small GTPases, which have a myriad of functions in regulating neuron morphology and function (Hall and Lalli, 2010). *PLEKHG5* is predominantly expressed in the nervous system and specifically activates Rab26, a small GTPase selectively controlling the delivery of synaptic vesicles into pre-autophagosomes (Binotti *et al.*, 2015). *PLEKHG5* depletion in mice results in swollen presynaptic nerve terminals accumulating synaptophysin and neurofilament-H, as well as disorganization of actin filaments (Luningschror *et al.*, 2017). The accumulation of synaptic vesicles at the presynaptic nerve terminal is a direct result of the reduced autophagosome formation in *PLEKHG5*-depleted motor neurons (Luningschror *et al.*, 2017). Rab26 activation is a crucial factor for recruitment of the autophagy machinery to synaptic vesicles. *PLEKHG5*-depleted cells demonstrated lack of Rab26 activation as a cause for the reduced autophagosome formation, a potential underlying pathomechanisms for *PLEKHG5*-related neuropathy (Luningschror *et al.*, 2017).

Transfer RNA aminoacylation

tRNA aminoacylation is the first step in the critical process of protein translation, wherein each amino acid is matched with its cognate tRNA in an ATP-dependent reaction by aminoacyl tRNA synthetases (aaRS) (Storkebaum, 2016). Subsequent capturing of the aminoacylated tRNA and processing by the ribosome constitutes the process of protein synthesis. Despite the tRNA aminoacylation process being critical for all cells, there seems to be specific neuronal susceptibility to impairment in this pathway, resulting in many genes involved in tRNA aminoacylation to be associated with neurological disorders and specifically HMN (Table 2) (Meyer-Schuman and Antonellis, 2017).

AARS1

AARS1 encodes alanyl-tRNA synthetase (AlaRS). As with all of its family members, it is directly involved in the charging of tRNA with their cognate amino acid, in this case alanine. Dominant mutations in *AARS1* were first associated with CMT2N, but subsequently dominant mutations were associated with HMN and recessive mutations with multisystem syndromes (Latour *et al.*, 2010; McLaughlin *et al*., 2012; Zhao *et al.*, 2012; Simons *et al.*, 2015; Weterman *et al.*, 2018). *AARS1* is one of the 10 enzymes within the aaRS family that, in addition to its catalytic domain, possesses an editing domain to compensate for the mischarging of amino acids with high structural similarity to alanine (Storkebaum, 2016). Pathogenic

mutations have been identified in both the catalytic domain and the editing domain. Several mutations have been found in more than one family, but the *R329H* mutation seems to be the most recurrent (Lin *et al.*, 2011; McLaughlin *et al.*, 2012; Bansagi *et al.*, 2015; Motley *et al.*, 2015). Retained catalytic activity for some mutants in combination with mutants located outside of the catalytic domain, suggests that loss of the catalytic activity is not a universal mechanism. As such, further functional investigations showed that both the *R329H* and *N71Y* are localized to punctate intracellular structures, but it remains unknown if this is related to aberrant intracellular signalling and could be underlying the CMT phenotype for some mutants (McLaughlin *et al.*, 2012). Most recently, *in vitro* studies on the N71Y-*AARS1* mutant showed that the altered intracellular localization could be reversed with valproic acid, although *in vivo* studies are necessary to assess its effectiveness as a potential therapy (Tatsumi *et al.*, 2019).

GARS1

Mutations in *GARS1*, glycyl-tRNA synthetase (GlyRS), were the first aaRS mutations to be associated with CMT2D (Antonellis *et al.*, 2003). Mutations in *GARS1* have since also been associated with HMN and severe distal SMA (Del Bo *et al.*, 2006; James *et al.*, 2006; Eskuri *et al.*, 2012). Generally, *GARS1* mutations cause an upper limb-predominant neuropathy phenotype which may vary in terms of sensory involvement and severity (Forrester *et al.*, 2020). *GARS1* is one of the two aaRS genes that encodes both the cytoplasmic and the mitochondrial form of GlyRS, which are separately generated using either alternative translational start sites or by alternative mRNA splicing (Turner *et al.*, 2000). There are currently over 20 different neuropathy-associated *GARS1* mutations, all of which are missense mutations found throughout the protein (Antonellis *et al.*, 2003; Motley *et al.*, 2010; Lee *et al.*, 2019; Nan *et al.*, 2019; Yalcouye *et al.*, 2019). Initially, loss of tRNA charging function was thought to be the sole pathological mechanism, but several mutations including *E71G*, *P234KY*, *D500N*, and *S581L* have been shown to still be active, although pathogenicity of the *S581L* variant has since been questioned (Antonellis *et al.*, 2006; Nangle *et al.*, 2007; Xie *et al.*, 2007; Griffin *et al.*, 2014). As such, loss of tRNA charging capabilities does not seem a uniform mechanism for *GARS1*-associated neuropathy. Subsequent studies have included GlyRS dimerization as a potential mechanism, demonstrating that many mutations are localized to the dimer interface of the protein with varying effects on dimerization (Cader *et al.*, 2007; Nangle *et al.*, 2007; Xie *et al.*, 2007). Similarly, no uniform effect of GlyRS mutants could be observed as mutations both reduced, increased or left GlyRS dimerization unchanged (Nangle *et al.*, 2007; Xie *et al.*, 2007; Malissovas *et al.*, 2016). Interestingly a spontaneous *GARS1* mouse mutant, equivalent to a dominant human *P234KY GARS1* mutation, causes a severe motor and sensory neuropathy in mice (Seburn *et al.*, 2006). Since then, several *GARS1* mouse models have been made, including heterozygous

loss-of-function models without a neuropathy phenotype. This model indicates that simple loss of GlyRS is not responsible for the disease (Achilli *et al.*, 2009). Several different hypotheses exist around the pathomechanisms of *GARS1* mutations, some of which may occur in combination. It is thought that loss of tRNA charging function may in some cases contribute to the pathogenesis, despite it not being a uniform occurrence (Storkebaum, 2016). In addition, both dimerization and non-canonical GlyRS functions, such as formation of mRNA 3′-ends, are thought to contribute to some extent (Johanson *et al.*, 2003). A true conclusion to the discussion of whether *GARS1*-associated neuropathy is caused by loss-of-function or dominant-negative or toxic gain-of-function effects remains to be found. Encouragingly, a recent study used AAV9 technology to successfully attain allele-specific mutant GlyRS knockdown preventing onset of neuropathy in GARS*1* mouse models (Morelli *et al.*, 2019).

