Chapter

The Extraocular Muscles Are Selectively Spared in ALS

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Abstract

The extraocular muscles differ from other skeletal muscles in many respects but most strikingly in their response to neuromuscular diseases expected to affect the whole body. Oculomotor disturbances are not typical features of ALS. Recent data ascribe the muscle tissue an important role in the pathophysiology of ALS, with early involvement of the neuromuscular junctions and loss of axonal contact. We show that the extraocular muscles of terminal ALS donors and also of mice models of ALS maintain their morphology and well-preserved neuromuscular junctions until the end stages of the disease, whereas the limb muscles are severely affected and their neuromuscular junctions start losing contact with the supplying axons early in the course of ALS. There are intrinsic differences between the extraocular and limb muscles with respect to neurotrophic factors and Wnt isoforms and fundamental differences in their response to ALS that cannot be explained by the aging process. We propose that these differences may be instrumental in the selective sparing of the extraocular muscles in ALS.

Keywords: extraocular muscle, ALS, pathophysiology, neurotrophic factor, neuromuscular junction, Wnt, BDNF, GDNF, NT-3, NT-4, S-100B, synaptophysin, p75, neurofilament, Wnt1, Wnt3a, Wnt5a, Wnt7

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a late-onset progressive neurodegenerative disorder affecting both the upper and lower motor neurons. It is characterized by increasing muscle weakness, paresis, and paralysis, and although the course of the disease may vary considerably, death usually occurs within 3–5 years due to respiratory failure. While almost all skeletal muscles, including the cranially innervated muscles responsible for speech and swallowing, are affected in ALS, oculomotor disturbances are not dominant or typical features of this disease, not even in patients with long-duration ALS, and eye movements may remain the only form of communication available to these patients at the end stages of the disease. The cranial nerve nuclei supplying the eye muscles appear rather normal in terminal ALS patients [1], and abnormalities in eye motility in ALS patients, when present, have been ascribed to supranuclear deficits [2].

The pathophysiology of the neurodegenerative process in ALS has been proposed to be due to a number of different causes. The traditional point of view has been that ALS is a motor neuron disease that progresses to the periphery where it affects the muscles, due to the typical loss of motor neurons in the anterior horns of the spinal cord and sclerosis of the lateral corticospinal tracts. However, a number

of studies suggest an inverse course of events, with changes starting at the periphery and progressing along the motor neurons toward the CNS, reviewed by [3–5]. In SOD1G93A transgenic mice, it has been shown that peripheral denervation occurs in the limb muscles before the animals become clinically weak or motor neuron loss is detected [6]. Similar results were also reported for a patient with ALS who died unexpectedly, leading the authors to propose that motor neuron pathology in ALS begins at the distal axon end and proceeds in a "dying back" pattern [6]. Later, it has been shown that muscle-restricted expression of three different SOD1 gene variants can initiate the non-autonomous degeneration of motor neurons typical of ALS [7], truly showing that the muscle itself may be a major player in the early stages of ALS.

A unifying model compiling extensive experimental data from SOD1 transgenic models of ALS [3] proposes that although motor neuron degeneration and death are the primary causes of the progressive paralysis, neighboring nonneuronal cells are also involved in the toxicity and damage development. In this model, the earliest event is the retraction of motor axons from their muscle synapses, before any symptoms of the disease are present.

The extraocular muscles (EOMs) are unique in many respects and are considered a separate muscle class, allotype. They are both the fastest and the most fatigue-resistant muscles in the body, and their fiber-type composition is very complex [8, 9]. The unique properties of the EOMs are reflected in their expression profile that differs from that of limb muscle in over 300 genes [10]. However, the most striking property of EOMs is their varied behavior in disease. The EOMs are known to be selectively involved in certain diseases like mitochondrial myopathies, myasthenia gravis, and Miller Fisher syndrome, but preferentially spared in other devastating diseases like Duchenne muscular dystrophy and merosin-deficient muscular dystrophy, that affect all other muscles in the body [11–13].

