BRAIN
REVIEW ARTICLE

Skeletal muscle in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS), the major adult-onset motor neuron disease, has been viewed almost exclusively as a disease of upper and lower motor neurons, with muscle changes interpreted as a consequence of the progressive loss of motor neurons and neuromuscular junctions. This has led to the prevailing view that the involvement of muscle in ALS is only secondary to motor neuron loss. Skeletal muscle and motor neurons reciprocally influence their respective development and constitute a single functional unit. In ALS, multiple studies indicate that skeletal muscle dysfunction might contribute to progressive muscle weakness, as well as to the final demise of neuromuscular junctions and motor neurons. Furthermore, skeletal muscle has been shown to participate in disease pathogenesis of several monogenic diseases closely related to ALS.

Here, we move the narrative towards a better appreciation of muscle as a contributor of disease in ALS. We review the various potential roles of skeletal muscle cells in ALS, from passive bystanders to active players in ALS pathophysiology. We also compare ALS to other motor neuron diseases and draw perspectives for future research and treatment.

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Introduction

Amyotrophic lateral sclerosis (ALS), as originally described by Charcot in the 19th century, connotes a disease involving the degeneration of cortical motor neurons and their axons concurrent with the degeneration of alpha motor neurons.[1–3] The resultant clinical syndrome thus combines signs and symptoms of upper motor neuron loss (e.g. spasticity, pseudobulbar affect, pathological reflexes) with lower motor neuron loss (e.g. weakness, muscle atrophy). Given this definition, ALS has been viewed for most of the 20th century almost exclusively as a disease of cortical and lower motor neurons (MNs), with muscle changes interpreted exclusively as the consequence of the progressive loss of MNs, their axons and neuromuscular junctions. The prevailing view is thus that muscle tissue and myocytes are secondarily involved in ALS (Fig. 1).

The last 30 years of research have shown that ALS is highly heterogenous, with clinical, pathological and/or genetic overlap to several primary muscle diseases. Indeed, multiple studies have demonstrated that skeletal muscle and MNs mutually influence their respective development and function and that these two cell types, together with Schwann cells (glial cells that myelinate peripheral axons, and present at neuromuscular junctions), should be considered together as a single neuromuscular unit and not isolated entities. This is particularly illustrated in neuromuscular embryological development in which both myocytes and MNs play major roles in proper development and differentiation of the neuromuscular junction.[4,5] While the dialogue between the different cell types involved in neuromuscular transmission is important for proper development, it might be expected that disease conditions, including ALS, would also actively involve these different cell types (Fig. 1).

Here, we review the various potential roles of skeletal muscle cells in ALS, from passive bystanders to active players in ALS pathophysiology. We also compare ALS to other MN diseases and draw perspectives for future research and treatment.

Muscle as an innocent bystander: muscle pathology is secondary to lower motor neuron degeneration

An undisputed fact is that skeletal muscle of patients with ALS is subject to chronic neurogenic changes, similar to other diseases of peripheral motor nerve loss, but contrasting with primary muscle diseases. It is thus clear that a large part of muscle alterations related to ALS occur subsequent to MN degeneration. This is illustrated by early clinical neurophysiological investigations on patients with ALS, such as the classic study by Erminio *et al.*[6] in 1959 that characterized motor unit territories of patients with peripheral nerve injury and ALS. In contrast to modern EMG, this study employed a novel needle electrode in which 14 active recording ports were placed 2.5 mm apart along the shaft of the needle. The needle was 1 mm in diameter and inserted orthogonal to the long axis of muscle fibres, making this a heroic study for participants. Participants were asked to contract the muscle in question; an attempt was made to localize the needle so the middle electrode displayed activity of a single motor unit producing larger amplitude waveforms than the neighbouring electrodes, which recorded from the same MN at varying distances from its motor point. In this way, both the maximum electrical amplitude and the spatial extent of the motor unit potential (MUP) could be examined. Figure 2A shows the pattern seen for four motor units from patients with ALS, judged severe to mild, along with a MUP from a normal subject. The ALS patient MUP was, in general, higher in amplitude. However, the strikingly dispersed spatial extent suggested that muscle fibres not previously innervated by the motor unit being recorded were now innervated by that axon. Similar patterns were seen in other diseases of peripheral nerve loss, strengthening the contention that ALS had similar impacts on muscle. With the advent of more traditional EMG techniques, ALS was felt to show changes emblematic of peripheral nerve loss, with MUPs that were larger in amplitude, longer in duration, and more polyphasic than normal MUPs.[7]

The development of motor unit number estimation techniques provided data supporting the idea that muscle in ALS was impacted similarly to other diseases of peripheral MN loss. Motor unit numbers were reduced in ALS, with an increase in individual motor unit size.[8,9] Longitudinal studies suggested progressive loss of units,[10–12] with motor unit territory remaining increased until late in the disease.

The above studies all suggested that, from a physiological point of view, muscle from ALS patients responded as if the only impact of ALS on muscle were a reduced number of nerve fibres innervating muscles. One study suggesting that ALS could have at least a somewhat different signature in muscle was that of Killian *et al.*,[13] who studied neuromuscular junction transmission using repetitive nerve stimulation in ALS patients; their observations found that more than half of muscles studied had an abnormal decrement of at least borderline significance. As this finding has not been replicated in patients with neuropathy, there was a suggestion that the neuromuscular junction in ALS is distinctly impacted, at least quantitatively, from other nerve diseases.