HARS1

HARS1, histidyl-tRNA (HisRS) was the fifth aaRS gene to be associated with axonal peripheral neuropathy (Vester *et al.*, 2013). The initial report in 2013, demonstrated the pathogenicity of the CMT2W-associated *R137Q* mutant by loss-of-function effects in yeast and *Caenorhabditis elegans* (Vester *et al.*, 2013). Since then the phenotypic spectrum has expanded to include HMN and intermediate CMT (Vester *et al.*, 2013; Safka Brozkova *et al.*, 2015; Abbott *et al.*, 2018; Royer-Bertrand *et al.*, 2019). A recent study reports a novel mutation, *V133F*, present closely to other mutations in a patient with demyelinating CMT with cerebellar atrophy, and cognitive deficits, expanding the spectrum further (Royer-Bertrand *et al.*, 2019). *HARS1* mutations generally cause a loss-of-function effect in yeast, inhibiting yeast growth, and show reduced tRNA charging capabilities (Safka Brozkova *et al.*, 2015; Abbott *et al.*, 2018; Royer-Bertrand *et al.*, 2019). In contrast to pathogenic *GARS1* mutations, published *HARS1* mutations do not seem to interfere with *HARS1* dimerization, although reduced histidine substrate binding was noted for the *Y330C* and *V155G* mutations (Abbott *et al.*, 2018). Recently, *in vitro* studies of HisRS mutants demonstrated pathomechanisms depending on the protein conformation rather than loss of tRNA charging activity (Blocquel *et al.*, 2019).

WARS1

Mutations in *WARS1*, tryptophanyl-tRNA synthetase (TrpRS), were first associated to the HMN phenotype when the same heterozygous mutation *H257R* was identified in HMN patients in three separate families (Tsai *et al.*, 2017). Recently, two novel heterozygous *WARS1* mutations, *F138Y* and *D314G*, were reported in HMN families (Li *et al.*, 2019; Wang *et al.*, 2019*a*). The most in-depth functional assessment was performed for the *H257R* mutation, which demonstrated reduced

aminoacylation activity of TrpRS, resulting in reduced neurite outgrowth and increased neurite degeneration. The *H257R* mutation also changes the translational machinery properties of TrpRS in yeast and acquires an enhanced angiostatic activity (Tsai *et al.*, 2017). Due to the location of the *D314G* mutation in the catalytic domain and near the critical binding domain of Trp and ATP, a similar disturbance of aminoacylation activity could be the mechanism (Wang *et al.*, 2019*a*). In contrast, the *F138Y* mutation seems to reduce overall TrpRS protein expression, which was not evident for the *H257R* mutation (Li *et al.*, 2019). Although overall decreased TrpRS expression might similarly result in a failing translational machinery unable to meet the demand for high protein synthesis in axons.

HINT1

Recessive mutations in *HINT1* were first associated with axonal neuropathy with neuromyotonia (Zimon *et al.*, 2012). Further associations with CMT2 have arisen since then (Caetano *et al.*, 2014; Zhao *et al.*, 2014; Boaretto *et al.*, 2015; Lassuthova *et al.*, 2015; Zimon *et al.*, 2015; Rauchenzauner *et al.*, 2016; Meng *et al.*, 2018; Scarpini *et al.*, 2019; Wang *et al.*, 2019*c*). HINT1 is a ubiquitously expressed protein, whose functions have remained largely unknown despite the account of several different presumed functions. HINT1 is capable of hydrolyzing aminoacyl adenylates, which are the intermediary products of the reaction in which tRNAs are charged with their cognate amino acids by the previously mentioned ARS proteins (Chou and Wagner, 2007; Wang *et al.*, 2012; Zhou *et al.*, 2013). It is thought that HINT1 thus mediates aaRS activity and influences the overall level of tRNA aminoacylation (Wang *et al.*, 2012; Peeters *et al.*, 2017). Several other roles have been attributed to HINT1, such as a transcriptional suppressor, an adaptor coupling protein kinase C gamma, enabling desulphurization of nucleoside 5′-*O*-monophosphorothioates (NMPS) (Krakowiak *et al.*, 2014). Despite these presumed functions, it remains unclear which failing mechanism is the cause for the neuropathy and neuromyotonia phenotype.

