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Biological networks and complexity in early-onset motor neuron diseases

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Motor neuron diseases (MNDs) are neuromuscular disorders where the spinal motor neurons–either the cell bodies themselves or their axons–are the primary cells affected. To date, there are 120 different genes that are lost or mutated in pediatric-onset MNDs. Most of these childhood-onset disorders, aside from spinal muscular atrophy (SMA), lack viable therapeutic options. Previous research on MNDs has focused on understanding the pathobiology of a single, specific gene mutation and targeting therapies to that pathobiology. This reductionist approach has yielded therapeutic options for a specific disorder, in this case SMA. Unfortunately, therapies specific for SMA have not been effective against other pediatric-onset MNDs. Pursuing the same approach for the other defined MNDs would require development of at least 120 independent treatments raising feasibility issues. We propose an alternative to this this type of reductionist approach by conceptualizing MNDs in a complex adaptive systems framework that will allow identification of common molecular and cellular pathways which form biological networks that are adversely affected in early-onset MNDs and thus MNDs with similar phenotypes despite diverse genotypes. This systems biology approach highlights the complexity and self-organization of the motor system as well as the ways in which it can be affected by these genetic disorders. Using this integrated approach to understand early-onset MNDs, we would be better poised to expand the therapeutic repertoire for multiple MNDs.

KEYWORDS

motor neuron disease, network biology, complexity, therapeutics development, spinal muscular atrophy, peripheral neuropathy, amyotrophic lateral sclerosis

Introduction

Neuromuscular diseases (NMDs) result in chronic, severe disability as well as early mortality and a high burden of disease. They also display clinical and genetic heterogeneity; in fact, there are currently 1,079 distinct NMDs and 608 known genes associated with these disorders (1). While each NMD is rare, they collectively have a prevalence of about 1 in 700. Motor neuron diseases (MNDs) are a group of NMDs where the spinal motor neurons–either the cell bodies themselves or their axons–are

the primary cells affected (2). Those MNDs with early onset include spinal muscular atrophies (SMAs), juvenile forms of amyotrophic lateral sclerosis (ALS), distal hereditary motor neuronopathies (dHMNs) and axonal–or type 2–Charcot-Marie-Tooth (CMT) disease. There are approximately 120 genes to date that are lost or mutated in pediatric-onset MNDs. Most of these childhood-onset disorders, aside from proximal SMA, lack viable therapeutic options.

The majority of research on MNDs has focused on a single disorder, like SMA, and to develop a single-target therapeutic strategy specific for that disorder. This reductionist approach has been applied to understanding disease pathogenesis and therapeutics development as well as for care (3). This approach to SMA has been successful in that there are currently 3 therapies–nusinersen (4, 5), risdiplam (6) and onasemnogene abeparvovec (7)–that are approved for clinical use which target *SMN* expression, either by *SMN1* gene replacement or modulating the alternative splicing of the orthologous *SMN2* gene. While these options are likely to have significant positive impacts on the quality of life of many patients with SMA, not all SMA patients respond positively to these clinically approved therapies and the length of therapeutic benefit in these patients is not known (8). For maximal therapeutic benefits these agents need to be administered at the early stages of disease progression. These approved therapies lessen the severity of disease in SMA but, unfortunately, do not completely cure children with this disorder raising the issue of whether combination therapies could improve outcomes further. Importantly, therapies specific for SMA have not been effective against other pediatric-onset MNDs thereby limiting their benefits for early-onset MNDs in general. These issues suggest that there needs to be a broadening of concepts related to pathophysiology of MNDs with a view that this will lead to alternative research approaches and ultimately the development of novel therapies.

While each disorder is caused by a loss of or mutation in a specific gene, there are commonalities with respect to mechanisms of disease within the various early-onset MNDs as well as within other neurodegenerative diseases (9). Motor units are functionally linked entities composed of spinal motor neurons that innervate skeletal muscles as well as regulatory interneurons projecting from these target muscles. The question we pose in this article is whether motor units can be conceptualized as complex adaptive systems and whether there are pathophysiologic and therapeutic advantages to a complex adaptive system conceptualization.