Muscle biopsies from patients with ALS also exhibit abnormalities caused by neurogenic processes, with pathology similar to that seen in other diseases of denervation. In particular, the hallmark abnormalities of ALS muscle biopsies include type II fibre dropout and fibre type grouping, with angulated fibres representing those that have lost their innervation.[14,15] While neurogenic changes are obvious in ALS muscle biopsies, some differences between ALS and other denervation-related processes have been noted. Some studies have suggested that there are differences between different denervating conditions, perhaps reflective either of the speed of progression or the extent of nerve loss. Telerman-Toppet and Coers[16] studied muscle biopsies from patients with both/either ALS or Charcot-Marie-Tooth (CMT) type I, and found that the CMT patients had a greater innervation ratio, greater amount of type II muscle loss, and more fibre type grouping than did those with ALS. A greater number of angulated fibres were found in ALS, perhaps indicating a faster rate of nerve fibre loss. Other studies observed a more frequent pattern of mixed-fibre type atrophy in ALS as compared to other neurogenic atrophies, suggesting intrinsic differences in denervation/reinnervation patterns across these diseases.[17,18]

In summary, stated in classical terms, muscle abnormalities in ALS are reflective of the many processes involving MN loss and do not suggest a primary locus of disease in the muscle that might provide a therapeutic target. Differences between muscle in ALS

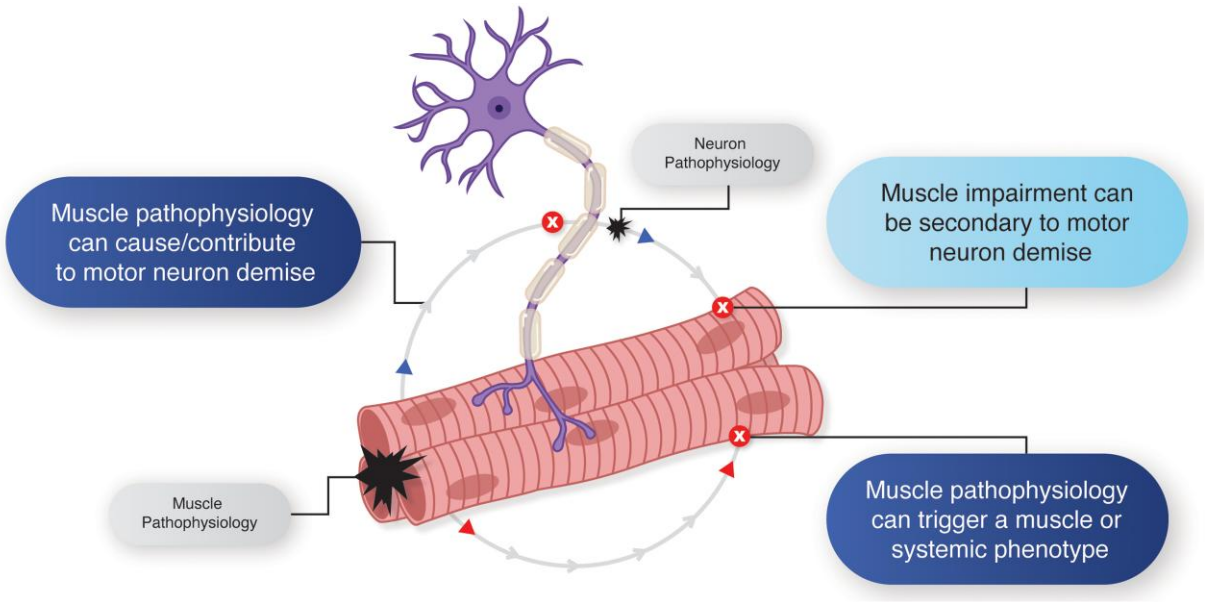

Figure 1 Possible levels of skeletal muscle involvement in ALS. Skeletal muscle involvement in amyotrophic lateral sclerosis (ALS) is generally viewed as the secondary consequence of motor neuron (MN) death. However, muscle pathophysiology has been shown to lead to muscle autonomous defects, while also contributing to MN loss.

and in other denervating diseases have been interpreted as being the result of either the pace of degeneration, the severity of degeneration, or both.

Muscle autonomous phenotype in ALS

While MN degeneration clearly causes muscle weakness and atrophy, there is also evidence supporting the hypothesis of a more active role of skeletal muscle in disease progression. Muscle fibres are indeed precociously affected in ALS disease independently of MN influence or denervation. These muscle autonomous defects appear to affect muscle differentiation or muscle energy metabolism leading to an overall decrease in muscle strength (Fig. 3).

Muscle differentiation

If skeletal muscle were only affected indirectly through MN demise, it would be expected that muscle cells from ALS patients would grow and differentiate normally *in vitro*. This is, however, not the case as satellite cells, the muscle stem cells that participate in muscle regeneration and homeostasis, display altered behaviour when cultured from ALS patients' biopsies, and are either unable to proliferate *in vitro* or display striking senescence-related abnormalities.[19] Similar abnormalities are seen in myoblasts from *SOD1*^{G93A} mice[20] or when studying skeletal muscle-derived induced pluripotent stem cells (iPSCs) from ALS patients, which show altered differentiation, reduced fusion efficiency, and reduced expression of acetylcholine receptor (AChR) compared to non-ALS iPSC-derived muscle.[21,22] In addition, TAR DNA-binding protein-43 (TDP-43), or fused in sarcoma (FUS), regulate expression of muscle genes, including contractile proteins, but also key myogenic transcription factors.[23–25] These studies, performed in muscle cells carrying either ALS-associated *SOD1*, *TARDBP* or *FUS* mutations, show that ALS-related mutations alter muscle differentiation properties through intrinsic expression in muscle (Fig. 3).

