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# **Skeletal muscle in motor neuron diseases : therapeutic target and delivery route for potential treatments**

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**Abstract (237 words):**

Lower motor neuron (LMN) degeneration occurs in several diseases that affect patients from neonates to elderly and can either be genetically transmitted or occur sporadically. Among diseases involving LMN degeneration, spinal muscular atrophy (SMA) and spinal bulbar muscular atrophy (Kennedy's disease, SBMA) are pure genetic diseases linked to loss of the SMN gene (SMA) or expansion of a polyglutamine tract in the androgen receptor gene (SBMA) while amyotrophic lateral sclerosis (ALS) can either be of genetic origin or occur sporadically. In this review, our aim is to put forward the hypothesis that muscle fiber atrophy and weakness might not be a simple collateral damage of LMN degeneration, but instead that muscle fibers may be the site of crucial pathogenic events in these diseases. In SMA, the SMN gene was shown to be required for muscle structure and strength as well as for neuromuscular junction formation, and a subset of SMA patients develop myopathic pathology. In SBMA, the occurrence of myopathic histopathology in patients and animal models, along with neuromuscular phenotype of animal models expressing the androgen receptor in muscle only has led to the proposal that SBMA may indeed be a muscle disease. Lastly, in ALS, at least part of the phenotype might be explained by pathogenic events occurring in skeletal muscle. Apart from its potential pathogenic role, skeletal muscle pathophysiological events might be a target for treatments and/or be a preferential route for targeting motor neurons.

## **1) Introduction: lower motor neuron degeneration affects first neuromuscular junctions**

Voluntary locomotion is governed by motor units, composed of lower motor neurons (LMNs) (alpha-motor neurons) that innervate skeletal muscle fibers and control their contraction. Myelinated axons of the LMNs contact muscle fibers on a discrete region of the fiber, highly specialized, called the neuromuscular junction (NMJ). A number of pathologies target this critical region. First, myasthenic syndromes affect the efficacy of synaptic transmission through either immunological or genetic processes. In these pathologies, the absolute number of NMJs remain roughly the same but their efficacy to trigger a muscle action potential in response to motor neuron stimulation is decreased leading to fatigable muscle weakness. NMJs are also affected during lower motor neuron degeneration. In this review, we will focus on pathologies involving LMN degeneration, but sparing sensory innervation. This group of diseases affects patients from neonates to elderly people and can either be genetically transmitted or occur sporadically in a family. The table 1 summarizes the clinical features of the diseases we will focus on in this review, namely spinal muscular atrophy (SMA)[1], spinal bulbar muscular atrophy (Kennedy's disease, SBMA) [2] and amyotrophic lateral sclerosis (ALS)[3].

During LMN degeneration, NMJs are progressively destroyed, leading to fully denervated muscle fibers and neurogenic figures in histopathology. The figure 1 presents typical histopathological and electrophysiological results obtained in patients afflicted with a LMN degeneration. One is able to observe that muscle fibers of these patients display spontaneous denervation activities as judged in electromyography (figure 1A). Histopathology shows the occurrence of small grouped, angulated fibers (figure 1B-D). It is thought that reinnervation of muscle fibers by the same motor neuron leads to these grouped fibers. NMJ destruction during LMN degeneration is associated with the degeneration of lower motor neuron cell bodies, hence their name and a widely accepted paradigm was that LMN death was the cause of NMJ destruction in these pathologies. Indeed, muscle denervation by nerve transection leads to roughly similar histopathological figures, suggesting that in LMN degeneration, the same cause (disruption of the nerve to muscle communication) leads to the same consequence (muscle denervation). However, recent histopathological evidences suggest that

grouped atrophic fibers are highly homogenous in type in polyneuropathies, but heterogenous in LMN degeneration [4]. The reason for this discrepancy is unknown, but might indeed reflect profound differences in the mechanisms of denervation/reinnervation between purely denervating diseases and diseases with LMN degeneration.

In this review, we would like to put forward the hypothesis that muscle fibers might not be the collateral damages of LMN degeneration, but rather, one of their murderers. For this, we will first describe how muscle is involved in the patterning and development of fully functional NMJs and how muscle participates in the pathology of ALS, SBMA and SMA. We will also give insights into how drugs might target muscle pathophysiological events or target motor neurons but be delivered through muscles to treat LMN degeneration.

## **2) Muscle and motor neuron : a peer to peer dialog to establish neuromuscular junctions**

The establishment of neuromuscular synapses is a highly controlled developmental event. Motor axons, emerging from the embryonic spinal cord, contact newly formed myofibers around E13. It was long known that motor neurons and muscles are interdependent for their development. Indeed, during development, motor neurons die if deprived of their target muscle[5-8]. Conversely, muscle development is arrested in the absence of motor neurons. A neurocentric view of the development of NMJs long prevailed and postulated that this process was mainly driven by motor neurons, with muscle fibers being passive players of NMJ development. Indeed, motor neurons secrete a very active isoform of the glycoprotein agrin (neural agrin), that is sufficient to cluster nicotinic acetylcholine receptors on the myofiber [9, 10] through a complex receptor pathway involving the tyrosine kinase Musk and a co-receptor LRP4 [11, 12]. In this model, the presence of agrin secreted by the nerve is sufficient to cluster AchR and form NMJs. However, in the absence of nerves, clusters of nicotinic acetylcholine receptors still form in the correct, central, region of the developing muscle[13, 14]. Moreover, diaphragm muscles from E14 embryos cultured in the presence of recombinant agrin as well as muscles from transgenic mice overexpressing a miniaturised form of agrin in the

whole muscle fiber retain the formation of AchR clusters in their central region[15]. Conversely, the transient muscle overexpression of a constitutively active form of ErbB2 widened the zone of AchR transcription and of the region where NMJs formed[16]. Altogether, these data show that the site where NMJs form in skeletal muscle is pre-patterned by muscle cells, independantly of nerve derived cues such as agrin. In the current model, NMJs thus form through a dialog between muscle and motor neurons, muscle driving the zone where NMJs will form and neurons strengthening the newly formed contacts by the secretion of agrin.

This pathway of NMJ development has been largely studied in the context of congenital myasthenic syndromes in which mutations in Musk[17], or more recently in agrin[18] were found. Up to now, there is no documented association between this pathway and LMN degeneration. However, it is striking to note that a mutation in Musk, when introduced in knockin mice, not only leads to the full phenotypic spectrum of myasthenic syndrome, but also to denervation of endplates and molecular features of denervation [19]. In line with this, the recently described mutation in agrin in a patient with congenital myastheniav[18] leads to not only to post-synaptic defects but also to pre-synaptic pathology suggesting that the Agrin/Musk pathway might also be involved in the maintenance of NMJs in adults and, thus potentially in LMN degeneration.

### **3) Muscle pathogenic role in spinal muscular atrophy**

#### 3-1) spinal muscular atrophy

Childhood spinal muscular atrophy (hereafter called spinal muscular atrophy, SMA) is a child-onset motor neuron disease involving mutations in the survival of motor neuron gene[20]. SMA patients are divided in three clinical groups according to the severity of their disease[1]. Type I SMA is characterized by severe, generalized muscle weakness and hypotonia at birth or within the first 6 months, with death usually occuring within the first 2 years. Type II children are able to sit, although they cannot stand or walk unaided, and they survive beyond 2 years. In type III SMA patients have proximal muscle weakness, starting after the age of 18 months.