Motor neuron diseases and biological networks

The commonalities amongst the genetically distinct early-onset MNDs can be understood holistically using a network biology, or network medicine, approach. Network biology examines the interactions and interdependencies between biomolecular components within a system, as opposed to the components themselves (10). Examination of these networks within motor neurons provides important insights into their function as well the consequences of a disease-associated mutation within one of the components. This characterization can be completed at different levels including gene regulatory networks, protein-protein interaction networks as well as metabolite analysis (11, 12).

Interrogating the changes in gene regulatory networks has provided important insights into how motor neurons are affected in early-onset MNDs. A genetic screen of modifiers of *Smn* dependent lethality in *Drosophila melanogaster* identified pathways involved in alternative splicing, neuronal differentiation, axonal guidance and neuromuscular junction maturation as modifier of the SMA-like phenotype (13). Using a genetic screen on a fruit fly model of ALS8–caused by loss of functional *VAMP-associated protein B* (*VAPB*), genes associated with vesicular trafficking, endosome trafficking and cell proliferation were identified as modifiers of disease phenotype (14). Pathways involved in cell proliferation and neuronal development were differentially affected in SMA mouse motor neurons derived from embryonic stem cells (15). Network analysis of presymptomatic SMA mouse spinal cord transcriptomes showed impairment of neurotrophin signaling pathways (16). Comparing the transcriptomes of motor neurons vulnerable to neurodegeneration against those which are resistant to neurodegeneration in wild-type as well as SMA mice revealed enrichment in pathways associated with ribosome biosynthesis, translation, the extracellular matrix and cell adhesion (17, 18). Kline et al. (18) compared their list of transcripts that were differentially vulnerable to neurodegeneration with independent screens for motor neuron vulnerability transcripts (19–21) and identified 595 transcripts altered in the same direction between at least two of the modifier screens. Network analysis showed enrichment of pathways involved in neuronal function and synaptogenesis. A comparison of the transcriptomes of SMA spinal motor neurons that are vulnerable to neurodegeneration against those motor neurons which are resistant to degeneration (ocular motor neurons) revealed networks associated with neurotransmitter release, neuronal survival, oxidative stress and apoptosis as modifiers of neurodegeneration associated with the loss of functional SMN (22).

Weighted correlation network analysis (WGCNA) is an approach to identify disease-related networks and hubs from different datasets (23). When comparing the transcriptomes from SMA patients with varying degrees of phenotypic severities, WGCNA identified transcripts associated with tumor necrosis factor α (TNFα)-mediated regulation of neural, cardiac and bone development (24). These target transcripts showed similar disease severity-dependent differential expression in mouse models for SMA. Large-scale WGCNA of blood

transcriptomes from ALS patients revealed enrichment of pathways involved in neurodegeneration and inflammation (25). The complement activator *dehydrogenase/reductase (SDR family) 4* (*DHRS4*) was identified as a hub gene for ALS1 by comparing the transcriptomes of *SOD1 (G93A)* mouse spinal cords using WGCNA (26). This analysis suggests the role of the complement cascade in motor neuron degeneration in ALS.

WGCNA can also be used to distinguish disease-specific network regulation between allelic disorders. For examples ALS4 and ataxia with oculomotor ataxia type 2 (AOA2) are clinical distinct early-onset neurodegenerative diseases disorders but are caused by mutations in *senataxin* (*SETX*) (27, 28). WGCNA of fibroblast transcriptomes with ALS4-specific *SETX* mutation against those with an AOA2-specific *SETX* mutation identified networks which are unique to either disorder (29). Specifically, networks associated with the regulation of RNA metabolism were overrepresented in ALS4 transcriptomes. Comparison of spinal cord and cerebellum transcriptomes from two different mouse models for ALS4 [expressing either *SETX (R2136H)* or *SETX (L389S)* mutant transgenes] identified overrepresentation of pathways involved in synaptic signaling and nervous system development (30). Interestingly, these pathways were not enriched in a mouse model for AOA2.