RNA metabolism and DNA integrity

Both RNA processing mechanisms and DNA damage and integrity are commonly occurring themes within neurodegenerative diseases (Butti and Patten, 2018; Weskamp and Barmada, 2018; Nussbacher *et al.*, 2019). Aggregation-prone RNA binding proteins (RBPs) are possibly the most well-known mechanism for neurodegenerative disorders, with genes such as *TARDBP* (TDP-43), *HNRNPA1*, *FUS*, *TIA1* and *ATXN2* sharing a common mechanism of mislocalized and aggregated RBPs, with altered RNA metabolism in the form of delayed mRNA transport, altered mRNA splicing, (local) translation and decay as shared pathomechanisms (Butti and Patten, 2018; Weskamp and Barmada, 2018; Nussbacher *et al.*,

2019). Similarly, DNA damage has been noted as a common pathological mechanism in neurodegenerative diseases (Madabhushi *et al*., 2014; Thadathil *et al*., 2019). Genes such as *ATM* and *FUS*, associated with ataxia telangiectasia and ALS (Wang *et al*., 2019*b*), respectively, have been shown to be crucial in maintaining genomic stability and DNA integrity (Baechtold *et al*., 1999; Shiloh and Ziv, 2013). Similarly, PARP1 and the process of ADP ribosylation, important for the DNA damage response, have been shown to be upregulated in neurodegenerative disorders including ALS and Alzheimer's disease (Love *et al*., 1999; Kim *et al*., 2004; McGurk *et al*., 2019). In addition to the broader association with neurodegenerative diseases, impairments in different parts of RNA metabolism and DNA integrity have been associated with HMN specifically (Table 3).

FBXO38

FBXO38 was first identified by yeast-two hybrid assay as modulator of KLF7 activity (MOKA), a member of the Krüppel-like transcription factor family, an important transcription factor for the developing nervous system (Smaldone *et al.*, 2004). In addition to its expession in several neurodevelopmental stages, FBXO38 is actively transcribed in both post-mitotic motor neurons and neural progenitor cells of the spinal cord in mice (Smaldone *et al.*, 2004; Sumner *et al.*, 2013). Confirmation of this expression in humans was obtained and expression was also detected in human skeletal-muscle tissue (Sumner *et al.*, 2013). The *C206R* heterozygous missense mutation in *FBXO38* was identified in two families with dominant HMN and was shown to impair activation of KLF7 target genes associated with a significant decrease in primary neurite length in primary motor neurons (Sumner *et al.*, 2013). Recently, a novel homozygous missense mutation *R526Q* was reported in association with a case of recessive HMN (Akcimen *et al.*, 2019). Despite the predicted deleterious effect for this novel mutation, functional validation of this variant was not performed. The identification of both dominant and recessive cases of *FBXO38* mutations fuels the discussion on the currently unknown underlying mutational effect: dominant-negative or loss-of-function.

SETX

Mutations in *SETX* are known to cause several neurodegenerative diseases, dominantly inherited *SETX* mutations are causative for ALS4 or HMN with upper motor neuron signs, while recessive loss-of-function mutations cause ataxia with oculomotor apraxia type 2 (AOA2) (Chen *et al.*, 2004; Moreia *et al.*, 2004; Tripolszki *et al.*, 2017; Grunseich *et al.*, 2020). *SETX* encodes senataxin, a RNA/DNA helicase that

provides protection against oxidative DNA damage, and is involved in transcription regulation, RNA maturation, R-loop resolution and localization at collision sites of the transcription and replication machinery (Chen *et al.*, 2004; Richard and Manley, 2014; Grunseich *et al.*, 2018). The effect of disease-causing *SETX* mutations seems to vary significantly for the dominant (ALS4) and recessive (AOA2) mutations. As increased senataxin helicase activity was noted for the ALS4-*L389S* mutant, whereas AOA2 *SETX* mutations prevent senataxin sumoylation (Richard and Manley, 2014; Grunseich *et al.*, 2018, 2020). Furthermore, high levels of R-loops were demonstrated in mitochondria of AOA2 neuronal progenitor cells, whereas reduction in R−loop levels has been demonstrated for both *L389S* and *E385K* ALS4-associated mutants (Grunseich *et al.*, 2018, 2020). R-loops are dynamic structures that have been associated with other neurological diseases, including disorders due to repeat expansions such as Friedreich ataxia, *c9orf72* ALS (Haeusler *et al.*, 2014; Groh *et al*., 2017; Kong *et al.*, 2017). Due to the location of R-loops in CpG island regions, it was thought that R-loops could have a transcription regulatory role. Using the ALS4-associated *L389S* mutant, it was shown that disruption of R-loop formation causes changes in DNA methylation, which results in altered gene expression of *BAMBI* and other genes in the TGF-β pathway (Grunseich *et al.*, 2018). Despite existing associations between the TGF-β pathway and neuronal degeneration, it remains to be elucidated whether the effect of ALS4 *SETX* mutations on TGF-β signalling activation is the causal mechanism for *SETX*-associated neuropathy (Mushtaq *et al.*, 2016; Grunseich *et al.*, 2018).

IGHMBP2

Recessive mutations in *IGHMBP2* were first associated with SMA with respiratory distress (SMARD), also known as HMN6 (Grohmann *et al.*, 2001). Since then, mutations have also been identified in association with CMT2 and DSMA1 (Guenther *et al.*, 2009*a*; Cottenie *et al.*, 2014). *IGHMPB2* encodes immunoglobulin μ-binding protein 2, a DNA helicase protein with transcriptional activating and repressing capabilities (de Planell-Saguer *et al.*, 2009; Lim *et al.*, 2012). *IGHMBP2*, like *SETX*, is part of the UPF1-like subfamily of helicases, capable of unwinding both DNA and RNA duplexes in the 3′–5′ direction (de Planell-Saguer *et al.*, 2009; Lim *et al.*, 2012; Perego *et al.*, 2020). IGHMBP2 is presumably involved in a large variety of cellular processes, such as pre-mRNA processing, immunoglobulin class switching, regulation of DNA replication and interactions with TATA binding proteins (Grohmann *et al.*, 2001; Lim *et al.*, 2012). The exact mechanisms by which mutations in *IGHMBP2* cause motor neuron degeneration remains unknown and there is not one single mechanism impaired in all disease-causing mutations. The majority of the HMN-related mutations are located within the helicase domain, resulting in changes in the ATPase and hydrolase activity or otherwise resulting in a reduced helicase motor stability (Guenther *et al.*, 2009*a*, *b*). In contrast, mutations outside the helicase domain, such as *T493I*, as well as several truncating mutations have been shown to result in reduced protein expression, possibly leading to an overall reduced activity (Eckart *et al.*, 2012). Lastly, mutations have been shown to affect

nucleic acid binding ability (*N583I* and *R603H*) uncoupling of ATPase activity from RNA unwinding (*D565N*) (Guenther *et al.*, 2009*a*, *b*). It seems that there might not be one single underlying mechanism relating to *IGHMBP2* motor neuron disorders (Perego *et al.*, 2020).