In the human genome, the *SMN* gene is duplicated with a highly homologous copy called *SMN2*. Both *SMN* and *SMN2* genes are expressed and only five nucleotides are different between both genes. We showed that even the promoter sequences and activities of these two genes were strikingly similar[21]. Importantly, only *SMN1* deletions cause SMA, while up to 5% of individuals are lacking the *SMN2* gene. The subtle difference in nucleotide sequence between *SMN1* and 2 has no effect on the encoded open reading frame but profound effects on *SMN2* splicing. Indeed, one of the nucleotides divergent between *SMN1* and *SMN2* creates a new splicing site in *SMN2* mRNA by skipping exon 7. Thus, the *SMN1* gene produces exclusively full length fully functional transcripts, while the transcripts derived from *SMN2* lack exon 7. Most importantly, the amount of *SMN2* protein are strikingly inversely correlated with the clinical severity of disease[22, 23], suggesting that *SMN2* is a modifying gene in SMA. Thus, SMA is caused by *SMN1* mutation and the severity of the disease is linked to the potential compensation by *SMN2* protein products. That SMA is caused by the lack of full-length *SMN* proteins is largely substantiated by the evidence that ablating specifically the exon 7 in motor neurons lead to motor neuron degeneration in mice[24-26].

### 3-2) a housekeeping function for SMN protein ?

What is the function of *SMN*, lost in SMA, that might lead to motor neuron degeneration ? *SMN* forms a large multiprotein complex with at least 7 other proteins (called gemins) both in the cytoplasm and in the nucleus where it is concentrated in a structure called gems (for "gemini of coiled bodies"), associated with Cajal bodies[27]. This complex including *SMN* and gemins appears crucial for the biogenesis of small nuclear ribonucleoprotein particles (snRNPs) [28-30] that are involved in the splicing of pre-mRNA. Consistent with a key role in RNA metabolism, the ablation of exon 7 of *SMN* in either muscle or neuron leads to strong up-regulation of a number of genes involved in pre-mRNA splicing, ribosomal RNA processing, or RNA decay [31]. Furthermore, a recent study used exon microarray in SMA mice and observed widely distributed splicing defects in numerous mRNAs[32]. Interestingly, while splicing defects were prominently observed in all the tissues studied, the genes that were abnormally spliced were different between tissues, suggesting that the selectivity of SMA for the neuromuscular system is explained by tissue specific alterations in mRNA splicing.

### 3-3) SMN is required for muscle function

The SMN protein is ubiquitously expressed and the tissue specific ablation of SMN leads to drastic alterations in the physiology of the targeted tissue[24-26, 33]. A number of studies have convincingly shown that *SMN* expression was required for muscle structure and function. In vitro, Shafey and collaborators demonstrated that *SMN* knockdown in C2C12 myoblasts lead to decreased myoblast proliferation and impaired myotube fusion[34]. Consistently, in vivo, exon 7 ablation of the *SMN* gene in mouse skeletal muscle leads to a massive muscle dystrophy and death of the animals [24] and RNAi knockdown of *SMN* in drosophila muscles is lethal in drosophila in a much more severe way than neuronal knockdown [35]. These studies were all based on the knock-down or knock-out of the *SMN* gene, a situation much more drastic of what occurs in real pathological situations. Their relevance for the human pathology is however strengthened by the work of Arnold and collaborators[36] that had previously observed decreased proliferation and impaired fusion of type I SMA myoblasts. More recently, Martínez-Hernández and collaborators showed that myotubes of fetuses affected with type I SMA were smaller and with abnormal arrangements suggesting delayed developmental maturation[37]. Importantly, about a quarter of SMA type 3 are reported to have a dystrophic phenotype with high serum creatine kinase (CK) levels and “myopathic” histopathology [38]. Thus, *SMN* is required for normal muscle structure and development either in experimental situations or in SMA patients.

The function of SMN in muscle is likely to be related to sarcomeric structure. In drosophila bearing an hypomorphic allele of *SMN*, Rajendra and collaborators in 2007 observed a severe disorganization of muscle filaments associated with decreased actin expression[39]. Indeed, *SMN* hypomorph displayed a similar phenotype than flies with ablation of one muscle isoform of actin. These authors suggested a function for *SMN* in sarcomere formation since both endogenous and transgenic SMN localized to the sarcomeric region of fly muscle. Such a function was further suggested by the work of Walker and collaborators[40]. Indeed, not only SMN, but also its associated proteins localize to the sarcomeric Z-disc in both cardiac and skeletal myofibrils of the mouse. This localization of the SMN complex appeared independent of its role in pre-mRNA splicing since snRNPs themselves were not found in the sarcomere. SMA mutant muscles exhibit numerous morphological defects, including vacuoles and altered Z-disc spacing



and an overall loss of sarcomeric uniformity and alignment. Whether this phenotype is primarily myopathic or if it is a secondary consequence of denervation is not known but these results establish that myofibrils from SMA type I mice display defects that are consistent with those observed in other myopathies. Furthermore, these observations are consistent with the occurrence of congenital heart defects in SMA. This function of SMN in sarcomere formation might be related to the postulated role of SMN in beta-actin mRNA translation[41]. In all, the data available point to a key function for *SMN* in muscle development and function.

### 3-4) SMA is a developmental pathology of NMJs

Recent evidence suggest that the SMA pathology begins at NMJs. Indeed, Kariya and collaborators used severe- and mild-*SMN2* expressing mouse models of SMA as well as material from human patients to understand the initial stages of neurodegeneration in the human disease[42]. In these studies, the earliest defects appear at the NMJ. Indeed, lack of SMN protein lead to the failure of post-natal development of the NMJ. In particular, NMJs of SMA mice showed impaired maturation of acetylcholine receptor (AChR) clusters into 'pretzels' that were reflected in functional deficits at the NMJ characterized by intermittent neurotransmission failures. Similar results were also reported by Kong and collaborators which observed immaturity of NMJs in SMA mice as assessed for instance by persistence of embryonic gene expression, reduced post-synaptic apparatus and electrophysiological abnormalities[43]. Importantly, the pre-synaptic pathology of severe SMA mice is dissociated from the post-synaptic pathology, suggesting that both events might be independent[44]. These data are indeed consistent with earlier studies showing reduced expression of AchR genes in SMA myocytes[36]. Altogether, SMA should now be viewed as a developmental NMJ synaptopathy

### 3-5) SMA as a global neuromuscular pathology

Given that *SMN* has a crucial function in muscle, and that SMA pathology is initiated at the neuromuscular synapse, one could hypothesize that SMA is indeed a muscle disease spreading to the motor neuron. Indeed, muscle knockdown of *SMN* leads to a more drastic phenotype than neuronal knockdown in *Drosophila* [35]. This is however not true in all animal models, since *SMN* ablation is partially rescued by neuronal but not

muscle transgenic expression in the nematode[45]. Furthermore, overexpression of SMN in skeletal muscles did not allow the rescue of the phenotype of severe SMA mice, while a more widespread overexpression including high levels in neurons and low levels in muscle strongly alleviated the phenotype of these mice [46]. However, it should be kept in mind that the transgenic overexpression of SMN2 in these animals might be sufficient on its own to fully rescue the muscle phenotype. In this situation, one should not expect an even more increased survival of the mice after increasing SMN expression. In any case, the bunch of litterature currently available strongly suggests that SMA is a global neuromuscular pathology, involving pathological events in muscle and neurons, and potentially a number of other cell types (figure 2).