In addition to gene regulatory network analysis, important insights into the pathogenesis of early-onset motor neuron diseases can be gained from the analysis of disease-associated protein-protein interaction (PPI) networks. When comparing the protein-protein interactomes between ALS and ALS linked with frontotemporal dementia (ALS-FTD), there were common hub proteins between these two types of motor neuron disease but the enrichment profiles around these hubs were distinct between ALS and ALS-FTD (31). This PPI network analysis identified ubiquitin-C as a common downstream target amongst these disorders. Jensen et al. (32) recently compiled a PPI network analysis for ALS and identified key modules associated with disease phenotype–oxidative stress, energy metabolism, proteosome dysfunction and mRNA processing defects. Some of these modules are commonly observed in other forms of neurodegeneration but other networks, like oxidative stress-induced protein misfolding, were found to be unique to ALS. A similar analysis of the ALS PPI networks showed similar modules and identified the cell cycle regulatory protein CDC5L as a stabilizer of these interactomes (33).

Biological networks have also been identified amongst disorders affecting motor neuron axons, such as type 2 CMTs and hereditary spastic paraplegias (HSPs). Network analysis of all CMT-related genes–including those linked to demyelinating peripheral neuropathy–identified pathways involved in myelination, axonal function, mitochondrial metabolism and autophagy as key pathways in CMT pathogenesis (34). Bis-Brewer et al. (35) identified common modules between the PPI networks of these axonopathies. These common modules include protein misfolding (endoplasmic reticulum stress) response, spliceosome assembly and function as well as mRNA processing. They also found that energy metabolism processes, mainly those related to glycolysis and gluconeogenesis, were unique to the type 2 CMTs. Comparison of the transcriptomes of different cellular models of type 2 CMT motor neurons {CMT2A2A [*MFN2(R94Q)]*, CMT2E [*NEFL(P8R)*], CMT2F [*HSPB1(G84R)* and *HSPB1(P182L)*] and CMT2L [*HSPB8(K141N)*]} revealed overrepresentation of mitochondrial respiratory chain and axon guidance networks (36). Interestingly, the differential overrepresentation of these networks is unique to motor neurons.

There is strong overlap and interconnectivity between the SMA and ALS PPI networks (37). One of the first common links between PPIs for SMA and ALS was described in 2017 where survival motor neuron 1 (SMN1) and the ALS-associated proteins FUS RNA binding protein (FUS), TAR DNA binding protein 43 (TDP43) and senataxin (SETX) were identified as shared components with the RNA metabolism network (38). Interactome analyses of the ALS-associated proteins FUS, EWS RNA binding protein 1 (EWSR1), TAT-box binding protein associated factor 15 (TAF15) and matrin-3 (MATR3) revealed a common association with the U1 snRNP assembly network as well as the RNA polymerase II network (39). The U1 small ribonucleoprotein (snRNP) assembly network is also linked to SMA. This group also demonstrated five proteins linked to SMA– SMN1, activating signal cointegrator 1 complex component 1 (ASCC1), exosome component 8 (EXOSC8) and heat shock protein B1 (HSPB1)–were integral components of this RNA polymerase II network (40). Comparative proteomics between SMA and ALS biosamples revealed alterations in energy homeostasis, protein metabolism and oxidative stress response that were present in both disorders (41). Kubinski and Claus (42) used network analysis to show that the ALS-linked protein Cu/Mn-dependent superoxide dismutase (SOD1) and profilin-1 (PFN1) serve as intermodular nodes connecting motor neuron disease pathogenesis and actin cytoskeleton dynamics.