Muscle metabolism

Beyond altered differentiation of cultured muscle cells, functions of mature muscles are also affected in ALS, particularly in relation to mitochondrial metabolism. Skeletal muscle maintains its pool of healthy mitochondria through multiple cellular processes to ensure that sufficient energy (as adenosine triphosphate, ATP) is produced to support function (reviewed in Pickles *et al.*[26]). Multiple studies have observed alterations in skeletal muscle metabolic pathways, including altered expression of mitochondrial uncoupling proteins[27,28] in mouse models and human biopsies associated with modulation of metabolic flexibility (i.e. the balance between the use of glucose and fatty acids as a fuel substrate).[29–31] Building on this observation, as well as emerging reports of systemic hypermetabolism in ALS, subsequent studies have shown that an increase in whole body energy expenditure in ALS occurs in parallel with alterations in skeletal muscle metabolism.[29,31] Interestingly, modulation of skeletal muscle metabolic flux in *SOD1*^{G93A} mice leads to an attenuation of MN death.[29,30]

While muscle metabolism appears broadly impacted in ALS, two major questions remain: first, are current results restricted to *SOD1* mutations? Second, are muscle changes purely the result of ongoing denervation?

As most of this work has been restricted to *SOD1* mice, it could be argued that outcomes are purely a consequence of mutations in the *SOD1* gene. This is a reasonable caveat given that SOD1 plays an integral role in buffering superoxide radicals that are produced by mitochondria.[32] However, *CHCHD10*$^{S59L/+}$ mice expressing an ALS-FTD (frontotemporal dementia) relevant mutation[33–36] show deficiencies in mitochondrial oxidative phosphorylation preceding MN degeneration.[37] Moreover, disruption of TDP-43 (through *TARDBP* knock-out) leads to numerous muscle-specific cryptic exons,[38] many of them related to mitochondria and energy metabolism, and prominently alters muscle metabolism, in particular through disruption of TBC1D1, a key protein involved in insulin signalling.[39,40] Last, ALS patients carrying a *FUS* mutation displayed