#### **4) Muscle pathogenic role in Kennedy's disease**

##### 4-1) Kennedy's disease

Spinal and bulbar muscular atrophy (SBMA), or Kennedy's disease, is a purely genetic LMN degeneration caused by expansion of a CAG repeat in the first exon of the androgen receptor (AR) gene, leading to an AR protein with an expanded polyglutamine (polyQ) tract [47]. Like in other triplet repeat disorder, including Huntington's disease, the disease develops only in individuals bearing an expansion of the polyQ tract over a certain threshold (>36 Gln) and the length of the polyQ is correlated with a number of clinical parameters[48, 49]. SBMA is an X-linked, gender specific disease since only male carriers of a pathogenic mutation are affected. The phenotype of females carrying a pathogenic allele is absent or very mild even if homozygous for an expanded CAG repeat [50, 51]. SBMA patients develop proximal muscle weakness, fasciculations, and atrophy, along with lower motor neuron degeneration in the brainstem and spinal cord [2, 52]. A subset of patients also display additional features associated with androgen dysfunction such as androgen insensitivity, oligozoospermia or azoospermia, testicular atrophy, feminized skin changes, and gynecomastia[53, 54].

Different animal models of SBMA have been generated in the recent years. In particular, expansions of CAG repeats have been introduced in knock-in mice[55-57] or in transgenic mice[58-61]. For instance, male mice with a 113 polyglutamine tract in the

endogenous AR developed an SBMA-like neuromuscular pathology, with early muscle pathology but late spinal cord disease and relatively spared motor neurons. In this mouse model, there was prominent early death of males caused by obstruction of the urinary tract [55]. Interestingly, affected males display several signs of androgen insensitivity, as commonly observed in SBMA.

The physiological function of the mutant protein in SBMA is extremely well documented. The AR is a transcription factor, whose transcriptional activity is activated by testosterone or dihydrotestosterone[62]. After ligand activation, AR drives the expression of its target genes, including key factors for muscle growth, male reproductive function and male secondary sexual phenotypes[63]. The lack of functional AR protein leads to testicular feminization and complete infertility in both mice and humans[64, 65]. In skeletal muscle, AR is especially required for the maintenance in muscle mass and fiber type [66] as well as muscle strength in males [67].

Studies in animal models indicate that hormonal ligands are crucial for the development of SBMA. First, only male transgenic mice develop an SBMA-like phenotype. Second, castration of transgenic males alleviates the phenotype while testosterone-treated females develop the pathology [58, 60, 61]. This is reminiscent of the absence of phenotype of women carriers of SBMA mutations [50, 51]. Consistently, a phase 2 clinical trial has recently suggested that androgen deprivation by leuporelin acetate may be beneficial to patients [68] Thus, SBMA is due to a toxic gain of function in mutant AR unmasked by the presence of the ligand.

#### 4-2) Myopathic pathology in SBMA

Studies in both patients and mice have shown that, apart from lower motor neuron degeneration, SBMA patients display myopathic features. In particular, SBMA patients showed CK elevation in muscle biopsies [69]. Recently, we described a family with early onset and rapidly progressive SBMA mimicking muscle dystrophy [70]. Three out of seven patients in this family displayed increased CK levels indicating rhabdomyolysis, suggesting that the occurrence of such muscle abnormalities is indeed very common in SBMA. It should however be noted that CK elevation is a poor indicator of myopathy since it is subject to large variations and increased in a number of conditions. Apart

from plasma CK levels more elevated than expected in denervative diseases, SBMA patients also often show myopathic abnormalities in muscle biopsies with centrally nucleated fibers or other myopathic features [69] and such abnormalities do not appear to be the sole consequence of denervation. In the same line, Yu and collaborators have shown that muscle pathology long precedes spinal cord pathology in an animal model of SBMA. Furthermore, these animals display mixed features of myopathy and denervation and showed prominent and lethal myotonic discharges [55]. Thus, while SBMA was considered as a pure motor neuron disorder, recent research reevaluated this notion and showed that SBMA is a mixed condition involving not only neurogenic denervation, but also myopathic features.

#### 4-3) A myogenic origin for KD related motor neuron degeneration ?

These findings prompted to test the hypothesis that LMN pathology of SBMA patients indeed resulted from a myogenic pathology. This idea is further supported by the crucial role of AR in the skeletal muscle [63-65] and by the intriguing observation that AR accumulates at NMJs[71]. Indeed, the pathology of animal models of SBMA is primarily muscular, with both neurogenic and myogenic pathology occurring long before motor neuron degeneration is observed [55]. Indeed, in most SBMA animal models, there is no detectable motor neuron degeneration [55, 58, 59]. Most importantly, the muscle specific overexpression of a non-expanded AR leads to a SBMA-like phenotype. In this model, the phenotype was androgen dependent, with a pathology strictly affecting males but alleviated by castration[72]. Importantly, mice present with a pathology not restricted to muscle (muscle necrosis) but also show axonal loss and denervation related changes in gene expression but not loss of MN cell bodies [72]. Last, the pathology did not affect females except when treated with testosterone, and the cessation of testosterone treatment in females allows complete recovery of the phenotype [73, 74]. These studies thus provide the convincing proof of principle that SBMA might be a LMN degeneration of muscle origin [74] (figure 3). Further work is needed to delineate the mechanisms linking muscle AR and NMJ denervation, as well as the pathogenic role of polyglutamine expansion.

## 5) Muscle pathogenic role in amyotrophic lateral sclerosis

### 5-1) Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the most frequent LMN degeneration of adult onset. ALS worldwide incidence is estimated to be of 1–3 new cases per 100 000 individuals, which ranks ALS as the most frequent neurodegenerative disease after Alzheimer's and Parkinson's diseases[3]. Like SMA and SBMA, ALS presents with a progressive paralysis affecting first either limb muscles (spinal onset) or cranial muscles (bulbar onset). A difference between ALS and other diseases with LMN degeneration is that LMNs are not the only neuronal cell type to be affected. Indeed, in parallel to LMN involvement, upper motor neurons (corticospinal motor neurons) also degenerate. Most of the ALS patients die within two to five years after the diagnosis but the disease is heterogeneous in its duration and clinical presentation. No current treatment is able to stop the disease process. Riluzole remains the only FDA approved drug and increases the survival of the patients by a few months.

Most ALS cases are not associated with a family history and are, hence, termed sporadic; the remainders (20%) are of genetic origin, generally transmitted with an autosomal dominant inheritance. Sporadic and familial ALS are clinically indistinguishable. Indeed, even in a single affected family, the clinical presentation of the patients may vary, strongly suggesting that genetic and/or environmental cues are of pathological importance[75-77]. Several genes including *angiogenin*[78], *vapb*[79], *dynactin*[80, 81], and more recently *tdp43*[82-86], *fus* [87, 88] and *fig4* [89] have been genetically linked to familial forms of ALS, but how these specific mutations lead to ALS is currently unknown[90]. The recent description of a mouse model overexpressing mutant TDP-43 will hopefully lead to new insights in the field[91]. On the contrary, the mechanisms underlying ALS linked to mutations in the *sod1* gene, the first and major gene linked to familial ALS in 1993, have been extensively studied[75-77]. It is interesting to note that variations in the SMN1 and 2 genes involved in SMA have been reported to be a risk factor in ALS also[92-97].

