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Sporadic amyotrophic lateral sclerosis: is SMN-Gemins protein complex of importance for the relative resistance of oculomotor nucleus motoneurons to degeneration?

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Abstract

Lower motoneurons (MNs) show varied vulnerability in amyotrophic lateral sclerosis (ALS): those of non-ocular brainstem nuclei and most of those of the spinal cord are highly vulnerable, while those of extraocular brainstem nuclei are quite resistant. Results of our former study on the immunoexpression of the survival of motor neuron protein (SMN) and Gemins 2-4 in cervical spinal cord anterior horn α-MNs of sporadic ALS patients suggested that a relative deficit in Gemin2 may play some role in the pathomechanism of the disease. Here, we tested this idea further by comparing immunoexpression patterns of SMN and Gemins 2-8 between MNs of the oculomotor nucleus and α-MNs of the cervical spinal cord anterior horns in autopsy material from sALS patients and controls. In the latter, no considerable difference in any studied protein was found between these structures except that SMN expression was slightly but significantly lower ($p < 0.01$) in the oculomotor MNs. In the sporadic ALS patients, the expression of SMN, Gemin4 and Gemin7 was significantly weaker ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively), while that of Gemin8 was stronger ($p < 0.001$) in the MNs of the oculomotor nucleus than in the examined cervical spinal cord anterior horn α-MNs. The immunoexpression of Gemin3 and Gemin6 in the spinal cord correlated strongly negatively with ALS duration (Spearman's correlation coefficient: $R_S = –0.84$, $p < 0.001$, and $R_S = –0.86$, $p = 0.002$, respectively). In the oculomotor nucleus MNs, no studied protein immunoexpression correlated significantly with ALS duration, but there was a tendency for such negative correlation for Gemin2 ($R_S = –0.56$, $p = 0.07$). There was an apparent relative deficit of Gemin2 and Gemin8 in the spinal cord α-MNs and of Gemins 2, 4 and 7 in the oculomotor nucleus MNs. These data do not support the hypothesis that the diverse ALS vulnerability of the two MN subsets is related to their disparate expression patterns of SMN and Gemins 2-8. The differences in these patterns may result from ALS-related epiphenomena, or from intrinsic differences in the structure and function between the MN subsets, or both.

Key words: *amyotrophic lateral sclerosis, gemin, oculomotor nucleus, spinal cord, survival motor neuron.*

Introduction

Despite the extensive progress made over the last 20 years, the etiology and pathogenesis of amyotrophic lateral sclerosis (ALS) are not fully elucidated. This is due to the intricacy of the interplay between many underlying genetic, neurometabolic, developmental, age-related, environmental, and stochastic factors (reviewed in [59,61,62]). Customarily, 5-10% of all ALS cases were classified as familial based

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on family history, while the remaining 90-95% that seemed to occur occasionally were termed sporadic (sALS). However, this categorization appears spurious now since there is a sizeable genetic component also in the latter [61,62]. On the other hand, even the idea of ALS as one disease is increasingly disputed because of the diversity of its clinicopathological forms. Regardless of the phenotype, the disease causes a profound degeneration and loss of both the upper and the lower motoneurons (MNs), and the axial end-stage symptomatology includes atrophy of most skeletal muscles, bulbar palsy, tetraparesis or tetraplegia, and respiratory failure. However, oculomotor activity usually persists until late in the disease.

Detailed studies revealed a variety of oculomotor anomalies in sALS patients. Most are subtle deficits undetectable with a routine neurologic examination, some of which can emerge early in the course of ALS; the most common defect, mainly in long-term survivors, is ophthalmoparesis (reviewed in [18,49], for recent additions see [6,9,33]). However, the MNs of the brainstem oculomotor (ON), trochlear and abducens nuclei (also called jointly extraocular motor nuclei or oculomotor nuclei) are much more ALS-resistant than those of other cranial nerve nuclei and of the spinal cord (SC) anterior horns, and their degeneration and loss over the course of the disease is much slower [18,49].

An important role in sALS pathogenesis is attributed to excitotoxicity. Data from animal and human studies show alterations in both excitatory and inhibitory signaling in the affected CNS structures and in intrinsic excitability of the respective MNs (reviewed in [25]). Monkey and human studies [44,45] revealed that the abundance of parvalbumin in MNs, which protects them from excess intracellular Ca^{2+} ions, a key mediator of glutamate toxicity, is much higher in the aforesaid three extraocular motor nuclei than that in other brainstem MN nuclei. These data are generally in line with those from ALS autopsy material [1]. Rat studies showed also that oculomotor MNs (OMNs) and trochlear MNs express much less metabotropic GluR1a glutamate receptors than hypoglossal and spinal MNs [28] and there is a similar difference in the expression of NR2B subunit of the NMDA ionotropic glutamate receptor between the OMNs and hypoglossal MNs [22], which features also reduce possible glutamate toxicity. Notable differences between ALS-vulnerable and ALS-resistant brainstem MNs, supporting a better protection of the latter against such toxicity, exist in the respective patterns of $GABA_A$ receptor subunits expression as well [29]. MNs of the ALS-resistant brainstem extraocular motor nuclei show also, in both rodents and end-stage sALS patients, an enhanced (neuro)-trophic tone that was postulated to contribute to their higher resistance to ALS [2,24,50]. The resistance of MNs of these nuclei may also be attributed to the fact that they all lack direct, *i.e.*, monosynaptic, connections with cortical MNs [56,58].