The extraocular muscles are richly innervated, and their motor units are very small, with 7–25 muscle fibers, in contrast to the motor units of limb muscles, which typically contain hundreds of muscle fibers. The majority of the nerve fibers in the EOMs are large and myelinated and innervate a typical single "en plaque" endplate in the middle portion of each muscle fiber. The remaining 15–20% of the EOM nerve fibers are small and innervate many small, "en grappe" motor endplates along the length of a muscle fiber (the so-called multiply innervated muscle fibers, reviewed in [11]). This traditional description of the innervation of the EOMs has been significantly changed by the recent report of a subpopulation of muscle fibers that exhibit multiple large "en plaque" nerve endings [14]. The NMJs of the EOMs also differ from limb muscle in retaining expression of the fetal gamma subunit of the acetylcholine receptor (AChR), in addition to the adult epsilon subunit, typically present in mature motor endplates [14, 15]. We have shown that the NMJs of the human EOMs have a different immunoreactivity pattern than that seen in limb muscles for antibodies against gangliosides GQ1b, GT1a, and GD1b [16]. Gangliosides are a large family of sialylated glycosphingolipids highly enriched in neuronal and glial plasma membranes and important for the development, function, and maintenance of the nervous system. Complex gangliosides have neurotrophic factor-like activity and may regulate the expression levels of neurotrophic factors and their receptors [17]. Notably, there have been at least two case reports of ALS associated with antibodies against gangliosides [18, 19].

Given that the EOMs differ significantly from the limb and trunk muscles with respect to their response to disease and given the more recent view that the muscle itself may play a key role in the pathophysiology of ALS, we first investigated whether there are any major differences between the EOMs and the limb muscles of terminal ALS donors [20]. Thereafter, we investigated neuromuscular

junction integrity [21–23], neurotrophic factors (BDNF, NT-3, NT-4, GDNF) and their receptors (p75NTR, TrkB, TrkC) [24, 25], and relevant Wnt isoforms (Wnt1, Wnt3a, Wnt5a, Wnt7a) [26], as possible important puzzle pieces to better understand the pathophysiology of ALS and the selective sparing of the EOMs in the disease.

2. The extraocular muscles of terminal ALS donors are remarkably well preserved

We compared the general morphology, fiber-type content, fiber area and myosin heavy chain (MyHC) composition of EOM, biceps brachii, vastus lateralis, and tibialis anterior muscle samples collected from eight terminal ALS donors (age 58–80 years) and from three age-matched (age 72–86 years) and a younger (age 26 years) control, with ethical approval, using immunohistochemistry and SDS-PAGE [20]. There were five cases of sporadic ALS and three cases of familial ALS. The MyHC isoforms are the major determinants of key contractile and biochemical characteristics of the myofibers including shortening velocity and power and therefore very good markers of the physiological properties of myofibers. Our study showed that the morphology of the ALS limb muscle samples is clearly pathological. All limb muscle samples from terminal ALS donors exhibit typical signs of myofiber type grouping, myofiber splitting, necrosis, increased connective tissue, fatty replacement, and ongoing regeneration, with a very wide range of myofiber areas. In contrast, the EOM samples from the same donors show remarkably well-preserved morphology. In 18 out of the 43 EOM samples examined, larger variation in fiber area was apparent than in the control samples and irrespectively of being sporadic or familial ALS cases. In fact, areas that appear well preserved reveal a general atrophy of all myofiber types, whereas areas that appear more affected generally show atrophy of the myofibers containing MyHCslow and hypertrophy of the myofibers containing MyHCfast2A. In 20 EOM samples, increased connective tissue amounts were noted, either around individual myofibers or around groups of myofibers. The changes noticed vary among donors and among different EOM samples from a given donor. Centrally placed nuclei are rarely detected. The MyHC content, determined with SDS-PAGE of whole EOM extracts, varies among ALS donors, whereas it is rather similar among control EOM samples [20]. This variation in MyHC content at the whole muscle level can be interpreted as reflecting different levels of involvement, and, to a lesser degree, naturally occurring variation among subjects [8].