### 5-2) Myopathic features in ALS

Myopathic features have been rarely described in ALS and are certainly much less frequent than in SMA or SBMA. However, it was reported that a large number of ALS muscle biopsies displayed myopathic features[98, 99] and moderately elevated CK levels [100]. The extent of these findings is however very limited since similar changes in magnitude are observed upon denervation. Most interestingly, a number of case report studies suggest that even typical ALS patients might initially display myopathic features. For instance, a patient bearing a SOD1 mutation displayed CK elevation before EMG abnormalities [101]. Moreover, ALS patients might present ragged-red muscle fibers, indicative of mitochondrial pathology in their muscles[102]. It is noteworthy that mitochondrial alterations in skeletal muscle might contribute to the pathology[103]. However, this interpretation is complicated by the fact that detectable mitochondrial dysfunction in muscle is a rather late event [104, 105] that could indeed be the result of muscle denervation. Thus, ALS is also associated with myopathic changes, although milder than in SMA or SBMA and mitochondrial defects in muscle might be associated in at least a subset of ALS cases.

### 5-3) NMJ destruction is the critical pathogenic event in SOD1-linked ALS

Studies in ALS during the last ten years have been focused on the elucidation of SOD1-linked ALS, through the use of transgenic mice overexpressing mutant SOD1 (mSOD1 mice) isoforms. Between 3 and 12 months of age, mSOD1 mice develop muscle weakness linked to muscle denervation and both upper and lower motor neuron degeneration. The first event in this disease process is the destruction of the NMJ, more specifically of the postsynaptic apparatus, followed by axonal degeneration and late-onset degeneration of motor neuron cell body[106, 107]. In years 2000, the accepted paradigm was that neuronal expression of mSOD1 was responsible for motor neuron degeneration, leading to muscle denervation and paralysis. Thus, most research efforts sought to understand the mechanisms leading from mutant SOD1 expression in motor neurons to cell death. Indeed, apoptosis was shown to be the main mechanism of motor neuron death in ALS and multiple signaling pathways are now known to contribute *in vivo* to the degeneration of motor neuron cell body [108]. However, none of these

pathways did seem to contribute to overall survival of mSOD1 mice and all preclinical trials based on interference with cell death pathways only marginally affected mSOD1 mice lifespan. Most importantly, clinical trials based on those results, including the recent minocycline trial [109], not only did not improve patient outcome, but in some instances even worsen it.

On top of this, we and others have shown that even a complete rescue of motor neuron cell bodies does not cure mSOD1 mice. For instance, gene ablation of *bax*, a key player in motor neuron apoptosis, completely rescued mSOD1 mice motor neurons from apoptosis, while only modestly delaying muscle denervation and animal death [110]. Along the same line, sodium valproate, a drug inhibiting epigenetic chromatin remodeling during apoptosis, or an inhibitor of p38MAPK, a protein kinase involved in initiating cell death, are able to rescue the cell bodies but have no effects on muscle denervation and animal lifespan [111, 112]. Last, ablating mSOD1 from motor neurons, while delaying onset modestly, did not cure the pathology [113]. Thus, the primary pathogenic event, determining the survival of the animal, is not motor neuron death itself, but rather the loss of motor neuron/muscle contacts. Hence, preserving motor neuron cell bodies is therapeutically not sufficient since the rescued motor neurons are unable to recreate destroyed NMJs. In this model, motor neuron degeneration represents a late, secondary consequence of synaptic destruction

#### 5-4) Is muscle mutant SOD1 expression sufficient to trigger ALS ?

In SBMA, recent work suggested that the muscle overexpression of AR, even not bearing an expanded polyglutamine tract could lead to the pathology [72]. Could mSOD1 expression in muscle also lead to such a concept in ALS? Indeed, the transgenic overexpression of mSOD1 was sufficient to induce severe muscle atrophy associated with significant reduction in muscle strength, sarcomere disorganization, significant changes in mitochondria morphology and disposition, and disorganization of the sarcotubular system. The authors involved several signalling pathways, including autophagy and oxidative stress in the deleterious effects of muscle mSOD1 expression. However, while muscle-restricted mSOD1 expression promote spinal cord astrocytosis and inflammation, no motor neuron loss was observed. Thus, in this study, muscle mSOD1 expression was not sufficient to support full blown ALS [114]. A very recent

study confirmed and significantly extended this initial work. Wong and Martin created transgenic mice overexpressing either wild type or two mutations in the SOD1 cDNA under the control of the skeletal muscle actin promoter[115]. These authors, in a manner consistent with Dobrowolny and collaborators, found important clues for this overexpression triggering localized oxidative stress and subsequent muscle atrophy. Most interestingly, they found NMJ denervation and animals developed paresis and motor neuron degeneration. In this study, muscle SOD1 expression, either wild type or mutant was sufficient to trigger the full ALS phenotype. It is intriguing to note that there was no difference between wild type and mutant SOD1 overexpression, reminiscent of the effects of wild type AR overexpression in muscle[72].

Intriguingly, the knock-down of mSOD1 in muscle did not appear to be sufficient to slow down the pathology [116, 117]. This suggests either that muscle mSOD1 expression is not key in the pathology, or that even the small remaining amounts of mSOD1 in knocked down muscles were sufficient to lead to their toxic effects, or that the knock down occurred too late in animal lifespan to yield its protective potential. In all, muscle appears to be one of the sites of mSOD1 toxicity, although it remains elusive whether NMJ destruction is primarily driven by muscle mSOD1 expression.

#### 5-5) Muscle energy metabolism abnormalities as a cause of NMJ destruction

What could be the mechanisms underlying muscle toxicity to motor neurons in ALS? We have shown that the neurite outgrowth inhibitor Nogo-A is massively expressed in the skeletal muscle of mSOD1 mice and ALS patients [118-120]. Importantly, Nogo-A ablation increased mSOD1 mice lifespan while its overexpression in muscle fibers lead to NMJ shrinkage [119]. These findings provide the proof of principle that alterations in muscle gene expression are able to modulate the disease process in mSOD1 mice.

From a more general point of view, our work suggests that abnormalities in muscle energy metabolism might be the direct cause. We and others have observed that muscles of mSOD1 mice display decreased cellular levels of ATP[121, 122], along with increased expression of mitochondrial uncoupling proteins and of markers of both lipid and carbohydrate use[123, 124]. Indeed, as previously mentioned, mitochondrial dysfunction occurred in muscles of mSOD1 mice, and, to a lesser extent, in muscles of ALS patients[103]. These findings suggested that muscles of ALS patients and mSOD1 mice



show an exacerbation of their energy metabolism. To determine whether this was sufficient to lead to motorneuron degeneration, we studied the neuromuscular system of mice overexpressing the mitochondrial uncoupling protein 1 in their muscles, as a model of muscle restricted hypermetabolism. These animals displayed age-dependent deterioration of the NMJ that correlated with progressive signs of denervation and a mild late-onset motor neuron pathology[125]. Furthermore, NMJ regeneration and functional recovery were profoundly delayed following injury of the sciatic nerve and crossing these mice with mSOD1 mice exacerbated ALS-like pathology[125]. Thus, a muscle restricted mitochondrial defect is sufficient to generate motor neuron degeneration.