AIFM1

Recessive mutations in mitochondrial apoptosis-inducing factor 1, encoded by *AIFM1*, were initially associated with mitochondrial encephalomyopathy (Ghezzi *et al.*, 2010). Since then the phenotypes that are associated with X-linked recessive *AIFM1* mutations have broadened extensively, into e.g. HMN, CMT4, ataxia, Cowchock syndrome and spondylometaphyseal dysplasia (Rinaldi *et al.*, 2012; Ardissone *et al.*, 2015; Hu *et al.*, 2017; Sancho *et al.*, 2017; Wang *et al.*, 2018*a*). Mitochondria perform their essential role in energy production through the oxidative phosphorylation system (OXPHOS), which is comprised of protein complexes CI to CV (Susin *etal.*, 1999; Hu *et al.*, 2017). AIFM1 functions as a FAD-containing and NADH-specific oxidoreductase with an important function for energy metabolism (Sevrioukova, 2011; Ferreira *et al.*, 2014). Deficiency of *AIFM1* or indeed some disease-related mutations, such as *R201del*, *F210L*, *G308E*, results in misassembling of the OXPHOS complexes, or inhibition in catalyzing redox reactions (Sevrioukova, 2016; Hu *et al.*, 2017). AIFM1 performs another function however, as an apoptotic stimulus via PAR-related cell death, as AIFM1 translocates to the nucleus upon poly-ADPribose (PAR) accumulation where it promotes chromatin condensation and DNA degradation (Sevrioukova, 2011). However, the mechanistic effects of the *AIFM1* mutations remain diverse, some disease-associated mutations, such as *E493V*, seem to specifically enhance apoptogenic properties, possibly by increased DNA-binding affinity (Rinaldi *et al.*, 2012; Sevrioukova, 2016). In-depth assessment of the high-resolution X-ray structure of wild-type and mutant AIFM1 revealed that changes in MIA40 binding, dimerization of AIFM1, and alterations of the FAD binding domain are also capable of producing disease phenotypes (Sevrioukova, 2016). There seems to be a general trend that suggests that mild changes in the AIFM1 structure and function/expression correlate more with the axonal neuropathy phenotype, whereas the mutants that affect the energy metbolism and OXPHOS complex formation are more associated with the severe mitochondrial encephalomyopathy phenotypes (Sevrioukova, 2016; Hu *et al.*, 2017). In addition to these chemical and structural changes, mitochondrial morphology in *F210S* patient-derived fibroblasts was found to be severely fragmented, although replication in other *AIFM1* patient fibroblasts is needed to corroborate this finding (Sancho *et al.*, 2017).

Ion channels and transporters

Ion channels and other transporters can have a variety of different functions within a cell. However, their common purpose is to transport their clients from one cellular compartment to another, either activating specific processes or achieving and maintaining homeostasis. Malfunctioning ion channels and

transporters are associated with a multitude of different neurological diseases including epilepsy, ataxia and peripheral neuropathy (Persson *et al.*, 2016; Oyrer *et al.*, 2018; Bushart and Shakkottai, 2019). This section will discuss the diverse group of ion channels and transports that are directly associated with HMN (Table 4).

TRPV4

In 2010, mutations in *TRPV4* were first associated with CMT2C, HMN and scapuloperoneal neuropathy (SPSMA) (Auer-Grumbach *et al.*, 2010; Chen *et al.*, 2010; Deng et al., 2010; Landoure *et al.*, 2010). Interestingly, there are several relatively uncommon symptoms for other CMT types, that are more common for *TRPV4*-related neuropathy, such as vocal cord paralysis and phrenic nerve paralysis, scapular weakness and wasting, and hearing loss (Zimon *et al.*, 2010; Deng *et al.*, 2020). These additional features may be suggestive of *TRPV4*-related neuropathy and can be used in clinical practice as a clue for genetic testing. *TRPV4* encodes transient receptor potential subfamily vanilloid member 4, a ubiquitously expressed cation channel with weak selectivity for Ca^{2+}. Assessment of the physiological properties of the neuropathy-associated *R316C* and *R269C* mutations demonstrated an increased calcium-channel activity for these TRPV4 mutants (Deng *et al.*, 2010; Landoure *et al.*, 2010; Klein *et al.*, 2011). In addition, TRPV4-mutant channels have a higher chance of being in the open conformation (Fecto *et al.*, 2011). Based on these observations, the hypothesized mechanism of *TRPV4* mutation is a gain-of-function mechanism caused by increased intracellular calcium influx (Fecto *et al.*, 2011; Klein *et al.*, 2011; Sullivan *et al.*, 2015; Deng *et al.*, 2020).