Given that this first study clearly shows that the human EOMs are remarkably well preserved at the end stage of ALS [20] and because the EOMs are known to show a divergent behavior in disease, we decided to further investigate these muscles in ALS, comparing the EOMs and limb muscle samples from human donors and from mouse models of ALS, taking a "muscle perspective" to study the pathophysiology of the disease.

3. Maintained neuromuscular junction occupancy in the extraocular muscles at end stages of ALS

In the SOD1G93A mouse, the most widely used mouse model of ALS, it has been shown that loss of contact between the axons and the myofibers, at the neuromuscular junctions, occurs early in the course of the disease and indeed precedes detection of loss of motor neurons in the spinal cord [6]. Similar findings have been

reported in ALS patients who died of other reasons early in the course of ALS [6]. Furthermore, data show that muscle-specific alterations are sufficient to trigger neuromuscular pathology and distal degeneration of motor neurons [5, 7], suggesting that the muscle itself may play an important role in the pathophysiology of ALS.

We investigated [21] whether there are signs of loss of contact between the axons and the myofibers, at the neuromuscular junctions of the EOMs, extensor digitorum longus, tibialis anterior, and gastrocnemius and soleus muscles from seven SOD1G93A transgenic and six control mice. We used triple labeling immunohistochemistry with antibodies against neurofilament protein and synaptophysin to identify the axonal side of the neuromuscular junctions and their axons and alpha-bungarotoxin, to identify the muscle side of the neuromuscular junctions, in the same muscle section. Our study confirmed that, as described above for the human EOMs of terminal ALS donors, the eye muscles in this animal model of ALS also show rather well-preserved morphological features, in clear contrast to the limb muscles of the same animal, at the end stage of the disease. We could confirm previously reported findings [6] of significant loss of contact between the nerve and myofiber in approximately 33% of the neuromuscular junctions in limb muscles of the ALS mouse model, whereas in the limb muscles of the control animals, only approximately 4.6% of the neuromuscular junctions lack contact with the supplying nerve ($p = 0.0071$). In contrast, the number of neuromuscular junctions lacking nerve contacts in the EOMs is extremely low and similar between transgenic (1.9%) and control (1.7%) animals ($p = 0.659$) [21].

Similarly, the neuromuscular junctions of the EOMs of terminal ALS donors show maintenance of their integrity [22, 23]. In a study of 7 terminal ALS donors (age 58–80 years, three cases with SOD1D90A genotype) and 10 controls (adults aged 34–53 years and elderly aged 69–83 years), we compared the EOMs and the limb muscles with respect to the presence and distribution of neurofilament light subunit (marker of the axons in nerves and at the neuromuscular junction), synaptophysin (marker of synaptic vesicles and thereby of functioning neuromuscular junctions), S100B, $p75^{NTR}$ and glial fibrillary acidic protein (GFAP) which are markers of terminal Schwann cells at the neuromuscular junction, and alpha-bungarotoxin, which labels the muscle side of the synapse [23]. GFAP and $p75^{NTR}$ are upregulated in Schwann cells after injury [27–29]. Two groups of control donors (adult and elderly) were included in this and all our studies of ALS in order to distinguish between the effects of normal aging and those related to ALS. This pioneer study showed that there is a dramatic decrease in the number of neuromuscular junctions and nerve axons containing neurofilament light subunit in limb muscles of terminal ALS donors, whereas the EOMs maintain neurofilament light subunit in their neuromuscular junctions and nerves. There is also a significant decrease in neuromuscular junctions labeled with synaptophysin in the limb muscles but not in the EOMs of terminal ALS donors. Together, these results confirm that there is a marked loss of nerve-muscle contacts in the limb muscles of terminal ALS donors, whereas the EOMs succeed in maintaining their neuromuscular junctions until the end stage of the disease. The neuromuscular junctions of limb muscles show also massive loss of S100B and $p75^{NTR}$ in ALS, whereas the staining pattern of GAFP is maintained. In contrast, the neuromuscular junctions in the EOMs of terminal ALS donors also show loss of S100B but have an unchanged pattern regarding the other two Schwann cell markers, GFAP and $p75^{NTR}$. The loss of S100B can be interpreted as due to downregulation rather than a sign of loss of Schwann cells, given that the other two markers remain unchanged. It may also indicate a disturbed calcium homeostasis as an early step toward the loss of the neuromuscular junction in ALS. In summary, this study shows fundamental differences between the limb muscles and the EOMs of terminal ALS donors with a significant impact of the