#### 5-5) ALS as a systemic pathology

Muscle hypermetabolism of mSOD1 mice has broad consequences on the overall energetic physiology of these animals, and provides clues for a potential therapeutic strategy. Indeed, mSOD1 mice show body weight deficit as compared to wild types due to an increase in the basal metabolic rate, as a reflect of muscle hypermetabolism[123]. Furthermore, energy metabolism, especially lipid metabolism, was strikingly altered in these animals[126]. We sought to determine whether correcting energy deficit of mSOD1 mice could delay their pathology and fed these mice with a diet enriched in saturated fats. High fat fed mSOD1 mice lived longer and showed reduced muscle denervation associated with improved motor neuron survival, a finding recently confirmed by Mattson's group in another mSOD1 strain[123, 127].

ALS patients also show abnormalities in their systemic energy homeostasis. Couratier's group has found an increased energy expenditure in these patients, similar to what observed in mSOD1 mice[128-132]. Most importantly, ALS patients show increased blood lipid levels and hyperlipemia was associated with increased survival [133]and better respiratory capacity in these patients. However, the translation from mSOD1 mice to ALS patients is not straightforward since patients show an important trend to become insulin resistant[134]. In these conditions, the increase in energy intake, in the form of an high fat diet, might precipitate insulin resistance and worsen patient outcome. Potential therapeutic strategies based on nutrition should take this trend to insulin resistance into account.

In all, skeletal muscle is involved in ALS through an increase in its metabolic rate, precipitating systemic defects in energy homeostasis, such as leanness. Muscle might also directly influence NMJ stability through energy metabolism defects and yet unknown signalling pathways (figure 4).

## **6) Muscle as a drug target in lower motor neuron diseases**

The previous results in either SMA, SBMA or ALS point to muscle being an active contributor to these pathologies. Based on these studies, a number of therapeutic strategies have been proposed to target muscle pathology (figure 5).

In SMA mice, treatment with follistatin, an inhibitor of myostatin [135] that increases muscle mass, was reported to lessen disease severity in SMA mice [136] but not in ALS mice [116]. In SMA mice, but not in ALS mice, follistatin-treated mice performed better than their vehicle-treated littermates. The most widely documented factor that could be beneficial in LMN degeneration through a muscle action is IGF1. In 2002, Antonio Musaro and colleagues generated a transgenic mouse line overexpressing an isoform of IGF1 (termed mIGF1) that is retained locally in skeletal muscle and does not systematically diffuse [137]. Crossing these mice with either mSOD1 mice [138] or SBMA mice [139] potentially increased the lifespan of both models. In mSOD1 mice, mIGF1 expression delayed the disease, enhanced survival, stabilized NMJs and had also distant protective effects by decreasing astrocytosis[138]. In SBMA mice, mIGF1 increased AR phosphorylation, promoted the degradation of aggregated AR, rescued the muscle phenotype, increased motor neuron counts and potentially increased the lifespan of SBMA mice [139]. These data thus suggest that a pharmacological intervention solely targeted at muscles is able to provide global protection to motor neurons. Indeed, it should be noted that the protection mediated by exercise [140-142], high fat feeding[123, 127], creatine [143] or carnitine [144] in animal models of LMN degeneration might well be due to an action of these treatments on skeletal muscle rather than on motor neurons. Such an indirect effect is suggested by the profound effects of exercise on genes involved in NMJ maintenance [145].

## **7) Muscle as a delivery route to cure motor neurons in lower motor neuron diseases**

While skeletal muscle is involved in at least some of the key pathogenic events in LMN degeneration, one could also consider to use muscle as a way to target motor neurons (figure 6). For instance, local production of neurotrophic factors by the skeletal muscle might sustain neuronal survival and enhance muscle reinnervation. Such an example was provided by the study of Li and collaborators [146] showing that muscle production of GDNF, but not astrocyte derived-GDNF delayed disease onset and slowed down disease progression of mSOD1 mice.

In the same line, retrograde transport of a therapeutic gene through muscle injections of a virus could provide protection to motor neurons. Two different examples provide the proof of concept for such a strategy. First, delivery of IGF1 through AAV vectors in skeletal muscles leads to protection of motor neurons and delayed disease onset. This protective effect might be due to retrograde transport of viral particles in the axon, leading to IGF1 production in motor neurons and autocrine protection of these cells. Alternatively, and as suggested by A. Musaro and colleagues, IGF1 has profound local effects on skeletal muscle that might account for its potential for therapeutics in ALS [138]. A second example of a therapeutic strategy aiming at delivering a neurotrophic factor through skeletal muscle is VEGF. M. Azzouz and collaborators used a lentiviral vector to provide VEGF directly to motor neurons through retrograde transport. They observed that a single injection of a VEGF-expressing lentiviral vector into various muscles delayed onset and slowed progression of in mSOD1 mice [147]. Interestingly, either viral delivery of VEGF or IGF1 slowed disease progression even when treatment was initiated at the onset of disease, suggesting that these strategies might be relevant in ALS patients.

Viral delivery might prove useful not only for providing a neuroprotective factor, but also to restore the causative deficient gene. Indeed, multiple muscular injections of a lentiviral vector expressing SMN restored SMN expression in motor neurons and delayed the pathology of severe SMA mice [148]. Conversely, similar viral strategies might be used to silence the toxic gene when gain of function of the mutant protein is shown to lead to the pathogenic effects. In this line, targeting of SOD1 mutations through siRNA has been achieved by Ralph and collaborators in mSOD1 mice [149] through

muscle injections of viral vectors retrogradely transported to motor neurons. This mode of delivery achieved what remains probably the most impressive protective effect in mSOD1 mice. Thus, muscle might not only be a tissue to target, but also a route to deliver drugs or treatments.

## **8) Conclusion**

As a conclusion, when considering all the data discussed above, one may hypothesize that LMN degeneration may be “synaptopathies” rather than « motor neuron diseases ». As such, one should focus on designing treatments to strengthen and stabilize the remaining NMJs and/or to stimulate the generation of newly formed NMJs. To our knowledge, no treatment has been specifically designed to target NMJs in motor neuron diseases until now. It may worth to consider that a drug or treatment targeting the common final pathway of diseases with LMN degeneration i.e. the NMJ, would be equally beneficial for ALS, SMA and SBMA patients.

Lastly, it should be kept in mind that while skeletal muscle is a much more important actor in LMN degeneration than previously anticipated, these affections target motor neurons and involve other cell types, i.e. astrocytes, schwann cells and microglia[75, 150-157]. An efficient therapy should take all these cellular actors into account.

## **Figure and legends to figures :**

### **Figure 1 : electrophysiological and muscle pathological features of LMN degeneration in skeletal muscle**

A. Electrophysiological (EMG) recording of a patient with ALS demonstrating fibrillation potentials at rest, *i.e.* spontaneous denervation-related muscle activity.