Modifier genes can regulate disease phenotype without necessarily causing the early-onset MNDs. *SMN2* is an extremely well characterized modifier gene for SMA as increasing *SMN2* copy number lessens the disease severity [reviewed in (43)]. *Plastin-3* (*PLS3*)–one of the first *SMN2*-independent modifier genes identified for SMA–mRNA levels were found to be higher in females with milder SMA than those siblings with a more severe SMA clinical presentation (44–49). The modifier capability of *PLS3* for SMA was subsequently found to be incompletely penetrant within the SMA population as well as within animal models (47, 50–53). Other putative SMA modifier genes identified to date include *neurocalcin-D* (*NCALD*) (54), *Tolloid-like 2* (*TLL2*) (55) and *neuritin 1* (*NRN1*; cpg15) (48, 56). When comparing the clinical variability between CMT4K, *ganglioside-induced differentiation-associated protein 1* (*GDAP1*)-linked patients, *junctophilin-1* (*JPH1*) was identified as a modifier of disease phenotype by altering mitochondrial

calcium channel activity (57). Loss of or mutation in *neuropilin-1* (*NRP1*), the voltage-gated sodium channel NaV1.6 (*SCN8A*) or *neuronal cell adhesion molecule* (*NRCAM*) are linked to disease severity in CMT4C–which is mediated by mutation in the *glycyl-tRNA synthetase* (*GARS*) gene (58, 59).

In addition to identifying common molecular pathways amongst early-onset MNDs, network analysis can provide important insights into therapeutic options. Using this systems network approach, ubiquitin-C was identified as a common therapeutic target between the various genetic forms of ALS and ALS-FTD (31). Gene delivery of *UCHL1*–a key enzyme controlling the amount of ubiquitin-C present in polyubiquitin chains on proteins–to upper motor neurons improves motor neuron phenotype in multiple models of ALS (60). Future studies will demonstrate the therapeutic potential of *UCHL1* modulation in multiple early-onset MNDs as well as identify *via* network analysis additional common targets for therapeutic benefit.

Sensory inputs onto motor neurons are also affected in early-onset MNDs. Mentis et al. (61) showed that SMA motor neurons lose their proprioceptive sensory inputs, referred to as deafferentation, during early stages of disease progression in the SMNΔ7 SMA mouse model. Deafferentation of motor neurons has been confirmed in a different mouse model for SMA (62). This loss of sensory input to motor neurons is a result of misregulation of *ubiquitin-like modifier-activating enzyme-1* (*UBA1*) and *GARS* (63). Both genes have been implicated in early-onset MNDs. It remains to be determined whether loss of sensory inputs to motor neurons is universal to early-onset MNDs. The biological networks implicated in deafferentation of motor neurons need to be more fully characterized to determine how they are affected in early-onset MNDs.

Biological networks affected by early-onset MNDs involve glial cells in addition to motor neuron [reviewed in (64)]. Reactive gliosis was observed in the spinal cords of patients with type I SMA (65, 66) as well as in pathological specimen from CMT2A2 patients (67). Reactive astrogliosis occurs during the end stage of disease progression in SMA mouse models and restoration of SMN in SMA astrocytes reduces the release of proinflammatory cytokines (68, 69). SMA reactive astrocytes show altered calcium homeostasis and hyperactivation of Notch signaling (70, 71). SMN-deficient reactive astrocytes alter the release of regulatory agents like MCP-1 (monocyte chemoattractant protein-1) and miR-146a which promotes motor neuron loss (72, 73). SMA astrocytes also show diminished ability to form and maintain neuromuscular synapses (74).

Microglia are activated within the spinal cord during early-onset MNDs. Microglial activation has been observed in mouse models for SMA (68, 75) as well as for CMT2K (76). SMA microglia acquire a reactive phenotype that enhances inflammatory responses in the spinal cord (77). Classical complement C1q pathways are activated in SMA to mark damaged motor neurons for microglial degradation (78). Microglial activation can be attenuated in SMA mice with the sigma-1 receptor agonist PRE-084 (79). PRE-084 can also attenuation deafferentation in SMA motor neurons. Future studies need to fully characterize how alterations in glial–both astroglial and microglial–reactivity can interact with and affect motor neuron biological networks.