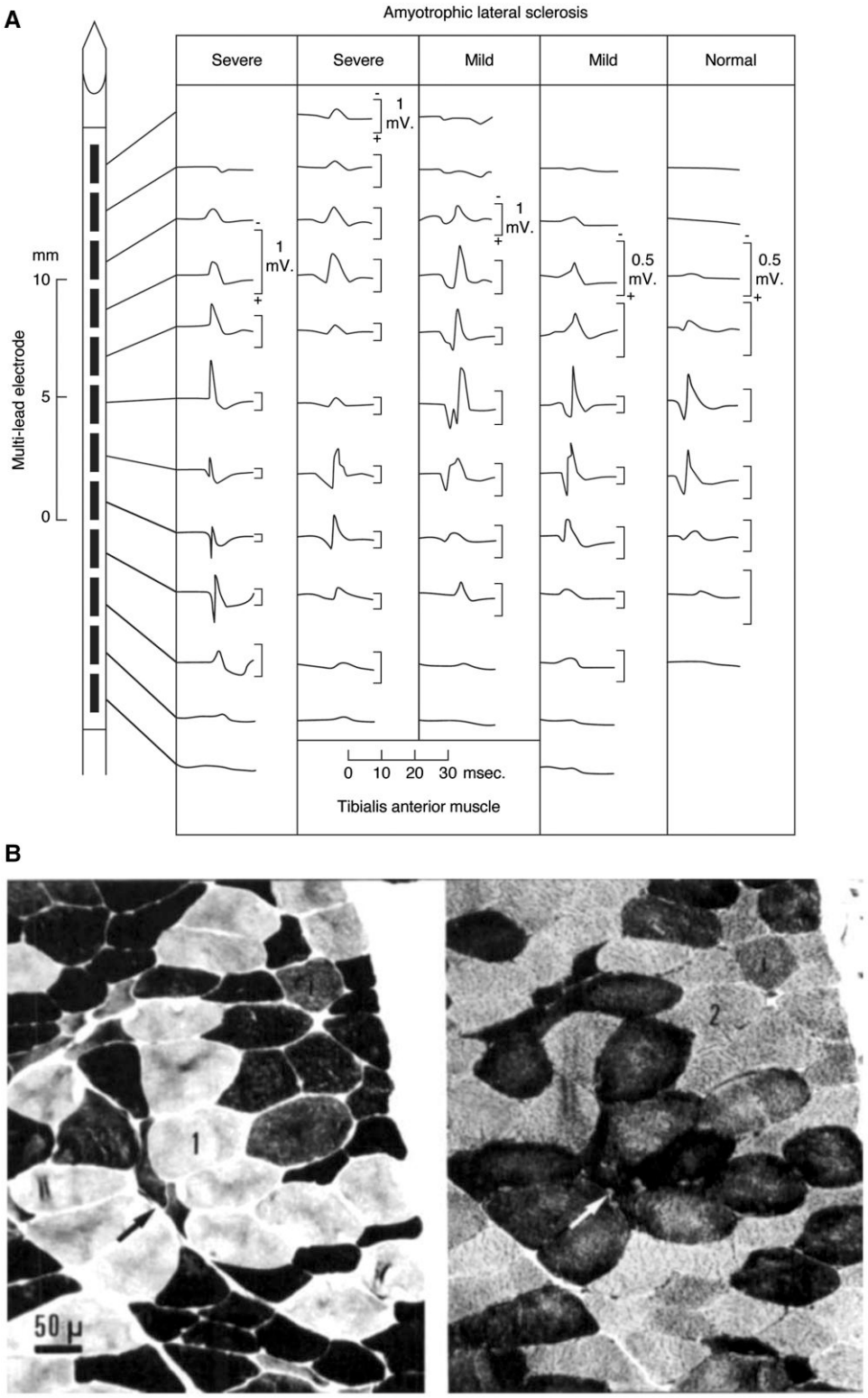

Figure 2 Neurophysiological and pathological alterations of ALS skeletal muscle resemble those observed in other diseases of peripheral motor nerve loss. (**A**) From Erminio *et al.*[6]: electrical spread of compound motor action potential across leads of a multielectrode probe. Spread is more extensive with more severe disease. (**B**) From Telerman-Toppet and Coers[16]: muscle biopsy sections with myosin ATPase (*left*), NADH diaphorase (*right*) showing grouping of type 1 and type 2 fibres.

severely increased lipid droplet accumulation in muscle fibres[25] while mutant FUS protein expression in muscle causes mitochondrial dysfunction, increase in ROS production, impaired lipolysis, inhibition of fatty acid β-oxidation, and reduction in ATP production.[25] Thus, muscle metabolism appears broadly altered in at least a substantial proportion of familial ALS cases.

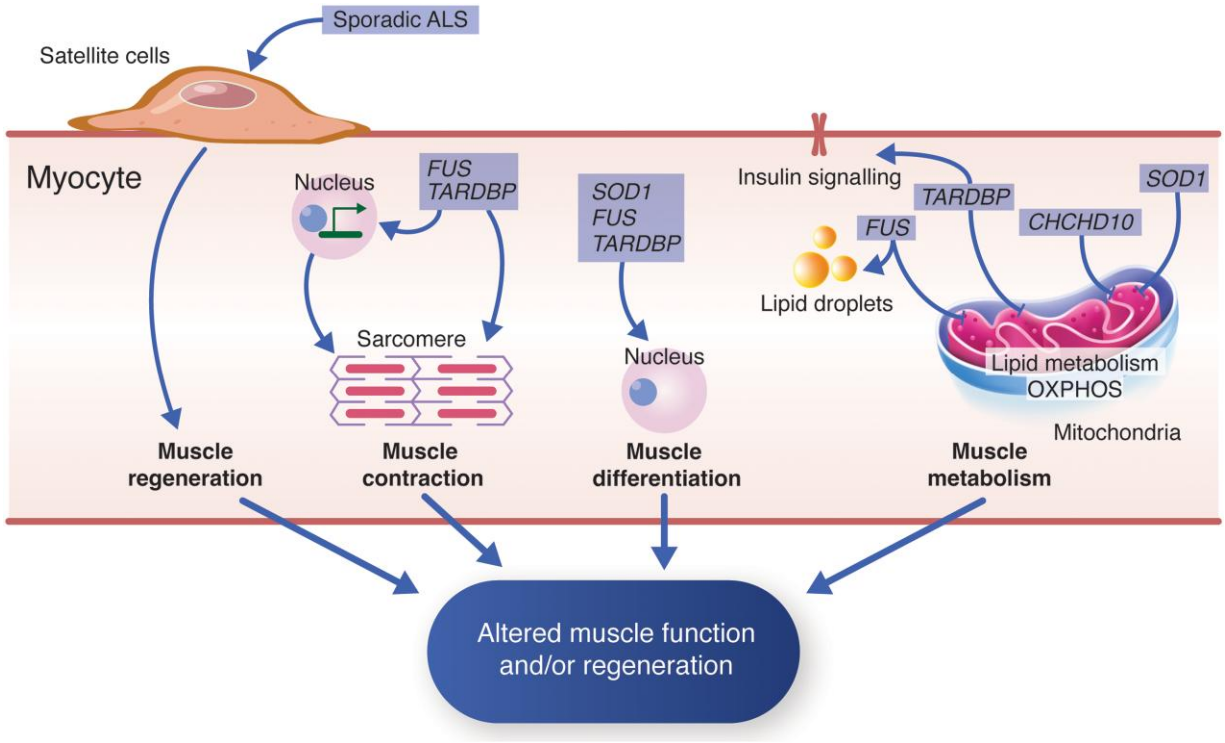

Figure 3 Muscle autonomous effects. A typical muscle fibre is shown, with satellite cell (*top left*). Mutations in ALS-related genes such as *SOD1*, *FUS*, *TARDBP* or *CHCHD10* (blue squares), have toxic cell autonomous effects. Satellite cells from sporadic ALS patients show decreased proliferation ability. *FUS*, *TARDBP* or *SOD1* mutations affect muscle contraction through regulation of muscle gene expression, altering either muscle contraction and/or muscle differentiation. Most ALS genes also alter muscle metabolism through modifying lipid metabolism and oxidative phosphorylation (OXPHOS) (e.g. *FUS*, *TARDBP*, *CHCHD10* or *SOD1*), while *TARDBP* loss affects insulin signalling. All of these events converge to impact muscle contractile function and muscle regeneration upon injury. ALS-related genes are shown in light blue squares. ALS = amyotrophic lateral sclerosis.