SLC5A7

The sodium-dependent high-affinity choline transporter (CHT), encoded by *SLC5A7*, is a critical component in the neuromuscular junction, where it constitutes the re-uptake of choline into the nerve terminals (Barwick *et al.*, 2012). Initially, a single heterozygous truncating variant was described in a family with HMN with vocal cord paralysis (Barwick *et al.*, 2012). Since then three additional HMN mutations have been reported, with variable vocal cord involvement between patients (Ingram *et al.*, 2016; Hamanaka *et al.*, 2018; Salter *et al.*, 2018). All of these HMN-associated mutations are truncating variants located in the last exon, presumably escaping NMD. Functional studies on the initial *K499Nfs*13* showed that this variant does indeed lead to a reduced choline uptake activity (Barwick *et al.*, 2012). However, this effect was aggravated in the presence of WT-CHT, suggesting a dominant-negative effect (Barwick et al., 2012). In contrast, recessive variants in *SLC5A7* are known to cause congenital myasthenic syndrome (Ohno *et al.*, 2001; Byring *et al.*, 2002; Bauche *et al.*, 2016; Wang *et al.*, 2017; Pardal-Fernandez *et al.*, 2018). Recessive *SLC5A7* mutations are associated with a loss of protein expression and/or loss of transporter activity. The most aggressive mutations, *S263F*, resulted in complete

loss of protein activity, which is thought to be the cause of the early lethality, as it is reminiscent of the phenotype associated with *SLC5A7*-null mice (Ferguson *et al.*, 2004; Bauche *et al.*, 2016). The *K499Nfs*13* has a reported activity of ~25%, which is higher than the reported CMS mutants (Barwick *et al.*, 2012). This seems to suggest that a relationship between the amount of CHT activity and the associated phenotype could exist, although this requires further investigation (Bauche *et al.*, 2016; Banerjee *et al.*, 2019).

SLC12A6

Recessive truncating mutations in *SLC12A6*, encoding KCC3, were first identified in four families with CMT and agenesis of the corpus callosum, also known as Andermann syndrome (ACCPN), by candidate gene sequencing of the known locus on chromosome 15q14 (Howard *et al.*, 2002*a*, *b*). These initial variants include the *T813fs*81* French-Canadian founder mutation (Howard *et al.*, 2002*b*). Subsequently, several additional recessive truncating and missense variants were identified in patients with Andermann syndrome (Uyanik *et al.*, 2006; Ding *et al.*, 2013; Park *et al.*, 2019). Dominant mutations in *SLC12A6* have since been identified with non-syndromic HMSN and a single variant *T99IA* has been associated with HMN (Kahle *et al.*, 2016). Based on functional assessment of several ACCPN mutations, it is thought that the ACCPN mutations result in non-functional protein with no activity (Howard *et al.*, 2002*b*; Ding *et al.*, 2013; Flores *et al.*, 2019). KCC3 is a cation-chloride co-transporter, which regulates efflux of K^+ and Cl^- across plasma membranes, maintaining intracellular Cl^- concentration, and as such regulating cell volume (MacAulay *et al.*, 2004; Cruz-Rangel *et al.*, 2011). Interestingly, studies have revealed that the average brain mass of individuals with KCC3 truncating variants was significantly greater when compared to matched controls, suggesting accumulation of fluid could be linked with the function of KCC3 (Auer *et al.*, 2016). Similarly, fluid accumulation together with specific nodal disruption was shown for sciatic nerves of KCC3-null mice, supporting the patient's findings (Byun and Delpire, 2007). It is interesting that the only HMN-associated mutation, *T991A*, occurs on one of the two critical phosphorylation-regulatory residues in KCC3 (*T991* and *T1048*) (Rinehart *et al.*, 2009; Kahle *et al.*, 2016). Under normal circumstances, KCC3 activity is silenced upon phosphorylation of *T991* and/or *T1048* (Rinehart *et al.*, 2009). The *T991A* mutation results in a lack of phosphorylation at the *T991* locus and causes constitutive activation of KCC3, which suggests an opposing gain-of-function mechanism, specifically for this variant (Kahle *et al.*, 2016). Indeed, an opposing effect on cell volume was also observed in the *T991A* mouse model, demonstrating a smaller axon diameter range and overall decrease in myelin thickness (Flores *et al.*, 2019).

SLC25A21

The first disease association for *SL25A21* (*OCD1*), was a mother and son with synpolydactyly in both

hands and feet, who both carried a heterozygous 14q13.3 deletion within the *SLC25A21* gene (Meyertholen *et al.*, 2012). Since then five cases of heterozygous *de novo* variants in patients with developmental delay, alopecia, and dysmorphic features have been reported (Bupp *et al.*, 2018; Rodan *et al.*, 2018). Specifically, for motor neuropathies, one recessive missense mutation, *K232R*, has been found in a patient with childhood-onset distal HMN (Boczonadi *et al.*, 2018). A muscle biopsy in the HMN case provided important clues linking with the mitochondrial function of *SLC25A21*, as the oxidative enzyme histochemistry revealed cytochrome *c* oxidase (COX)-deficient fibres as well as respiratory chain dysfunction by decreased complex I and IV activity and decreased mtDNA levels (Boczonadi *et al.*, 2018). Further characterization of the *K232R* mutation demonstrated impairment of mitochondrial oxodicarboxylate transport by reduced formation of the salt bridge network (Boczonadi *et al.*, 2018). The reduced *SLC25A21* activity leads to increased 2-oxoadipate, quinolinic acid, and pipecolic acid levels in patient urine samples, which selectively impairs mitochondrial respiratory chain complexes in neuronal cells (Boczonadi *et al.*, 2018).