Lower motor units as complex adaptable systems

The previous section highlights the observation that a genetic mutation alters multiple networks in a way in which there is biological convergence with similarities in emergent phenotypes. We suggest that the convergences are critically important components of disease pathophysiology that deserve explicit study. A strong theoretical framework underlying these sorts of studies is that of complex adaptive systems.

A generalizable description of complex adaptive systems (Figure 1A) is that they are collections of relatively simple agents that have the property that they can aggregate, so that collections of agents can form meta-agents (and meta-meta-agents etc…) with hierarchical higher-order structure (80–82). These aggregates interact non-linearly, so that aggregate behavior of a collection of agents is qualitatively different from the behavior of the individual agents, e.g., the behavior of a collection of genes is qualitatively different to the behavior of a collection of neurons. The interactions among agents mediate flows of materials or information. Finally, the agents are typically diverse with distinct specialties that are optimized through adaptation to selective pressures in their environments. There are an enormous number of system types that meet these criteria e.g., economies, ecologies, traffic networks and brains (83, 84). Given the commonalities in underlying structure of these systems, there are generalizable strategies for understanding and manipulating such systems. The complex adaptive systems approach to biology is rather new, but the ideas resonate with many scientists and therefore studies designed within this framework are becoming more common. Our aim is to operationalize these general concepts to MNDs and explore how this would change current paradigms.

Motor units can easily be seen as complex adaptive systems. At the lowest hierarchical layer there is molecular diversity amongst spinal motor neurons (85). Networks of gene expression defined by transcriptomics non-linearly influence the emergence of spinal motor neurons that differ by temporal development and differentiation as well as by spatial organization within the body (86). Importantly, it is not the function of a single gene that determines motor neuron fate, but rather the interactome of genes and the non-linear hierarchical interactions of proteins, neurons and networks that define the emergent phenotypes we define a motor function.

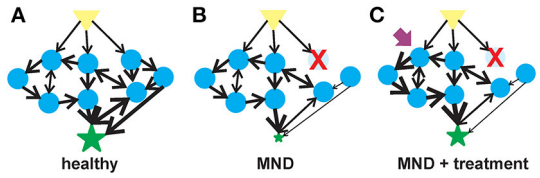

FIGURE 1
Flow of information in a complex system in motor neuron health, disease and therapeutic intervention. **(A)** In a healthy motor unit–lower motor neurons, interneurons and the muscle, activation of a signal (yellow triangle) leads to the stimulation of multiple agents (blue circles) that are interconnected into collection of aggregates known as meta-agents to form a hierarchical higher-order structure. The aggregates interact non-linearly to elicit a response (green star) whose behavior is quantitatively different from the individual agents. **(B)** Upon the loss of or alteration in an agent (in this case, an early-onset MND represented by the red X), the behavior of the aggregated meta-agents is altered and the flow of information is blunted. **(C)** A therapeutic agent (purple arrow) can activate one of the undamaged agents which would, in turn, activate the unaffected parts of the hierarchical aggregate and partially compensate for the diminished flow of information resulting from the altered agent.

During development, spinal motor neurons differentiate into compact anatomic units known as motor neuron pools that connect to specific and unique muscle (87). The composition of these motor neuron pools is heterogeneous based on the types of myofibers they innervate. The diversity of motor neuron subtypes as well as their formation into pools suggests multiplicity and self-organization. Mathematical modeling of the spinal monosynaptic reflex unit as well as the regulatory interneurons demonstrate functional self-organization of the motor unit (88, 89). Thus, motor units can be characterized by non-linear interactions between multiple components operating on multiple temporal and spatial scales (90). High-density surface electromyographic (EMG) recordings of synergistic muscles in the thigh demonstrate both shared synaptic drive amongst the neural inputs within the synergistic group as well as synaptic control specific to a muscle, depending on the frequency of the neural drive (91). This synchrony between spinal motor neurons and muscles involved in movement starts manifesting itself during neonatal development (92). While there are common inputs controlling groups of motor neurons to produce movement, there is not complete concordance between motor neuron input and muscle anatomy (93). This incomplete concordance suggests non-linearity and multi-scaling properties of complex adaptive systems.