The aforementioned findings regarding the differences in synaptic transmission-related cellular gear between ALS-resistant and ALS-vulnerable MNs were mostly confirmed by protein signature [17] and transcription profile studies [7] in human autopsy material. The latter study has revealed extensive differences between these MN types in the expression of about 1800 genes involved, *i.a.*, in ubiquitin-dependent proteolysis, mitochondrial function, extracellular matrix, and immune system.

Differences between the respective glial environments may contribute to the differences in ALS vulnerability as well. Astrocytes in ALS-vulnerable brainstem motor nuclei (facial, trigeminal, and hypoglossal) and spinal motor nuclei express much more of the glutamate transporter GLT-1 (EAAT2) than those in the ALS-resistant brainstem extraocular motor nuclei, whereas levels of the neuronal glutamate transporter EAAC1 in all the respective MN subsets are relatively low [31]. In the case of GLT-1 deficit that develops in sALS [47], extracellular glutamate may elevate in the vicinity of the various MN subsets. This should enhance Ca^{2+} influx and the related cell damage more in the ovalbumin-poor, ALS-vulnerable MNs located in the initially GLT-1-rich nerve nuclei, see [31] and references therein.

There is also a large and ever-growing body of evidence for anomalous RNA processing as a key driver of neurodegeneration in motor neuron diseases including ALS [3]. A major role in the latter is played by perturbed biogenesis of Sm-class small nuclear RNA-protein complexes (UsnRNPs) involved in the maturation of pre-mRNAs [54]; reviewed in [13]. In vertebrates, this process requires apt functioning of a protein complex formed by the survival of motor neuron protein (SMN, or Gemin1), Gemins 2-8 and the Unrip protein [10,11,20]; for review see [13,43]. The best-documented role of this canonical complex is specific cytoplasmic assembly of Sm protein cores

onto uridine-rich small nuclear RNAs to yield UsnRNPs that then enter the nucleus where they take part in pre-mRNA splicing [4,41]. Recent studies indicate that the SMN-Gemins complex is also a chaperone for nuclear and cytoplasmic small nuclear ribonucleoproteins [43], and SMN-Gemin2 complex is a versatile platform for ribonucleoprotein exchange [52]. SMN and at least some of Gemins, acting in the form of joint non-canonical complexes or complexes with an assortment of additional macromolecules, are essential players in a number of other RNA metabolism-related processes as well [51]. Some of these processes are specific for neurons and are of high importance for their function and viability, particularly in MNs [8,12,46]. Deficits in the expression or function of SMN and/or the other Gemins and their complexes may thus enhance vulnerability to and severity of motor neuron diseases [20,46,57].

Results of our initial study on the immunoexpression of SMN and Gemins 2-4 in α-MNs of cervical SC anterior horns in sALS patients [42] suggested that some deficits in these proteins may contribute to sALS pathogenesis; namely, Gemin2 was present at a very low level relative to SMN level. The question has arisen whether the expression of the SMN-Gemins complex proteins in the MNs of the ON differs from that in the SC α-MNs. The present study aimed at the comparison of the expression of SMN and Gemins2-8 between the two locations. The results of the comparison might help answer the question whether these proteins may play a role in the resistance of MNs of the ON in sALS.

Material and methods

All procedures concerning human material complied with the ethical principles for medical research involving human subjects as stipulated in the Helsinki Declaration and with the current laws of Poland regarding the use of human tissues and organs. The study protocol has been approved by the Medical University of Warsaw Bioethics Committee (permit No. AKBE/20/14).

The studied sALS material comprised archival paraffin blocks with formalin-fixed SC samples (C4-C8 level) or midbrain samples carrying the ON from 14 sALS patients. The initial ALS sign in the patients was a spastic/flaccid limb paresis or bulbar symptoms, but at the death all the patients showed severe bulbar syndrome and deep tetraparesis or tetraplegia.

The motoneurons from lateral and medial nucleus of the anterior spinal horn were examined. Since the autopsy material came from patients who at the moment of death had a similar neurological deficit, it means that in any case both of the above mentioned nuclei were damaged. The motoneurons of oculomotor nuclei in the midbrain were also studied.