ATP7A

ATP7A encodes a copper-transporting P-type ATPase crucially important for regulation of intracellular copper homeostasis (Tumer, 2013). X-linked mutations in *ATP7A* have been associated with three different phenotypes: Menkes disease, the less severe occipital horn syndrome, and distal HMN (Kennerson *et al.*, 2010; Tumer, 2013; Gualandi *et al.*, 2019). A great variety of *ATP7A* mutations, including nonsense, missense, and exon duplications/deletions, are linked to Menkes disease and occipital horn syndrome (Tumer, 2013). In contrast, the HMN mutations are limited to four missense mutations: *Y760C*, *A991D*, *T994I and P1386S (Kennerson et al., 2010; Bansagi et al., 2017; Gualandi et al., 2019). Mutations underlying Menkes disease and occipital horn syndrome are generally truncating variants, resulting in protein loss or other variants resulting in reduced protein activity and copper trafficking (Kaler et al., 2008; Tumer, 2013). Menkes disease and occipital horn syndrome versus HMN mutations seem to have diverting underlying mechanisms as patients with Menkes disease and occipital horn syndrome show poor copper absorption and low copper levels in blood and brain whereas HMN patients show normal blood copper levels. (Kaler et al., 2008; Kennerson et al., 2010; Gualandi et al., 2019). Both the T994I patient fibroblasts and T994I knock-in mouse model do show subtle reduction of protein expression. Interestingly, for the A991D mutation, while predominantly associated with HMN, the patients show mild symptoms associated with Menkes disease and occipital horn syndrome, the clinical phenotype might be a spectrum of disease (Gualandi et al., 2019). ATP7A typically resides in the trans-Golgi network (TGN), but is trafficked to the plasma membrane with elevated copper concentrations (Kaler, 2011). Rather than loss of activity, HMN-associated ATP7A mutations, T994I and P1386S, demonstrated alterations in the intracellular localization both in patient fibroblasts, and T994I induced pluripotent stem cell (iPSCs), as well as in a model using overexpression of tagged proteins (Kennerson*

et al., 2010; Yi et al., 2012; Perez-Siles et al., 2020). Implementation of trafficking assays on the overexpressed HMN mutant ATP7A, revealed limited retrieval of mutant ATP7A from the plasma membrane to the TGN, causing a more diffuse cellular distribution with only 20–30% of ATP7A located in the TGN, compared to 80–90% for wild-type ATP7A (Yi et al., 2012). Interestingly, for the T994I mutant, this aberrant localization is linked with altered protein-protein interaction with VCP, a gene with known involvement in motor neuron disorders and multisystem proteinopathy (Yi et al., 2012; Tang and Xia, 2016; Yi and Kaler, 2018). The altered ATP7A-VCP interaction is caused by conformational effects induced by the T994I mutation exposed to/span> a UBX domain and which allows interaction with p97/VCP (Yi and Kaler, 2018). It would be of importance to see whether this is a mechanism shared with the A991D mutation, due to their close proximity, or the other HMN-associated variants. Furthermore, it would be of interest to explore the altered interaction VCP-T994I in the existing knock-in mouse model, as well as the iPSC model (Perez-Siles et al., 2016, 2020).

Endoplasmic reticulum

The endoplasmic reticulum (ER) is a cellular organelle with an important function in protein folding, lipid synthesis and calcium storage. Altered ER homeostasis by perturbed ER network formation or enhanced ER stress induction has been repeatedly associated with neurodegenerative disorders (Renvoise and Blackstone, 2010; Xiang *et al.*, 2017). The altered ER stress response due to increased protein aggregation is a common mechanism, likely due to the increased activation of the unfolded protein response (UPR) (Xiang *et al.*, 2017). Several HMN-associated genes have a pathological mechanisms related to altered ER function or heightened ER stress (Table 5).

REEP1

Heterozygous mutations in *REEP1*, receptor expression enhancing protein 1, were first associated with HSP type 31 (SPG31) (Zuchner *et al.*, 2006). Since then the phenotypic spectrum has broadened to include HMN and congenital neuropathy with diaphragmatic palsy, although the latter was reported to be recessively inherited (Beetz *et al.*, 2012; Schottmann *et al.*, 2015). With the exception of a few missense mutations, including *A20E*, all HSP-associated mutations are truncating mutations causing a loss of protein (Zuchner *et al.*, 2006; Beetz *et al.*, 2008; Guglielmi, 2020). Although the *A20E* likely still conforms with the presumed loss-of-function mechanism for HSP-related *REEP1* mutations as the *A20E* mutant fails to localize to the ER and unavailability of REEP1 at the site of physiological function could effectively resemble absence of the protein (Beetz *et al.*, 2012). In contrast, the HMN-associated mutant *102_139del*, which is an in-frame deletion of exon 5, displays a different type of mislocalization problem than the *A20E* mutant (Beetz *et al.*, 2012). Wild-type REEP1 localizes to the tubular portion of the peripheral ER, showing overlapping localization as the ER marker protein calreticulin (Hurt *et al.*, 2014).

While some of the *102-139del* protein localizes to the ER, there is a striking presence of REEP1-positive large compact structures within the cytoplasm with a perinuclear localization (Beetz *et al.*, 2012). Furthermore, in addition to REEP1 mislocalization, the *102-139del* mutant also results in similar perinuclear mislocalization of ATL1, a known REEP1 interactor (Park *et al.*, 2010; Beetz *et al.*, 2012). Several HSP-associated genes are involved in ER membrane maintenance and membrane shaping, including *REEP1* and the previously mentioned *ATL1* (Park *et al.*, 2010). The loss of *REEP1* associated with HSP might be due to disruption of the ER network, ER stress and ER fragmentation, as was shown for *REEP1*-null *Drosophila* (Yalcin *et al.*, 2017). However, despite the indication that the HMN-associated *102_139del* mutant will influence ER function, the exact mechanism remains unknown.