disease on important synaptic proteins normally present in the motor axons and terminal Schwann cells in the limb muscles, whereas the neuromuscular junctions in the EOMs are only very mildly affected [23].

Another study has in addition revealed fragmentation of the postsynaptic membrane, decreased density of acetylcholine receptors, and absence of nerve sprouting in denervated neuromuscular junctions in the limb muscles of the SOD1G93A mouse model of ALS, whereas no such changes are present in the neuromuscular junctions of the EOMs of the same animals [30].

In the context of motor axon retraction from the muscle synapse being an early event in the pathogenesis of ALS, it is of particular interest to further investigate the cellular and molecular microenvironment of the neuromuscular junctions of the extraocular muscles, as they may provide cues to the unique resistance of the EOMs to ALS.

4. Important differences in neurotrophic factors between extraocular and limb muscles

Alterations in the expression of target-derived neurotrophic factors and in their signaling pathways have been reported in ALS. Neurotrophic factors are important for survival and maintenance of neurons [31–33]. Target-derived neurotrophic factors can be transported to lower motor neurons from other cells, including the muscle cells, by retrograde transport, and from upper motor neurons, by anterograde transport, and act as signaling molecules [31–33]. Among neurotrophic factors, brain-derived neurotrophic factor (BDNF) is of particular interest due to its capacity to promote motor neuron survival and motor axon growth [32–34]. Glial cell line-derived neurotrophic factor (GDNF) is also relevant as it is capable of rescuing motor neurons from injury-induced cell death [35], and neurothrophin-4 (NT-4) plays a role in growth and remodulation of adult motor neurons [36, 37], whereas NT-3 is of particular importance for sensory neurons.

Data on the SOD1G93A mouse model of ALS show important differences between the EOMs and the limb muscles over time regarding the levels of expression of neurotrophic factors [24]. The authors compared the expression levels of BDNF, GDNF, NT-3, and NT-4, determined with quantitative RT-PCR, in limb (soleus and gastrocnemius) and extraocular muscles at the early stage (50 days) and at the end stage (150 days) of the disease. Starting with the control animals, the EOMs differ from the limb muscles by having significantly lower levels of BDNF and NT-3 mRNA at age 50 days. At 150 days, the control EOMs have significantly lower levels of BDNF and NT-4 mRNA than the limb muscles from the same animals. In the limb muscles of control animals, mRNA levels of BDNF increase significantly from 50 to 150 days of age, whereas the levels of NT-3 decrease dramatically. In the control EOMs, the mRNA levels of BDNF, GDNF, NT-3, and NT-4 remain unchanged from 50 to 150 days of age [24].