The control material comprised paraffin blocks with same SC samples from 13 patients who died of non-CNS internal organ diseases; midbrain samples with the ON were available from only three of these patients. No one of the control group donors showed signs of a motor neuron disease, including ophthalmoplegia or ophthalmoparesis, and no such ophthalmic symptoms were apparent in the sALS group donors. For basic characteristics of both donor groups see Table I. All autopsies were performed 11-24 h *post mortem*.

The selected paraffin-embedded samples were cut transversely into 8-µm slices, deparaffinized and rehydrated by standard procedures and then were subject to routine histologic (hematoxylin-eosin and cresyl violet) staining and SMN/Gemin immunohistochemistry by the streptavidin-biotin-peroxidase method. Briefly, the rehydrated slices were microwaved in citrate buffer pH 6 for antigen retrieval and then incubated with the following primary antibodies (Santa Cruz Biotechnology, Dallas, TX, USA): 1) anti-SMN (rabbit polyclonal, cat. no. sc-15320, dilution 1 : 200), 2) anti-Gemin2 (mouse monoclonal, cat. no. sc-166187, dilution 1 : 50), 3) anti-Gemin3 (mouse monoclonal, cat. no. sc-271853, dilution 1 : 250), 4) anti-Gemin4 (mouse monoclonal, cat. no. sc-166418, dilution 1 : 250), 5) anti-Gemin5 (goat polyclonal, cat. no. sc-21440, dilution 1 : 50), 6) anti-Gemin6 (rabbit polyclonal, cat. no. sc-367218, dilution 1 : 50), 7) anti-Gemin7 (rabbit polyclonal, cat. no. sc-368684, dilution 1 : 200), and 8) anti-Gemin8 (mouse monoclonal, cat. no. sc-376419, dilution 1 : 50). Next, the slices were treated with biotinylated $F(ab)_2$ fragment of goat anti-mouse IgG (Beckman Coulter, cat. no. PN IM0816, dilution 1 : 1500), or biotinylated $F(ab)_2$ fragment of goat anti-rabbit IgG (Beckman Coulter, cat. no. PN IM0830, dilution 1 : 1500), or horseradish peroxidase-labeled horse anti-goat IgG (Vector Labs., cat. no. MP-7405, dilution 1 : 1500) as appropriate. The formed immunocomplexes, except those with the horse IgG, were then incubated with a streptavidin-horseradish peroxidase conjugate. All sections were next developed with diaminobenzidine as

Table I. Basic clinical characteristics of the spinal cord and midbrain sample donors

sALS patients				
Case#	Age [years]	Sex	ALS history [years]	Initial symptoms
1	59	F	1	Bulbar syndrome + lower limb weakness
2	52	M	1	Foot drop
3	73	M	1	Bulbar syndrome
4	73	F	2	Dysphagia
5	87	F	2	Limb weakness
6	78	F	2	Bulbar syndrome
7	73	F	3	Upper limb weakness
8	70	F	3	Lower limb weakness
9	67	F	4	Gait disturbances
10	64	M	4	Bulbar syndrome + upper left limb weakness
11	55	M	4	Upper left limb weakness
12	65	M	4	Lower limb paraparesis
13	74	M	8	Lower left limb weakness
14	76	F	9	Lower limb paraparesis
Mean age ± SD (range) [years]: 69.4 ± 9.6[a] (52-87)				
Controls				
Case#	Age [years]	Sex	Cause of death; other diseases	
1[b]	56	F	Circulatory insufficiency; Th10-Th11 meningioma	
2[b]	68	M	Circulatory insufficiency; cauda equina neuroma	
3[b]	82	F	Circulatory insufficiency; Th11-Th12 meningioma	
4[b]	59	M	Ischemic stroke	
5[b]	64	M	Acute respiratory failure; Th3-Th4 intraspinal abscess	
6[b]	60	F	Digestive tract hemorrhage	
7[b]	67	F	Heart infarct	
8[b]	54	M	Chronic liver insufficiency	
9[b]	64	M	Chronic liver insufficiency	
10[b]	37	M	Renal insufficiency; insulinoma	
11	75	F	Circulatory insufficiency; Alzheimer's disease	
12	61	M	Pulmonary embolism; chronic ethanol intoxication	
13	66	F	Breast cancer with liver metastasis	
Mean age ± SD (range) [years]: 62.5 ± 10.8 (37-82)				

[a]*Not significantly different from that for the control group (p = 0.09, Student's t test)*
[b]*Cases with midbrain sample missing.*

the chromogen, counterstained with hematoxylin, and coverslipped using DPX mountant. Immunostaining specificity was verified for each protein by running a negative control with the respective primary antibody absent; no sizable staining was found in any such control. Intensity of specific staining was assessed as follows: 0 – none, 1 – traces in some MNs, 2 – weak, 3 – medium, 4 – strong and uniform. Only α-MN staining was assessed in the SC slices. The assessment was done individually by two specialists blinded to sample identity, using a Nikon (Japan) light microscope equipped with a Nikon CCD camera. In case of divergent assessments, the results were averaged.