Muscle metabolic alterations could also be mere consequences of ongoing denervation. Very early studies have reported increases in localized oxygen consumption in denervated muscle of patients with ALS, when muscle is at rest. This was coupled with increased lactate output, and increased uptake of glucose and fatty acids from circulation, suggesting increased metabolic demand.[41] Moreover, muscle denervation potently influences metabolic gene expression and is thus a major confounder of metabolic defects. However, myoblasts derived from patients carrying ALS mutations show metabolic defects,[22,31] suggesting the existence of cell autonomous defects. In addition, muscle-restricted expression of the *SOD1*^{G93A} gene induces severe muscle atrophy, associated with a significant reduction in the functional performance of skeletal muscle, alterations in the contractile apparatus, mitochondrial dysfunction and a shift in the metabolic activity of muscle fibres.[42,43] Mechanistically, the local muscular expression of *SOD1*^{G93A} induces alteration of muscle glucose metabolism associated with the induction of phosphofructokinases, pyruvate dehydrogenase kinase 4 expression, and pyruvate dehydrogenase complex, indicating the inhibition of acetyl-CoA synthesis.[44] These changes suggest that the metabolic oxidative switch occurs independently from MN degeneration, along with neuromuscular instability and deficiency in mitochondrial chain function. The extent to which other ALS-associated genes might lead to muscle autonomous metabolic defects remains open for future investigation, and studies in presymptomatic gene carriers in the absence of denervation offer an opportunity to determine whether similar defects occur independently of denervation.

Muscle and motor neurons as partners in crime: muscle pathways that could affect motor neuron health

Skeletal muscle cells are not just the effector of MN signalling, allowing motor function through the passive reception of MN input. Myocytes are also dynamically sending signals to their local environment, including motor axons, which not only heavily influence neuromuscular efficacy, but could have effects on MN survival or whole body physiology (Fig. 4).

Altered neuromuscular junction-related signalling

In ALS, the degeneration of neuromuscular synapses is a central and early feature of the disease,[45] and dysfunction of endplates occurs through a dynamic denervation–reinnervation process,[46] which is observed before signs of motor units loss/reinnervation.[47] In this context, the contribution of skeletal muscle to neuromuscular junction (NMJ) dismantlement has been largely overlooked despite the observation that of bidirectional signalling between skeletal muscle and MNs ensuring that neuromuscular synapses are optimally established at endplate zones[48] in particular during development and maintained during adulthood.

Using *in vitro* systems, Picchiarelli *et al.*,[21] Badu-Mensah *et al.*,[49] and Ding *et al.*[50] demonstrated that intrinsic morpho-functional deficits of the ALS skeletal muscle affect human NMJ integrity and function. In these studies, *SOD1* or *FUS* mutant human skeletal