In the transgenic animals, the expression levels of BDNF increase significantly from 50 to 150 days, that is, from the early stage of ALS, when the limb muscle morphology is still normal, to the end stage of the disease, when the limb muscles are extremely affected. Compared to the controls, the limb muscles of the transgenic animals show significantly lower levels of NT-4 at 50 days and significantly higher levels of GDNF at 150 days. The transgenic EOMs show no change in the levels of expression of BDNF or GDNF and show significant decrease in NT-3 and increase in NT-4, over time between 50 and 150 days, and the morphology is comparable to that of the age-matched controls. Compared to the controls, the EOMs of the G93A animals show significantly higher levels of GDNF and NT-3 at 50 days, whereas

no significant differences are detectable at 150 days. Finally, comparison between the EOMs and limb muscles of transgenic animals shows no significant differences in the levels of expression of any of the four neurotrophic factors at 50 days but significantly higher levels of BDNF in the limb muscles at 150 days [24].

Because no significant changes in the levels of expression of BDNF detected with quantitative RT-PCR are apparent in either the limb or EOMs of the transgenic animals at any stage, the authors dismiss a possible role for this neurotrophic factors on ALS. The BDNF results obtained in the SOD1G93A mice are in agreement with a previous report from muscle samples of ALS patients [38]. In contrast, the GDNF mRNA levels are significantly upregulated in the EOMs of the SOD1G93A mice at 50 days, and the authors suggest that this early upregulation is triggered by the disease and seminal for the maintenance of the general morphology of the EOMs and in particular for their capacity of keeping intact neuromuscular junctions. They interpret the upregulation of GDNF in the end stage in the limb muscles as reflecting the advanced motor neuron degeneration. Similarly, the upregulation of NT-3 in the EOMs detected at 50 days is also proposed to be triggered by ALS and to protect the EOMs, whereas early downregulation of NT-4 in the limb muscles is suggested to be detrimental and contribute to the loss of axonal contact at the neuromuscular junctions [24].

At the cellular level, there are also important differences in the patterns of distribution of neurotrophic factors between the EOMs and limb muscles, both among controls and among SOD1G93A animals at 50 and 150 days [25]. In the limb muscles of control animals, GDNF, BDNF, NT-3, and NT-4 are present in the vast majority of the neuromuscular junctions at 50 and 150 days, whereas that is the case in only a little over half of the neuromuscular junctions in the control EOMs, at 50 and 150 days. The proportion of neuromuscular junctions containing BDNF and NT-4 is significantly higher in the limb muscles than in the EOMs of control animals at 50 days, and the same is true for the proportion of neuromuscular junctions containing BDNF and GDNF at 150 days. Particularly noteworthy is that the limb muscles of SOD1G93A animals show a significant decrease in the proportion of neuromuscular junctions containing BDNF, GDNF, and NT-4 at 150 days, whereas the EOMs maintain their pattern. The same is true for the presence of the receptors $p75^{NTR}$, TrkB, and TrkC, which decline significantly in the neuromuscular junctions of the limb muscles of the SOD1G93A animals at 150 days, but not in their EOMs. TrkB is a high-affinity tyrosine kinase receptor specific for BDNF and NT-4, TrkC is a high-affinity tyrosine kinase receptor specific for NT-3, and $p75^{NTR}$ is a low-affinity receptor for BDNF, NT-3, and NT-4 [39]. There are only discrete differences between the control and the SOD1G93A animals with respect to patterns of neurotrophic factors in the nerve axons and the myofibers of both EOM and limb muscles [25].

Taken together, these studies [24, 25] clearly show both at the mRNA level and immunohistochemically that the EOMs have lower neurotrophic factor levels than the limb muscles, in control mice. One possible explanation resides in the differences in innervation between these muscles, as the EOMs have a rich population of multiply innervated myofibers, in contrast to the exclusively twitch myofibers of limb muscles, and the developmental fates of these different types of axons are dependent upon target-derived neurotrophic factors [40]. They also show that the significant changes in neurotrophic factors in limb muscles of transgenic animals are not related to normal aging.