Disease states, like adult-onset MNDs and aging, arise from the breakdown of the structures underpinning physiologic complexity (Figure 1B) (94, 95). One manifestation of this breakdown of physiological complexity may be spontaneous or induced discharges of hyperexcitable motor units known as fasciculations (96). Fasciculations have been observed in adults with ALS as the disease progresses (97). There is a relationship between the frequency of these fasciculations and muscle type in ALS patients (98). Muscle fasciculations in ALS may be an early indicator of motor neuron dysfunction (99) although their usefulness as an indicator for motor unit dysfunction has been questioned (100, 101).

Are fasciculations observed in early-onset MNDs? Clinical characterization of SMA population found the presence of tremors in some patients (102–104). Muscle fasciculations have also been observed in men with spinal and bulbar muscular atrophy (SMAX1) with a possible relationship with disease severity, triplet repeat length with the *androgen receptor* (*AR*) gene and testosterone levels (105). As with cases of adult ALS, these fasciculations are not always present in patients with early-onset MNDs. Further detailed analyses of fasciculations in patients with early-onset MNDs will provide important insights into the network level drivers of disease progression.

Multi-modal and multi-indication therapeutics for motor neuron diseases

Therapeutics options for early-onset MNDs are limited and have focused on targeted replacement of the specific MND-associated gene that is lost or mutated. While these targeted therapeutic replacement strategies have significantly improved the quality of life for patients with early-onset MNDs, treated individual still have a partial disease phenotype. As early-onset MNDs can be considered complex adaptive systems, therapeutic agents that target another component of the motor unit–aside from the one lost by genetic mutations–could provide significant therapeutic benefit (Figure 1C). There is mounting evidence demonstrating the efficacy of SMN-independent targets for SMA (106) and, by extension, other early-onset motor neuron diseases. These neuroprotective agents have multiple targets for their therapeutic benefits. The development of therapeutic strategies to manage epilepsy demonstrates the value of multi-target therapeutic strategies to treat neurological diseases (107). As examples of multi-target therapies for motor neuron diseases, we will describe two neuroprotectants that have shown strong *in vivo* efficacy in different types of motor neuron diseases: 4-phenylbutyrate (4PBA) and inhibitors of histone deacetylase 6 (HDAC6) (Table 1).

4PBA is one of the first multimodal therapeutics proposed from multiple motor neuron diseases. In SMA, early studies demonstrated that 4PBA increases *SMN2* expression in SMA patient-derived cells (109, 110), Administration of 4PBA to SMNΔ7 SMA mice resulted in a significant improvement in survival and motor function (108). As 4PBA was approved for treating humans, pilot clinical trials for 4PBA in SMA was completed with modest ameliorative effects in patients (112, 113). These studies did not detect any significant changes

TABLE 1 Summary of 4-PBA and HDAC6 inhibitors demonstrating therapeutic benefit in model systems for early-onset motor neuron diseases.