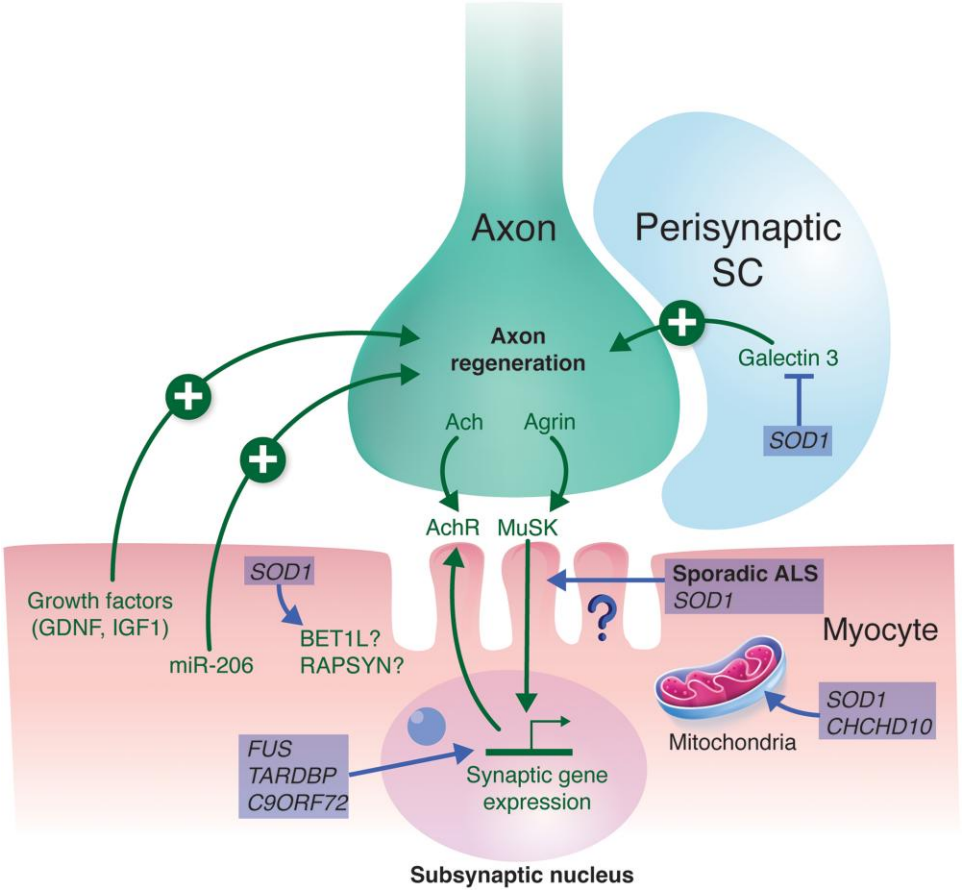

Figure 4 Muscle-dependent alterations in neuromuscular dialog in ALS. (**A**) Neuromuscular junction (NMJ) with its three major components; the terminal part of motor neuron (MN) axon (green), the subsynaptic region of myofibre (red) and the perisynaptic Schwann cell (SC). In ALS, muscles become progressively denervated, leading to compensatory muscle responses that serve to promote reinnervation of the denervated muscle fibre. Among these protective responses, growth factors (i.e. GDNF, IGF-1) and miR-206 related pathways have been shown to delay denervation. In addition, ALS mutations impact nerve-muscle dialogue at several levels. In muscle, *FUS*, *C9ORF72* or *TARDBP* mutations alter expression of synaptic genes in subsynaptic nuclei, which hampers NMJ differentiation. *SOD1* or sporadic ALS dampen the capacity of muscle to cluster acetylcholine receptors (AchRs) at NMJs (e.g. through rapsyn or other scaffolding proteins), and *SOD1* or *CHCHD10* mutations alter metabolism in mitochondria close to the NMJ. Perisynaptic Schwann cells are also affected by ALS-related mutations. ALS-related genes are shown in light blue squares. ALS = amyotrophic lateral sclerosis.

muscle cells, as well as sporadic ALS patient muscle cells, showed altered NMJ parameters, including AChR expression, NMJ density, NMJ stability, contraction fidelity and fatigue index. More recently, comparative transcriptome of myocytes derived from ALS iPSCs identified a common loss of BET1L, a protein located at the NMJ basal lamina across several ALS-related mutations.[51] Collectively, these studies suggest that ALS patient-derived skeletal muscle has inherent defective properties that detrimentally affect NMJ integrity and function independently of MN influence.

One explanation for this phenotype is that signalling that directs neuromuscular synapse maintenance is affected in muscle cells of ALS patients. Indeed, muscle cells derived from biopsies of sporadic ALS patients show impaired response to agrin, accompanied by altered expression levels of a number of the agrin/MuSK signalling pathway intermediates.[50] Interestingly, stimulating MuSK with a therapeutic agonist antibody preserved NMJs in $SOD1^{G93A}$ mice.[52,53] Consistent with this, FUS is required for transcriptional coactivation of NMJ genes in subsynaptic nuclei, and this is disrupted in muscles of mice carrying a *FUS* mutation or in myotubes derived from *FUS* mutant iPSC.[21] A similar function for TDP-43 in directing muscle post-synaptic formation has also been demonstrated in *Drosophila* models[54] and TDP-43 regulates expression of acetylcholine esterase in zebrafish.[55] In addition, muscle expression of $SOD1^{G93A}$ triggers the dismantlement of NMJ via PKCθ, which is functionally involved in the reduction of synapses that are normally generated in excessive and redundant numbers and resulting activity-dependent synapse modulation and loss by $SOD1^{G93A}$ -mediated toxicity,[56] and muscle SOD1 interacts with rapsyn, a protein critical in NMJ stability.[57]

In contrast, skeletal muscle responses to denervation might slow down axonal demise and, for some time, compensate for abnormal neuromuscular signalling. A prototypical example is denervation-induced expression of miR-206, that enables a compensatory mechanism stimulating reinnervation.[58–60] Consistently, $SOD1^{G93A}$ mice genetically deficient in miR206 showed a severe ALS symptomatology such as muscle atrophy, NMJ loss and shorter survival.[59] This regenerative response of the NMJ also normally involves the perisynaptic Schwann cell that participates in NMJ repair.[61] However, perisynaptic Schwann cells show maladapted properties that prevent them from ensuring the maintenance and the repair of NMJs in $SOD1^{G37R}$ mice, which might contribute to overall NMJ defect and instability.[46,62,63] Thus, the dialogue between myocytes and MNs is dysfunctional in

ALS, in part due to abnormal muscle signalling, that is compensated by protective pathways until collapse of the system.

Altered growth factor production

Muscle is an important source of growth factors, often in response to denervation (Fig. 4). Denervation of muscle, in general but also in ALS, is associated with the upregulation of transcripts for glial cell line-derived neurotrophic factor (GDNF), leading to reinnervation.[64] Delivery of GDNF to muscle (using human mesenchymal stem cells) in the *SOD1*^{G93A} rat model of ALS resulted in improved innervation, a delay in disease progression and an extension of survival.[65] GDNF is neuroprotective[66] for both nerve and muscle. Treatment with encapsulated GDNF-secreting cells in *pmn/pmn* mice (a model of motor neuropathy) slowed MN loss but did not improve axonal degeneration or delay death.[67]