Studies of neurotrophic factors in muscle tissue pose a technical challenge and clinical trials in ALS have not led to the desired effects, but the early detection of alterations in the levels of expression of neurotrophic factors and the distinct patterns observed in the EOMs versus limb muscles indicate that it is too early to

dismiss their possible roles in the pathophysiology of ALS and in particular in the selective capacity of the EOMs to maintain neuromuscular junctions and remain rather unaffected in the disease.

5. Differences in Wnt profiles between extraocular and limb muscles

Several Wnt proteins, including Wnt1, Wnt3a, Wnt 5a, and Wnt7a, are highly expressed at the neuromuscular junction, in muscle fibers, and in motor neurons, and abnormal Wnt signaling has been reported in ALS and a number of other neuromuscular conditions [41–46]. In particular, Wnt1 has been assigned important roles in muscle regeneration and in synaptic plasticity, acting on both sides of the synapse [41, 47]. Wnt3a modulates the formation of neuromuscular junctions and promotes nerve outgrowth [48–50], whereas Wnt5a is important for motor neuron survival during development [51]. Wnt7a plays an important role in synaptic assembly and plasticity and remodeling of incoming axons [52] and has also been shown to have positive effects on the myofibers [53, 54]. Furthermore, Riluzole, the only medical treatment available for ALS, enhances Wnt/beta catenin signaling.

We have performed a comprehensive immunohistochemical study comparing the distribution of Wnt1, Wnt3a, Wnt5a, and Wnt7a in the EOMs and limb muscle samples (biceps brachii, vastus lateralis, and tibialis anterior) from six terminal ALS donors, younger controls (mean age 41 years for EOMs and 33 years for limb muscles, referred to as adult controls), and older controls (mean age 75 years for EOM and 76 years for limb, referred to as elderly controls), and we have reported significant differences and suggested a role for Wnts in the different behaviors of EOMs and limb muscles in ALS [26].

In the adult control EOMs, roughly 71% of the axons are Wnt1 positive, and in the elderly controls, merely 12% of the axons contain Wnt1, whereas in the EOMs of terminal ALS donors, 43% of axons are labeled for Wnt1, clearly showing that they preferentially maintain Wnt1 in their nerves. The limb muscles of terminal ALS donors contain significantly less Wnt1-positive axons than the EOMs, with extremely high variation among the different donors. No statistically significant differences between the three limb muscle groups are detectable regarding the presence of Wnt1 in the motor nerves. Wnt1 is present in almost 40% of the myofibers in the EOMs of adult controls and almost in all myofibers of terminal ALS donors, whereas it is not present in limb myofibers of adult controls or terminal ALS donors.

With regard to Wnt3a, it is present in the vast majority of axons in both the EOMs of adult controls and ALS terminal donors, but again it is significantly lower in the elderly control EOMs. In the limb muscles of all groups, the percentage of axons containing Wnt3a is significantly lower than in the EOMs, with approximately 75% fewer Wnt3a axons. Approximately 17% of the myofibers contain Wnt3a in adult control EOM, whereas almost all myofibers in the EOMs of terminal ALS donors are positive. Higher levels of Wnt3a are present in adult control limb myofibers, but in contrast Wnt3a is practically absent in the limb muscle fibers of terminal ALS donors.

Wnt5a is present in practically all EOM axons both in adult controls and terminal ALS donors and is somewhat lower in the EOMs of elderly controls. Similarly, practically all limb muscle axons, irrespective of group, are Wnt5a positive. Wnt5a, seen as weak immunostaining, is present in almost all myofibers of control adult EOMs and terminal ALS donors, whereas it is not present in limb myofibers of adult controls or terminal ALS donors.

A very large proportion of the axons in the EOMs of adult controls and terminal ALS donors are Wnt7a positive, whereas a significantly lower number is found in the EOMs of the elderly controls. A similar proportion of Wnt7a-positive axons

are present in the limb muscles of both adult controls and terminal ALS donors, but a significantly higher number of positive axons are found in the limb muscles of elderly controls. Only approximately 20% of the myofibers in the adult control EOMs contain Wnt7a, but in the EOMs of terminal ALS donors, this number increases significantly almost 60%. In the limb muscles, Wnt7a is present in more than half of the myofibers of the adult controls and practically in all myofibers in the elderly controls. The differences found between adult controls and terminal ALS donors regarding Wnt7a in limb myofibers are not significant, probably due to large interindividual variation among the donors.