Compound	Disease model	Effect	Reference(s)
4-PBA	SMNΔ7 mouse	Improvement in survival and phenotype	(108)
4-PBA	SMA patient-derived fibroblasts	Modest increase in FL-SMN expression; interindividual variability	(109–111)
4-PBA	SMA patients	Modest improvement in motor function	(112, 113)
4-PBA	*SOD1 (G93A)* mouse	Marked improvement in survival and motor function	(114)
4-PBA + riluzole	*SOD1 (G93A)* mouse	Additive ameliorative effects relative to 4PBA or riluzole alone	(115)
4-PBA + AEOL-10150	*SOD1 (G93A)* mouse	Additive ameliorative effects relative to 4PBA or AEOL10150 alone	(116)
4-PBA	Postsymptomatic ALS patients	Improved motor function	(117)
4-PBA + taurursodiol	Postsymptomatic ALS patients	Additive ameliorative effected related to 4PBA alone	(118)
Tubastatin A	*FUS (R521H)* and *FUS (P525L)* cultured motor neurons (ALS6)	Improves motor neurite outgrowth and function	(119, 120)
Tubastatin A	*TARDBP (A382T)*, *TARDBP (G287S)*, and *TARDBP (N390S)* cultured motor neurons (ALS10)	Improves neurite outgrowth and mitochondrial transport along axons	(121)
Tubastatin A	*HSPB1 (S135F)* mouse (CMT2F)	Markedly improves motor neuron and muscle innervation	(122)
Tubastatin A	*GARS (R234KY)* and *Gars (C201R)* mice (CMT2D)	Improves motor function	(123)
Tubastatin A	*GARS1 (P724H)* cultured motor neurons (CMT2D)	Improves electrophysiologic misfiring and axonal transport defects	(124)
Tubastatin A	*Gars* knocked-down zebrafish embryos	Improve deficient neurite outgrowth and defective neuromuscular junction maturation	(125)
SW-100	*MFN2 (R94Q)* mouse (CMT2A1)	Improves motor phenotype	(126)
CKD-504	*GARS1 (P724H)* cultured motor neurons (CMT2D)	Improves electrophysiologic misfiring and axonal transport defects	(124)
CKD-504	*Gars* knocked-down zebrafish embryos	Improve deficient neurite outgrowth and defective neuromuscular junction maturation	(125)
ACY-738	*FUS (R521H)* and *FUS (P525L)* cultured motor neurons	Improves motor neurite outgrowth and function	(119)
ACY-738	*FUS* overexpressing mice	Improves motor function and diminishes neurodegeneration	(127)
ACY-738	*HSPB1 (S135M)* mice (CMT2F)	Improves survival and ameliorates the motor phenotype	(128, 129)
T3796106	Cultured *HSPB1 (S135M)* CMT motor neurons (CMT2F)	Improves motor neuron phenotype	(130)
T3793168	Cultured *HSPB1 (S135M)* CMT motor neurons (CMT2F)	Improves motor neuron phenotype	(130)

in *SMN2* expression in SMA patients treated with 4PBA. Additional testing of patient-derived SMA cell lines showed a high degree of interindividual variability in responsiveness to 4PBA (111). In the SMNΔ7 SMA mice, the protective effects of 4PBA—as well as of other butyrate prodrugs–were independent of *SMN2* induction (108). 4PBA significantly improves motor dysfunction and increases lifespan in the *SOD1 (G93A)* mouse model for ALS (114). In the same ALS mouse model, 4PBA displayed additive effects when co-administered with other neuroprotectants–such as riluzole and AEOL-10150–that have shown efficacy on the motor neurons of ALS mice (115, 116). In a clinical trial, 4PBA improved

motor outcomes in a cohort of ALS patients who received the therapeutic after disease onset (117). Postsymptomatic ALS patients showed some additive improvement in disease progression when treated with 4PBA and taurursodiol (118). Taken together, these studies demonstrate that 4PBA exerts neuroprotective effects in genetically different forms of motor neuron disease *via* an unknown mechanism, although we anticipate that the mechanism is mediated through network interactions. Furthermore, it is unclear at present if the neuroprotective mechanisms are disease-specific or share a common modality.

HDAC6 is highly expressed in the central nervous system and functions by removing acetyl groups from a variety of proteins aside from histones (131). HDAC6 has been implicated in the pathogenesis of many neurodegenerative diseases. Inhibitors of HDAC6 have demonstrated efficacy in ameliorating disease phenotypes in multiple motor neuron diseases. Ablation of *Hdac6* in transgenic mice harboring the ALS-linked *SOD1 (G93A)* mutation improves motor phenotype as well as increases lifespan in these mice (132). Tubastatin A and ACY-738 improve neurite outgrowth and motor neuron function in ALS-linked mutant *FUS* [*FUS (R521H)* and *FUS (P525L)*] motor neurons (119). Tubastatin A improves motor neuron function in ALS-linked *FUS* [*FUS (R521H)* and *FUS (P525L)*] motor neurons cultured in microfluidic devices (120). ACY-738 ameliorates the motor neuron degeneration observed in transgenic mice overexpressing wild-type FUS (127). Tubastatin A improves neurite outgrowth in patient-derived cultured motor neurons harboring different ALS-associated TDP43 mutations [*TARDBP (A382T)*; *TARDBP (G287S)* and *TARDBP (N390S)*] (121). The subcellular distribution of mutant TDP-43 as well as mitochondrial transport along axons were restored by tubastatin A.