Insulin-like growth factor-1 (IGF-1), while perhaps best recognized for its anabolic actions alongside growth hormone (GH),[68] is a key regulator of axonal sprouting[69] and a growth factor that supports motor nerve regeneration.[70] Moreover, IGF-1 is shown to be protective in MNs[71] and in muscle.[72] Produced in muscle in response to GH signalling, IGF-1 has been proposed as a treatment in ALS, acting through anabolic actions directly at the level of muscle, while also promoting the reinnervation of muscle following MN loss. GH secretion is thought to be impaired in patients with ALS.[73] While it is not clear if GH deficiency contributes to reductions on muscle-IGF-1, low plasma IGF-1 levels in patients with ALS is associated with faster disease progression and shorter survival.[74] Importantly, while declining with disease progression, GH and IGF-1 levels are increased early in the course of disease in *SOD1*^{G93A} mice, with levels of GH and IGF-1 correlating with the innervation of muscle and not MN survival.[75,76] As such, a physiological upregulation of IGF-1 in muscle during early disease is thought to promote reinnervation following MN loss. Increases in endogenous IGF-1 production through transgenic overexpression or viral delivery of IGF-1 in muscle and the CNS prolongs survival and slows disease progression of these mice.[77–79] Treatment outcomes with IGF-1 in patients with ALS are, however, mixed; while some benefit is observed, this does not delay death.[80] Differences in duration of exposure to IGF-1 could explain these discrepancies. In mouse studies, increases in IGF-1 levels were sustained and directed to the CNS[77] and muscle[78] via genetic overexpression, whereas patient studies are mostly limited to daily subcutaneous injections.[80] Subcutaneous IGF-1 treatment does not benefit *SOD1*^{G93A} mice, whereas intrathecal injections delay the onset of disease and extends survival.[81]

Besides GDNF and IGF-1, other muscle-derived growth factors could be involved in mitigating MN degeneration in ALS. These include VEGF,[73,82] or BDNF, neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), that regulate a range of neuronal and glial functions via their collective action on p75NTR, TrkA, TrkB and TrkC receptors.[83,84] Whether muscle-derived production of these different growth factors facilitate reinnervation following muscle-nerve damage in general or in ALS specifically is, however, not clear. In general, translation of growth factors (either muscle-derived or not) into clinical care in ALS is limited by current inability to promote widespread and sustained increases in production to target disease-relevant tissues and more specifically engage bidirectional signalling between the muscle and nerve. In addition, growth factor functions are widely documented in development yet their functions in adulthood, notwithstanding during denervation, remain largely unexplored.

One more theoretical consideration would be the prospect that muscle directly impacts CNS health. Indeed, there has been increasing interest in the role of 'myokines'—endocrine factors secreted by muscle—directly or indirectly impacting the brain. Such factors, including irisin,[85] cathepsin B and interleukin-6, have all been implicated in playing a role in Alzheimer's disease, another neurodegenerative condition.[86] In addition, skeletal muscle also releases extracellular vesicles, which could contribute to MN death in ALS.[87] Whether these or other factors could play some kind of role in the CNS during disease progression in ALS, and in upper MN dysfunction and loss remains, to our knowledge, entirely unstudied.

A mechanistic relationship between altered mitochondrial function and dysfunctional nerve/muscle crosstalk?

How can ALS-related injuries in muscle mediate damage to NMJ? They can do so by impairing muscle differentiation pathways. Some ALS-related proteins could indirectly impair adult maintenance or regeneration of NMJs. Indeed, TDP-43 is essential for skeletal muscle formation and forms cytoplasmic, amyloid-like oligomeric assemblies called myo-granules, during muscle regeneration.[88] In addition, FUS is required for proper expression of subsynaptic gene program, including AChR, and its alteration impairs NMJ structure and physiology.[21] It is also possible that muscle mitochondrial defects could secondarily affect neuromuscular maintenance. As a proof-of-concept, muscle restricted overexpression of UCP1, while leading to muscle weakness, progressively destroyed NMJ, and led to limited MN death.[89] In mutant *SOD1*^{G93A} mice, alterations in mitochondrial potential near NMJs occur prior to disease onset.[90] Supporting this possible mechanistic relationship between mitochondrial function and neuromuscular strength, there exists common pathways between mitochondrial biogenesis and NMJ gene expression, including the transcriptional coactivator PGC-1alpha,[91–93] FUS[21] or the androgen receptor.[94,95]

A viewpoint from other motor neuron disorders

Recent evidence from diseases closely related to ALS further tightens the relationship between MN disorders and skeletal muscle, which also share intrinsic-myopathic changes.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a childhood MN disease caused by mutations in the survival motor neuron 1 (*SMN1*) gene. Despite MNs being specifically affected in SMA, it remains unclear whether disease-initiating insults occur in neurons, skeletal muscle, or both tissues. Indeed, muscle from severe SMA patients shows widespread small myofibres, with developmentally arrested appearance[96] and immature expression profile of myofibrillary proteins.[97] Using a system of nerve-muscle co-culture with myogenic cells derived from healthy or SMA muscle, SMA muscle cells are sufficient to lead to the degeneration of innervated cocultures.[98] These results are consistent with animal studies as SMN deficiency appears to cause intrinsic defects in muscle development by impairing myofibre growth and regeneration before spinal MN damage. Similar to what has been observed in ALS mice, mice with SMN-deficient muscles are characterized by muscle fibre defects, NMJ abnormalities, compromised motor performance, and premature death. SMN protein is localized at NMJ[98] and is involved in NMJ

development,[99] just like FUS,[21] one of its interaction partners.[100] However, restoring SMN in skeletal muscle alone is insufficient to correct disease pathology in SMA mice.[101,102] In all, SMN is expressed in muscle and probably plays a role in maintenance of a functional motor unit.