Data collected in controls and in the SOD1G93A mouse model of ALS [26] indicates that practically all neuromuscular junctions in control EOMs and limb muscles express Wnt1, Wnt3a, Wnt5a, and Wnt7 at 50 and 150 days. The same is true for the transgenic EOMs at both 50 and 150 days and the transgenic limb muscles at 50 days, whereas a significantly decrease in the proportion of neuromuscular junctions co-expressing the different Wnt isoforms is clearly apparent in the transgenic limb samples at 150 days.

6. Summary

In summary, the EOMs remain remarkably well preserved until the end stages of ALS, both in human terminal donors and in mouse models of ALS. They maintain the composition of their neuromuscular junctions and do not loose contact with the supplying axon. Furthermore, important differences exist between the EOMs and the limb muscles with respect to neurotrophic factors and Wnts:

- The mRNA levels of BDNF, NT-4, and NT-3 and the detection of BDNF, NT-4, NT-3, and GDNF at the protein level are lower in the EOMs than in the limb muscles of control animals at 50 and/or 150 days.

- NT-3 is upregulated in the EOMs of SOD1G93A mice at 50 days and may be protective. A similar difference in GDNF may also be advantageous for the EOMs and protect their motor neurons.

- There is early downregulation of NT-4 in the limb muscles of SOD1G93A mice compared to the controls at the same age, which coincides with the timing of early loss of motor axon occupancy at the neuromuscular junctions of limb muscles in this ALS model [6].

- The proportion of neuromuscular junctions in the EOMs positive for BDNF, GDNF, and NT-4 is not changed between the controls and the transgenic mice at 150 days, whereas there is a significant decrease in the limb muscles of the SOD1G93A animals compared to the controls at late stage and there is also a significant decrease over the course of the disease in the limb muscles of the transgenic mice. Again, these changes in neurotrophic factors parallel the loss of axonal occupancy at the neuromuscular junctions of the limb muscles of transgenic animals and these neurotrophic factors are known to play crucial roles in regulating the synapses [55, 56].

- There is a significant decrease in the neurotrophic factor receptors p75NTR, TrkB, TrkC, and GFR-alpha1 in the neuromuscular junctions of terminal SOD1G93A mice, whereas they are maintained in the neuromuscular junctions of terminal transgenic EOMs.

- Wnt1 and Wnt3a are present in a larger proportion of axons in the human control EOMs than in the limb muscles. The myofibers in the control EOMs contain higher Wnt1 and Wnt5a and lower Wnt3a and Wnt7a than the myofibers of limb muscles, and although the exact implications of these intrinsic differences in Wnt isoform profiles remain to be determined, they may be relevant for the selective sparing of the EOMs in ALS.

- Wnt1 and Wnt3a are present at significantly higher levels in the axons and the muscle fibers of the EOMs than in the axons or muscle fibers of the limb muscles of terminal ALS donors. Because Wnt1 plays a role in synaptic plasticity and muscle regeneration and in neuromuscular junction formation during fetal development and Wnt3a has similar roles, these differences between EOMs and limb muscles are likely to be of importance for the selective sparing of the EOMs, and in particular of their neuromuscular junctions, in ALS.

Altogether, these data show important differences between the EOMs and limb muscles with regard to neurotrophic factors and four different Wnt isoforms, both at base line and in ALS, that likely play a role in the selective sparing of the EOMs in ALS. These data also suggest that muscle itself may provide a door for the development of new treatment strategies in the future.

Conflict of interest

There are no conflicts of interest.

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