B-D. Muscle biopsy of a patient with ALS. All the features associated with neurogenic disorders, *i.e.* grouping of atrophic fibers (B,C), predominance of one type of fiber (D), and presence of angulated fibers (D), are observed.

(B: semi-thin section, X 250; C: H-E staining, X 200; D: NADH-TR staining, X 100).

### **Figure 2 : potential mechanisms involving skeletal muscle in SMA.**

Loss of SMN1 leads to two consequences in skeletal muscle. First, SMN1 loss leads to abnormalities in sarcomere structure, which are a likely cause of muscle weakness. Second, SMN1 loss decreases the potential of muscle to produce mature AchR subunits, leading to an abnormal development of NMJs. SMN1 loss in motor neurons has also profound effects on NMJ development. See text (section 3) for further details.

### **Figure 3 : potential mechanisms involving skeletal muscle in SBMA.**

The mutant AR toxicity is unmasked by testosterone. This leads to myopathic features, such as myotonic discharges and elevation of blood CK, contributing to muscle weakness and lethality. On the other hand, mutant AR toxicity (or wild type AR overexpression) dismantles NMJs through yet unknown mechanisms. A pathogenic function of mutant AR in motor neurons has also been documented. See text (section 4) for further details.

### **Figure 4 : potential mechanisms involving skeletal muscle in SOD1-linked ALS**

Mutant SOD1 expression in skeletal muscle leads to oxidative stress, muscle atrophy and weakness. mSOD1 mice skeletal muscles are also hypermetabolic but whether mSOD1 expression in muscle or in other cell types is responsible of this phenotype is unknown. Muscle hypermetabolism is sufficient to drive NMJ destruction and systemic energy deficit. mSOD1 expression in both motor neurons and glial cells is also involved in the overall ALS phenotype of mSOD1 mice. See text (section 5) for further details.

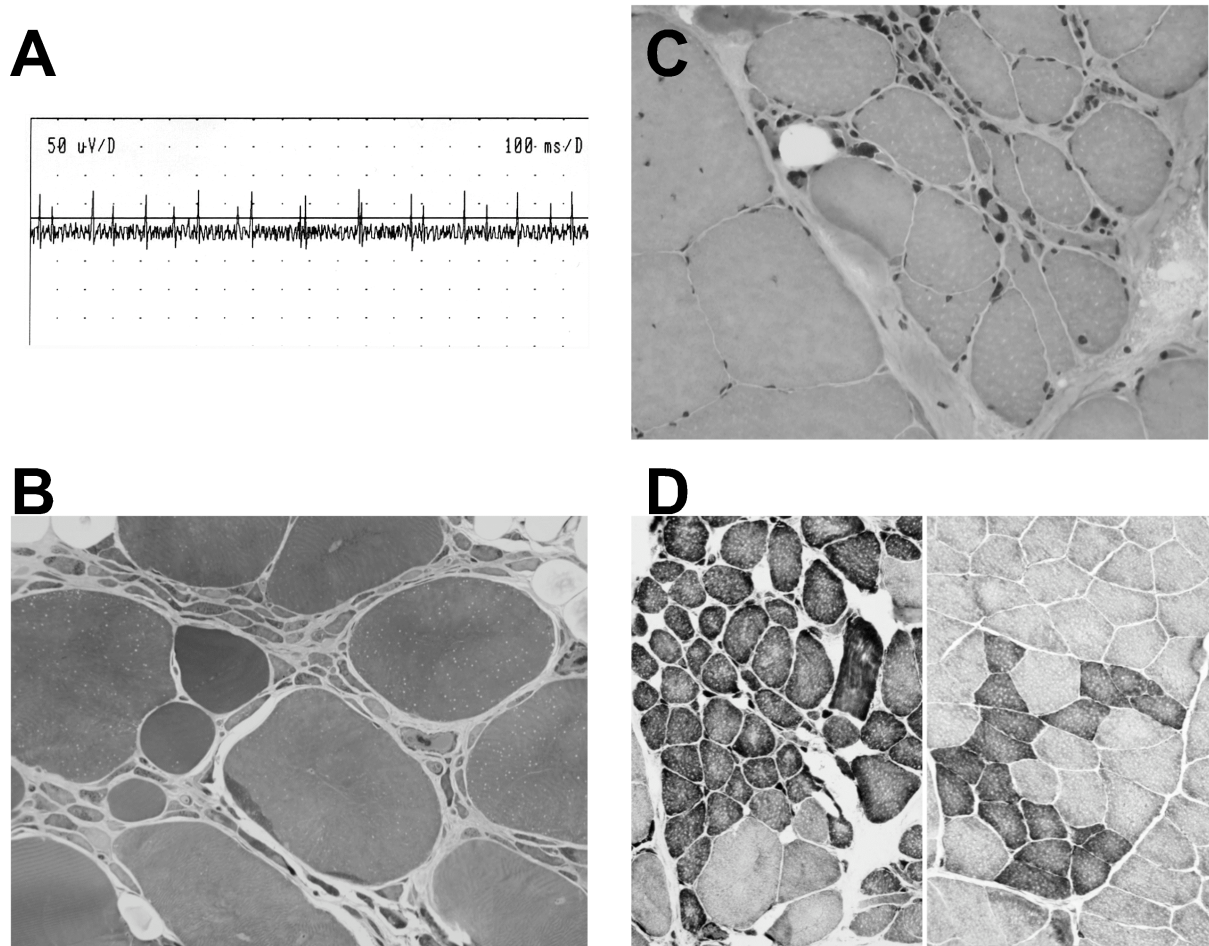
Figure 5 : targeting skeletal muscle in LMN degeneration

A potential therapeutic treatment for LMN degeneration might target deleterious processes occurring in muscle itself.