BSCL2

Recessive truncating *BSCL2* mutations are causative of Berardinelli-Seip congenital lipodystrophy via a loss-of-function mechanism (Magre *et al.*, 2001). In contrast, dominant mutations in *BSCL2* are associated with a variety of different neuromuscular disorders, including HMN, Silver syndrome, CMT2 and HSP (Windpassinger *et al.*, 2004; Auer-Grumbach *et al.*, 2005; Guillen-Navarro *et al.*, 2013; Musacchio *et al.*, 2017). Currently, two recurrent *BSCL2* missense mutations are associated with HMN, *N88S* and *S90L* (Irobi *et al.*, 2004*a*; Windpassinger *et al.*, 2004). *BSCL2* encodes an integral ER membrane protein called seipin, which possesses important functions in regulation of lipid droplet formation and metabolism (Fan *et al.*, 2015). The *N88S* and *S90L* mutations are located in the *N*–glycosylation motif of seipin and result in inclusion bodies within the ER, subsequently triggering ER stress (Windpassinger *et al.*, 2004; Ito and Suzuki, 2007, 2009). Seipin mutants *N88S* and *S90L* also result in increased lipid droplet size and fusion, which points to the function of the *N*-glycosylation domain in lipid droplet morphology (Fan *et al.*, 2015). Interestingly, the autophagic pathway was activated to adapt to the increased and supersized lipid droplets (Fan *et al.*, 2015). Upon inhibition of the autophagic pathway, the adaptive response to the enlarged lipid droplets was unable to be degraded, demonstrating the interplay between autophagy and lipid droplet formation and size (Fan *et al.*, 2015). It remains to be seen whether autophagy plays a causal role in *BSCL2*-associated HMN, or whether it is a downstream response due to ER stress and aberrant lipid droplet formation.

SIGMAR1

Recessive mutations in *SIGMAR1* are associated with a number of neurological disorders, including ALS, frontotemporal dementia, HMN and Silver-like syndrome (Luty *et al.*, 2010; Al-Saif *et al.*, 2011; Belzil *et al.*, 2013; Li et al., 2015; Horga *et al.*, 2016). *SIGMAR1* mutations linked with HMN are generally loss-of-function variants, either by truncating variants or large deletions as well as missense variants resulting in reduced protein expression, *N167I* (Li *et al.*, 2015; Almendra *et al.*, 2018; Nandhagopal *et al.*, 2018;

Ververis *et al.*, 2020). Similar to REEP1 and seipin, SIGMAR1 is localized to the ER and is thought to modulate a variety of signalling pathways including ion channels, GPCRs, lipid rafts, and ER stress response (Kourrich *et al.*, 2012; Roca-Agujetas *et al.*, 2019; Yang *et al.*, 2019). Furthermore, depletion of SIGMAR1, as would be the case for most of the disease-associated mutations, has been shown to compromise autophagy, possibly by impairing autophagosome-lysosome fusion (Roca-Agujetas *et al.*, 2019; Yang *et al.*, 2019). Regarding the mechanism for the specific HMN mutations, both the *N167I* and the *31-50del* mutant have been shown to reduce the protein expression, likely by proteasome degradation. The mutant proteins are also aberrantly located, showing a more diffuse ER localization. Lastly, similar to *BSCL2* mutations, the expression of these *SIGMAR1* mutants induces ER stress and apoptosis (Li *et al., 2015; Ververis et al., 2020). Despite these insights, the function of SIGMAR1 and the exact pathomechanism for SIGMAR1 mutants remains largely unknown.*

Identification of novel pathomechanisms

While candidate gene approaches are valuable tools to identify additional causal genes within known pathomechanistic clusters, by for instance screening for pathogenic variants in known interaction partners, the unbiased methodology of NGS also allows us to broaden our perspective by identifying causal genes in pathways previously not associated with neuropathy. Such is the case for pathogenic variants in *SORD*, the most recent addition to the HMN spectrum of genetics (Table 5).

SORD

SORD encodes the sorbitol dehydrogenase enzyme, involved in the two-step polyol pathway, converting glucose into sorbitol and, subsequently into fructose. Recessive mutations in *SORD* (both homozygous and compound heterozygous) are associated with both CMT2 and HMN (Cortese *et al.*, 2020). While a variety of mutations in *SORD* are reported in the initial study, including several frameshifts and missense variants, the *A253Qfs*27* seems particularly common, with an estimated carrier frequency of 0.004 in the general population (Cortese *et al.*, 2020). Functionally it was shown that the truncating *SORD* mutations cause loss of protein expression in patient fibroblasts, and result in increased serum fastig sorbitol levels (Cortese *et al.*, 2020). However, similar effects for the missense mutations have not yet been demonstrated. A SORD-deficient *Drosophila* model mirrors some of the key phenotypic aspects of patients, with normal lifespans, progressive and age-dependent synaptic degeneration and locomotor deficiency (Cortese *et al.*, 2020). The development of this animal model already allowed testing of a potential therapy inhibiting aldose reductase, the enzyme upstream of SORD, targeting the sorbitol accumulation. This showed that pharmacological inhibition of aldose reductase indeed rescues the sorbitol accumulation and subsequent neurological phenotype in SORD-deficient *Drosophila*, providing a promising hypothesis for treatment of *SORD*-neuropathy patients.

Discussion

The widespread introduction of NGS over the last decade has dramatically accelerated novel gene identification in many fields of human genetics, including that of neuromuscular disorders. These advances have led to the identification of at least 100 genes for the whole category of inherited peripheral neuropathies and the 26 genes that are currently associated with HMN. Despite this broad spectrum of HMN causal genes, the genetic detection rate is low compared to other neuropathy subtypes such as CMT1 or CMT2. General consensus is that only ~20–40% of HMN cases can be explained with our current understanding of HMN genetics, demonstrating a substantial gap in our knowledge of the heritability of these disorders despite increasing efforts (Dierick *et al.*, 2008; Bansagi *et al.*, 2017; Bacquet *et al.*, 2018; Hartley *et al.*, 2018).