In addition to exerting neuroprotective effects in model systems for ALS, HDAC6 inhibitors have been shown to ameliorate motor neuronopathy phenotypes observed in multiple types of axonal peripheral neuropathies, i.e., type 2 CMT. The HDAC6-selective inhibitor SW-100 improves motor phenotype in *MFN2 (R94Q)* mouse model for CMT2A1 (126). Tubastatin A markedly improves motor neuron and muscle innervation in the *HSPB1 (S135F)* mouse model for CMT2F (122). ACY-738 improves survival and ameliorates the motor phenotype observed in CMT2F [*HSPB1 (S135M)*] mice (128, 129). HDAC6 inhibition *via* the novel small molecules T3796106 and T3793168 improves motor neuron phenotype in cultured *HSPB1 (S135M)* CMT motor neurons (130). Tubastatin A improves motor function in the mutant *GARS (R234KY)* as well as in *Gars (C201R)* transgenic mouse models for CMT2D (123). Electrophysiologic misfiring and axonal transport defects are corrected in CMT2D motor neurons by tubastatin A and CKD-504 (124). Tubastatin A and CKD-504 improve deficient neurite outgrowth and defective neuromuscular junction maturation that are observed in zebrafish embryos where *Gars* is knocked down with splice-blocking antisense morpholino oligonucleotides (125).

Combinatory therapeutics have shown promise in animal models for SMA. One of the first examples of a combinatory therapeutic strategy demonstrated additive benefit was treatment of SMA mice with a small-molecule inducer of *SMN2* expression (D156844) alongside a myoprotectant (follistatin) (133). Coadministration of a *SMN2* exon 7 splice-correcting oligonucleotide and RG7800–a small molecule that promotes exon 7 inclusion–provided more pronounced additive effects in SMA mice (134). In addition, combinatory, multi-target therapies using a *SMN2* exon 7 targeted splice correcting antisense oligonucleotide alongside either a broad spectrum histone deacetylase inhibitor (135) or a protein stabilizing agent (136) have shown additive benefits in cell culture and mouse models for SMA. The compounds described previously (4PBA and HDAC6 inhibitors) could potentially be considered as multi-target and multi-modal therapies for MNDs.

Conclusions and future directions

Lower motor neurons and their connections to skeletal muscles as well as to interneurons–or the motor unit–can be thought of as a network of agents (genes, metabolites and activities) organized into aggregate clusters that interact with each other non-linearly in a hierarchical organization. These agents and their interactions have distinct, adaptable specializations that respond to selective environmental pressure. The complex adaptive system mediates the flow of information or materials from the external input from the lower motor neuron to the neuromuscular junction. Early-onset MNDs can be viewed as consequences of a breakdown of complexity within the motor unit network. A genetic perturbation within an agent in this network would pathologically alter the entire network and the flow of information from the input to the output.

Current strategies for therapeutics development for early-onset MNDs focus on amelioration or replacement of a single perturbation. This reductionist approach has been successful in improving the quality of life and disease presentation in SMA by SMN replacement. While very successful, targeted SMN replacement therapy has provided variable therapeutic benefit within the SMA patient population. Using a complex system approach to understand early-onset MNDs would provide a path for better understanding how loss of a specific agent affects the entire motor system network as well as multiple avenues for therapeutic interventions. These neuroprotective interventions potentially would provide therapeutic benefit for many early-onset MNDs as opposed to a single genetic disorder.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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