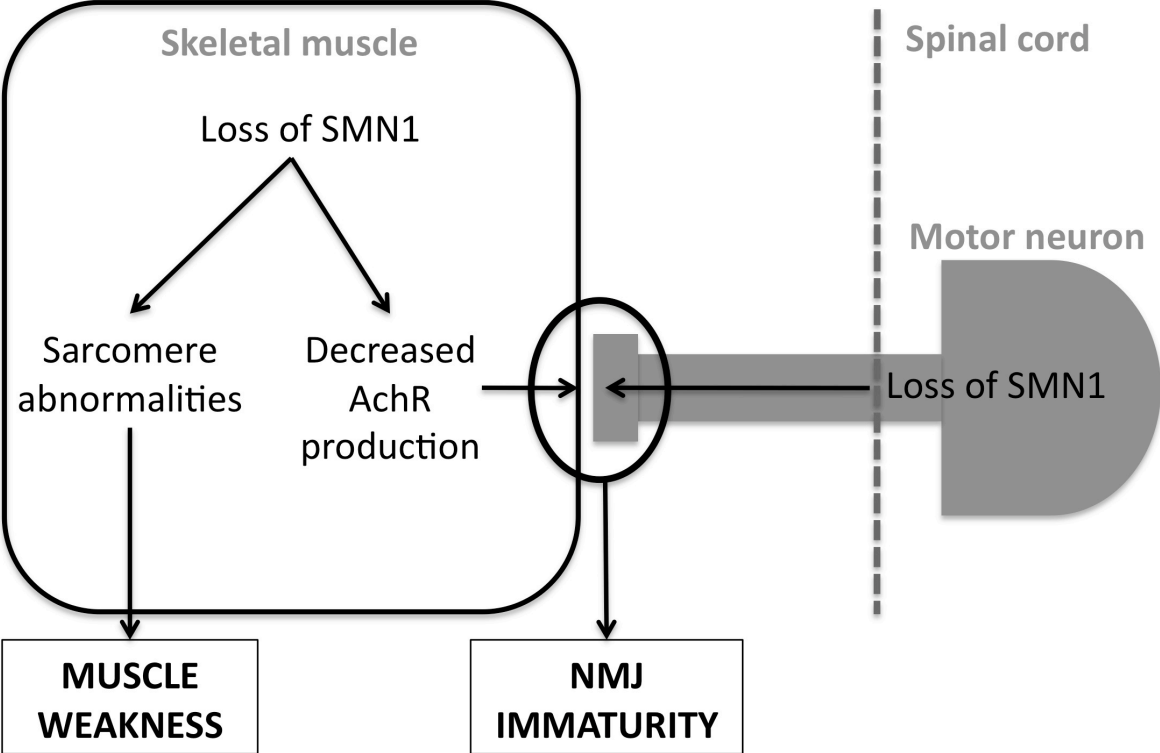
Figure 6 : skeletal muscle as a delivery route in LMN degeneration.

Skeletal muscle is also a privileged route to deliver drugs targeting motor neurons through retrograde transport.

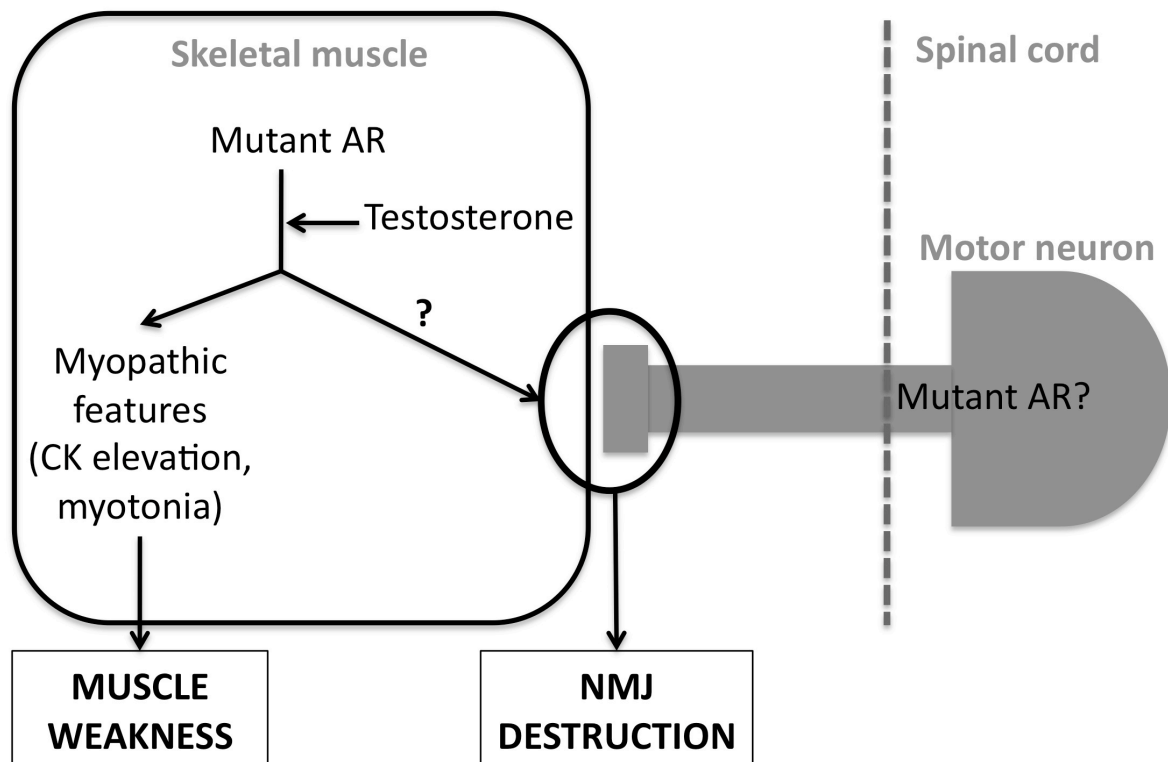
Figure 1 : electrophysiological and muscle pathological features of LMN degeneration in skeletal muscle