As is apparent from this overview, there is considerable genetic overlap with the other disease categories (Fig. 2 and Table 1). Of the 26 currently known genes for HMN, there are only three that remain specific to HMN (*FBXO38*, *HSPB3* and *WARS*). All other genes show overlap with other neuromuscular diseases, such as CMT, ALS, myopathy and SMA, and five genes have also been associated with neurological diseases beyond neuromuscular disorders.

In addition to the genetic overlap, there is significant clinical overlap between HMN and several other distinct neurological disorders with lower motor neuron involvement. Also, the similarity in clinical presentation of distal myopathies can be striking. Distal muscle weakness, the key clinical symptom of HMN, can have many different origins, both in terms of cause (genetic and environmental), and in terms of affected tissue (neurogenic and myopathic). Although the purpose of this review is not to discuss the details of clinical differential diagnosis of these disorders, it is clear that the phenotypic differentiation is not always straightforward based on the clinical presentation alone. The typical HMN phenotype of a relatively slowly progressive wasting of distal lower limb muscles as a result of axonal degeneration, is thought to be most similar to CMT2. While the cause of CMT2 similarly lies in axonal degeneration of peripheral nerves, in this case there is involvement of peripheral sensory nerves as well. For typical 5q-SMA, due to *SMN1* deletion, weakness is most pronounced for the proximal muscles. However, atypical SMA forms may present a distal wasting of muscles, although in contrast to HMN, the underlying primary cause is a degeneration of the neuronal cell body (neuronopathy), rather than the axon. Where ALS typically involves upper motor neuron degeneration in combination with lower motor neuron involvement, certain ALS subtypes may initially present with only lower motor neuron degeneration, sometimes in a predominant distal distribution. These atypical ALS forms can progress slower than classic ALS but still the pace of progression is ultimately often faster than it is for HMN, and bulbar involvement is more also pronounced as is the case for ensuing respiratory failure. Detailed knowledge of the genetic spectrum of HMN and associated disorders can, when combined with state-of-the-art molecular genetic analysis, be of great help in the often difficult (clinical) diagnostic process.

Over time, the boundaries, both clinical and genetic, between HMN and other neuromuscular and neurological disorders have become considerably less clear-cut. This seems to be driven mainly by the accelerating rate of genetic discovery which is—by virtue of the adopted technologies—also increasingly unbiased by prior knowledge and classification systems. As diagnostic sequencing increases, sufficient data will be produced to more accurately assess prevalence, phenotype and functional consequences of the rarer genetic HMN subtypes. It is clear that the increased use of NGS in future clinical and research settings will further broaden and augment the relationships between phenotypes and specific gene defects.

In this review, we have divided the known genes into five arbitrary, but functionally meaningful groups based on their known or postulated mechanisms, both in homeostatic and pathological conditions: axonal transport, tRNA aminoacylation, RNA metabolism and DNA integrity, ion channels and transporters, and endoplasmic reticulum. Most interesting is the observation that these pathways are often ubiquitous processes in the nervous system or even throughout the human body as a whole, yet impairments in these pathways can cause highly specific axonal degeneration of the peripheral motor neurons. It is likely that the extraordinary length of the peripheral motor neurons is a contributing factor to this specific vulnerability of lower motor neurons. If we consider axonal transport allowing movement of molecules and organelles within neurons over this tremendous distance, when disturbed this is one of the key pathomechanisms for HMN, and by extension, CMT (Beijer *et al.*, 2019*b*). Similarly, the extreme polarity of the peripheral motor neurons might make them more dependent on local protein translation, which has an obvious role in tRNA aminoacylation as the first step in protein synthesis, but also in terms of the function of the ER, an organelle we now know to also project into the far distal portions of axons (Renvoise and Blackstone, 2010). Disturbances of tightly regulated gene transcription and DNA integrity could render post-mitotic neurons specifically vulnerable, which is supported by the extensive involvement of these mechanisms in the broader group of neurodegenerative disorders. Lastly, both neuronal homeostasis as well as precise neuronal connectivity are heavily reliant on specific challenges to (ion) channel biology, as evidenced by the variety of neuronal disorders due to (ion)hannel disturbances.

The idea of tissue-specific vulnerability, in this case of lower motor neurons, is not an issue limited to peripheral neuropathies alone, but there are currently no examples of tissue-specific regulation, such as tissue-specific mutant expression or other mechanisms, in the pathology of HMN. However, as for other neuromuscular disorders, part of the future of HMN genetics may lie in the discovery of modifiers, transcript specificity, small RNA expression and other regulatory mechanisms (Hosseinibarkooie *et al.*, 2016; Tao *et al.*, 2019; Hekselman and Yeger-Lotem, 2020).

As demonstrated, the genetic spectrum of HMN spans many different genes and overlaps with a great diversity of neuromuscular and neurological disorders. Despite recent advances, there is still a considerable way to go in terms of diagnostic yield for HMN. However, the discovery of pathways in peripheral motor neurons might be a viable way to identify related genes in the same cascade of processes. Furthermore, discovery of novel genes as well as novel mutations for HMN will allow us to further establish and associate phenotypes, which will support genetic diagnosis and clinical care.

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Competing interests

The authors report no competing interests**.**

Figure legends

Figure 1 HMN and the underlying mechanisms. A schematic overview of a peripheral motor neuron with depiction of underlying processes of causal genes for HMN as divided into five subgroups: RNA metabolism and DNA integrity, endoplasmic reticulum, axonal transport, tRNA aminoacylation, and ion channels and transporters.

Figure 2 Striking genetic overlap between HMN and neurological and neuromuscular disorder. An overview of the overlap in causal genes between HMN and CMT (red), other neuromuscular disorders, including myopathy (NMD; yellow), ALS (pink), neurodevelopmental disorders (NDD; green) and HSP (light blue).

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