Spinal and bulbar muscular atrophy

Spinal and bulbar muscular atrophy (SBMA) is characterized by the degeneration and loss of lower MNs leading to proximal limb weakness and atrophy, fasciculations of bulbar, facial and limb muscles, cramps, atrophy of the tongue, dysphagia and nasal speech. SBMA is the consequence of an expansion of the CAG repeat sequence in the exon 1 of the androgen receptor (AR) gene coding for an elongated polyglutamine (polyQ) tract in the N-terminus of the AR protein.[103] The AR with the CAG expansion gains a neurotoxic function probably causative of the disease. The aberrant polyQ-protein misfolds and leads to the formation of aggregates that alter proteosomal and autophagic activities in affected cells. Although SBMA is a MN disease with accumulation of polyQ inclusions in spinal MNs associated with degeneration,[104] the muscle histopathology of patients is atypical for a pure MN disease. Both neurogenic and myopathic changes, such as splitting, presence of central nuclei and degeneration of fibres,[105] as well as high serum creatine kinase (CK) levels, are observed, suggesting that myopathic changes contribute to disease pathogenesis.

Evidence of mixed neurogenic and myopathic processes has also been reported in mouse models of SBMA.[106,107] In the knock-in mouse model of SBMA, muscle pathology is evident prior to the onset of spinal cord pathology, strongly supporting the view that muscle is a primary target of polyQ-AR toxicity and could contribute to the MN pathology.[108] An important piece of evidence supporting this hypothesis was provided by mouse studies as muscle restricted expression of wild-type AR leads to premature death upon birth or to the later onset of an SBMA-like phenotype. Furthermore, silencing the peripheral mutant ARpolyQ expression in different SBMA mouse models resulted in prolonged survival, thereby providing evidence for a direct effect of ARpolyQ on muscle atrophy.[109] Thus, SBMA provides a proof-of-concept that a MN disorder can be associated with or even triggered by muscle-specific toxicity that leads to MN degeneration.

What is the relevance of skeletal muscle in ALS?

Our literature review indicates that the view of skeletal muscle as being solely secondarily involved in ALS as a consequence of degeneration of MNs needs to be revised, at least when considering findings from animal models and/or *in vitro* cell models.

Direct mechanistic evidence for an active role of muscle in patients with ALS is lacking, especially as current trials either stimulating muscle contractile properties (e.g. reldesemtiv[110]) or muscle reinnervation (ozanezumab[111]) have failed. However, indirect evidence, mostly in cultured cells, suggests that the mechanisms elucidated in mouse models might, to some extent, translate to ALS patients. First, human ALS muscle cells, either satellite cells or iPSC-derived, show a number of cell intrinsic alterations, including altered cell proliferation[19] or metabolism,[22,25,31] reduced capacity to respond to innervation by MNs[50] or increased secretion of toxic extracellular vesicles.[87] In support of these observations, myopathic features have been reported in individuals or families harbouring ALS-associated gene mutations including *SOD1*,[112] *HNRNPA1/B2*,[113] *TARDBP*,[114] *ANXA11*,[115] as well as in rare ALS patients with sporadic disease.[116]

As a whole, skeletal muscle dysfunction, whether contractile or metabolic, likely contributes to progressive muscle weakness in ALS. This active role is beyond what would occur in response to progressive denervation due to MN loss. Furthermore, as skeletal muscle is actively involved in the maintenance and regeneration of NMJs, these pathways could contribute to the degeneration of MNs. While this new paradigmatic role of muscle in ALS emerges, it should be kept in mind that most of the current studies have focused on atypical forms of ALS (e.g. *SOD1* or *FUS*), that might share a higher myotoxic component than most other ALS forms, either sporadic or familial. In this respect, it will be important to evaluate muscle contribution in *C9ORF72* or *TBK1* ALS using appropriate models. Equally important would be the identification of mechanistic targets.

Skeletal muscle could constitute a valid therapeutic target in ALS, with at least two possible beneficial outcomes. First, muscle weakness is a cardinal symptom of ALS, and therapeutic strategies targeting muscle strength or muscle metabolism to improve muscle function, hence quality of life of patients, should be pursued, eventually as an add-on to neuroprotective therapeutics. Indeed, disease severity is directly linked to muscle atrophy that, in ALS, is primarily due to a loss of type II fibre muscles, as in sarcopenia and ageing. Thus, the description of common pathogenic pathways could define possible treatment strategies to counteract the loss of muscle in ALS but also in other muscle diseases. Improving muscle function could be achieved through pharmacological strategies. However, troponin activators (e.g. reldesemtiv) or neurite outgrowth stimulators (e.g. ozanezumab)[111] did not reach primary or secondary end points of efficacy despite *post hoc* analysis of the reldesemtiv trial indicating that possible benefits may be restricted to a subset of patients.[110,117] Such outcomes are likely to influence the conduct of future trials of muscle-directed therapeutics in the coming years as the focus on improving trial design in ALS grows exponentially.[118] Alternatively, improving mitochondrial function in muscle could also improve muscle strength, as was shown in $SOD1^{G93A}$ mice using PGC1-alpha overexpression.[93] The effect of already approved therapies, such as riluzole, edaravone or AMX0035, on muscle function should also be carefully evaluated as these drugs are known for their lack of specificity towards MN protective pathways.

Second, it is important to remember that skeletal muscle is also a preferred route to yield direct access to MNs and endplates. In mouse models, the stabilization of endplates yielded positive outcomes through targeting MuSK/agrin pathways[52,53] or axonal outgrowth inhibitors such as Nogo-A[119,120] or Semaphorin 3A/Nrp1.[121] Last, skeletal muscle could also be considered as a privileged route to MNs through endplates, for instance, to directly deliver growth factors via therapeutic viruses.[122,123] In all, it is critically important that the field of ALS considers skeletal muscle as an integral player in the complex pathophysiology of the disease, in addition to its classical secondary involvement due to MN loss.

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