**Figure 2: pathogenic roles of skeletal muscle in SMA**

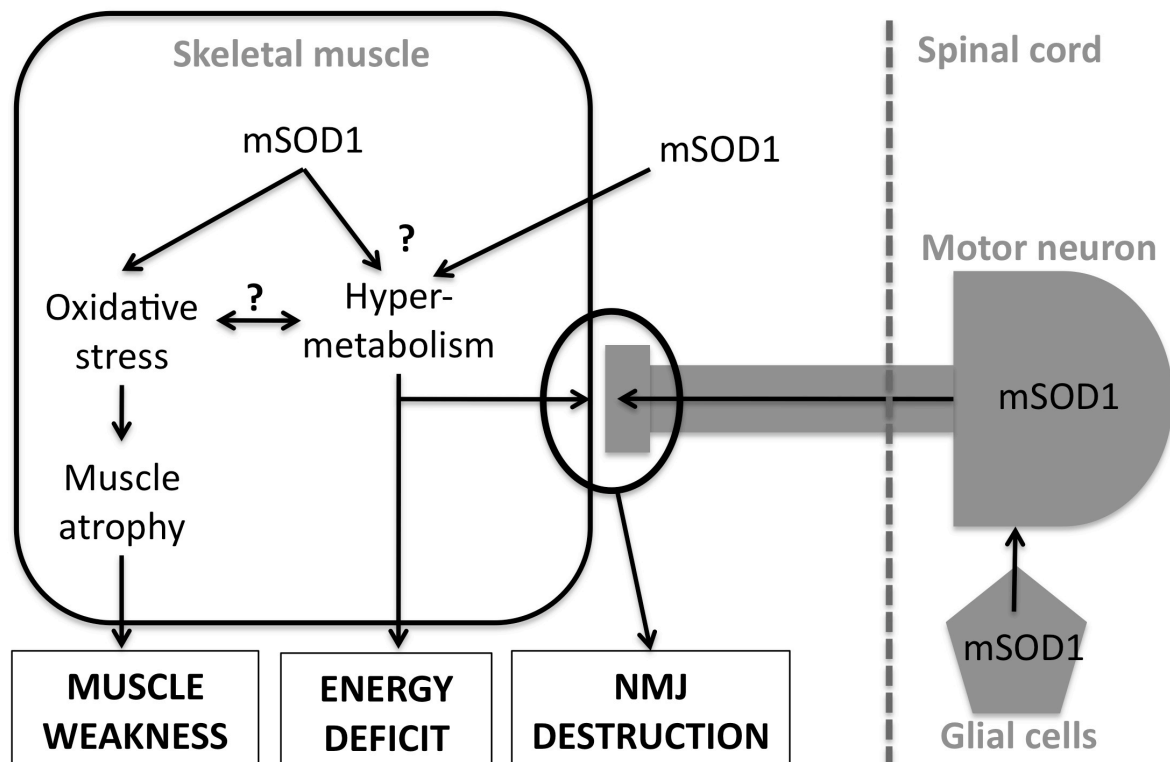


**Figure 3: pathogenic roles of skeletal muscle in SBMA**

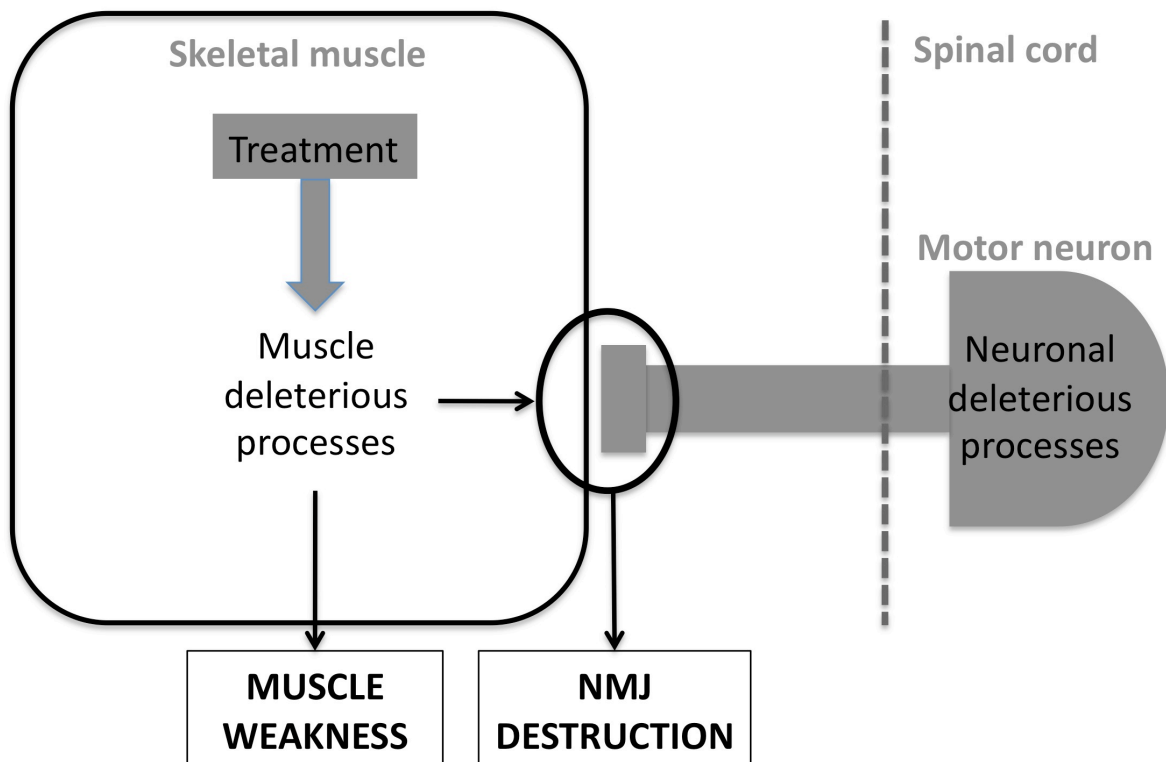




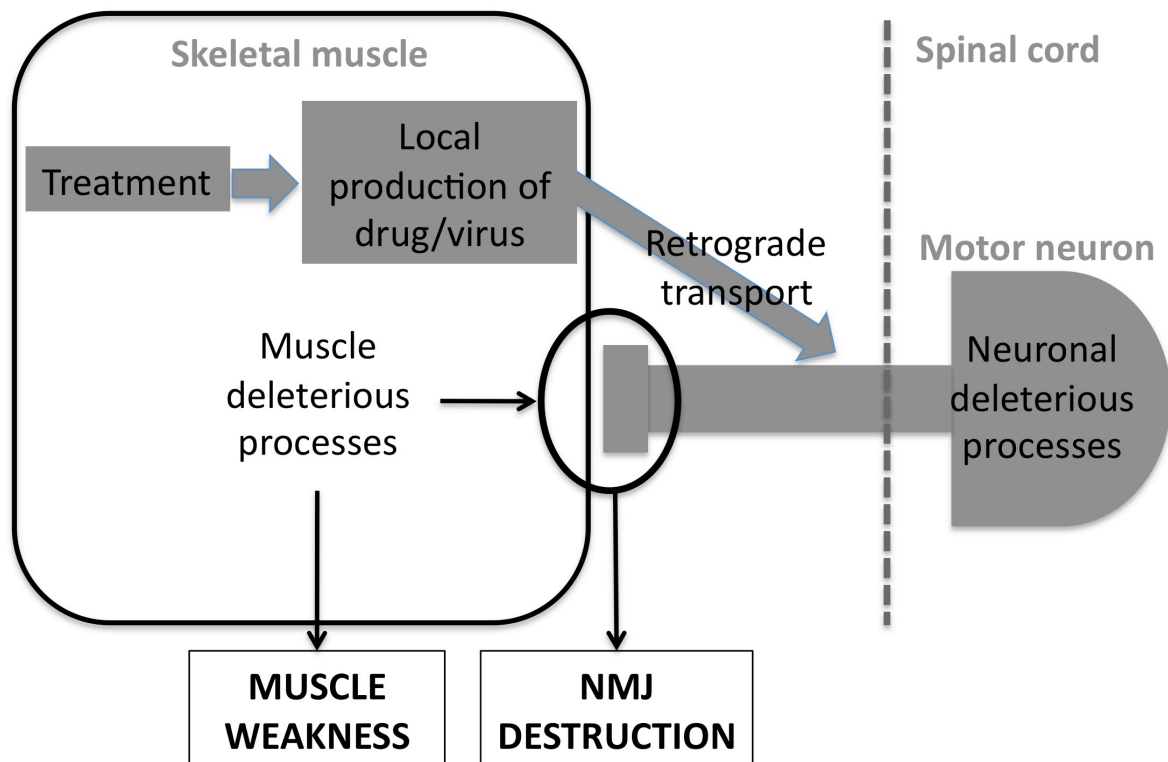
**Figure 4: pathogenic roles of skeletal muscle in ALS**



**Figure 5: targeting skeletal muscle in LMN degeneration**



**Figure 6: skeletal muscle as a delivery route in LMN degeneration**



**Table 1 :clinical presentation of diseases with LMN degeneration**

disease	onset	Cause	LMN involvement	UMN involvement	Myopathic features
<b>SMA</b>					
type I	birth	Loss of SMN1	+	-	+
type II	Before 18 mo	Loss of SMN1	+	-	+
type III	After 18 mo	Loss of SMN1	+	-	++
<b>SBMA</b>					
SBMA	Adult-onset	Expansion of the polyglutamine tract of AR	+	-	+++
<b>ALS</b>					
sALS	Adult-onset	Unknown (sporadic)	+	+	Occasionally reported
fALS	Adult onset	Genetic (multiple loci : sod1, tdp-43, fus, vapp...)	+	+	Occasionally reported

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