Statistics

Because of their semi-quantitative character, immunostaining data were analyzed by nonparametric methods. Between-group comparisons were run using the Mann-Whitney *U* test. Differences between data obtained from paired tissue samples and those between data obtained from overlapping donor subsets were tested with the Wilcoxon signed-rank test and the Mann-Whitney *U* test,

respectively. Differences in regional patterns of SMN and Gemins immunoexpression were assessed with the Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test. The association between variables was assessed by calculating Spearman's rank correlation coefficient (R_S). All the analyses were performed using Statistica v.11 software (StatSoft, Tulsa, OK, USA). In all cases, $p \leq 0.05$ was considered significant.

Results

Morphology

In the SC anterior horns from sALS patients, routine histological stainings (not shown) revealed a typical picture of this clinical entity. Compared to the respective control material, SC anterior horns of the sALS patients showed much less α-MNs per field of view, especially in cases with the longest disease history (8-9 years), which revealed the presence of but single surviving α-MNs. There was no such difference between the corresponding ON samples. In sALS patients, OMNs were morphologically normal except for the presence of vacuoles in the cases with the most rapid disease course. Fairly clear chromatin-poor α-MN nuclei were found in some sALS cases, but same changes were apparent in few control cases.

SMN and Gemins regional immunoexpression patterns

There was no significant correlation ($p \geq 0.12$) between donor age and the expression of any studied SMN-Gemins complex component in cervical SC α-MNs from the controls (data not shown). Control samples of the ON were too few ($n = 3$) for a meaningful test of the correlation between the OMNs' expression of SMN or Gemins 2-8 and either donor age, or the expression of these proteins in the respective cervical SC α-MNs.

In the sALS group, there was no significant correlation between the expression of any studied protein in cervical SC α-MNs ($p \geq 0.12$) and patients' age, but a moderate negative correlation was found between patients' age and Gemin8 expression in the OMNs (Spearman's rank correlation coefficient $R_S = –0.57$, $n = 13$, $p = 0.043$). There was also a high negative correlation between ALS duration and either Gemin3 or Gemin6 expression in the cervical SC anterior horn α-MNs ($R_S = –0.84$, $n = 12$, $p < 0.001$, and $R_S = –0.86$, $n = 10$, $p = 0.002$, respectively). In the OMNs, a tendency for negative correlation was only found between ALS duration and Gemin2 expression ($R_S = –0.56$, $n = 13$, $p = 0.073$).

In all the sALS patient samples, SMN immunoexpression was generally strong, whereas a high variability in Gemins 2-8 staining intensities of individual MNs was found both in the cervical SC and ON samples. Typical images of the immunostained cervical SC anterior horn sections and ON sections are shown in Figure 1 and Figure 2, respectively. The immunosignal for each studied Gemin was detected in the cytoplasm of MN perikarya, and sometimes also in the proximal part of the axon. In some MNs also nuclear localization of the immunosignals was observed (with the exception of Gemin5 showing only cytoplasmic presence).

Control midbrain samples were too few ($n = 3$) for a meaningful comparison of the immunoexpression of SMN and individual Gemins within OMNs or between the OMNs and the corresponding cervical SC anterior horn α-MNs. SMN expression in the OMNs from the controls ($n = 3$) was slightly but significantly ($p < 0.01$) lower than that in their SC counterparts ($n = 8$), whereas no significant difference was found for Gemins 2-8 ($p \geq 0.15$; data not shown).

In the cervical SC anterior horn α-MNs, the immunoexpression of SMN, Gemin3 and Gemin5, but not of the remaining Gemins, was significantly lower in the sALS patients than in the controls ($p < 0.05$, $p < 0.01$, $p < 0.05$, and $p \geq 0.18$, respectively; data not shown). In contrast, there was no significant difference between these groups in the immunoexpression of any of these proteins in the OMNs ($p \geq 0.15$; data not shown).

Statistical analysis showed a significantly lower expression of Gemin7 in the OMNs of the sALS patients than that in the respective cervical SC anterior horn α-MNs, and a similar tendency ($0.05 < p < 0.10$) for SMN and Gemin4 expression. In contrast, Gemin8 expression was significantly higher in the OMNs than in the respective SC α-MNs (Fig. 3). Similar analysis including the cases with data missing for any of the two MN subsets (using the Mann-Whitney *U* test) confirmed these findings at even lower *p* values (Fig. 4).

The analysis of Gemin immunostaining intensity in the material from sALS patients has shown considerable differences between Gemin expression

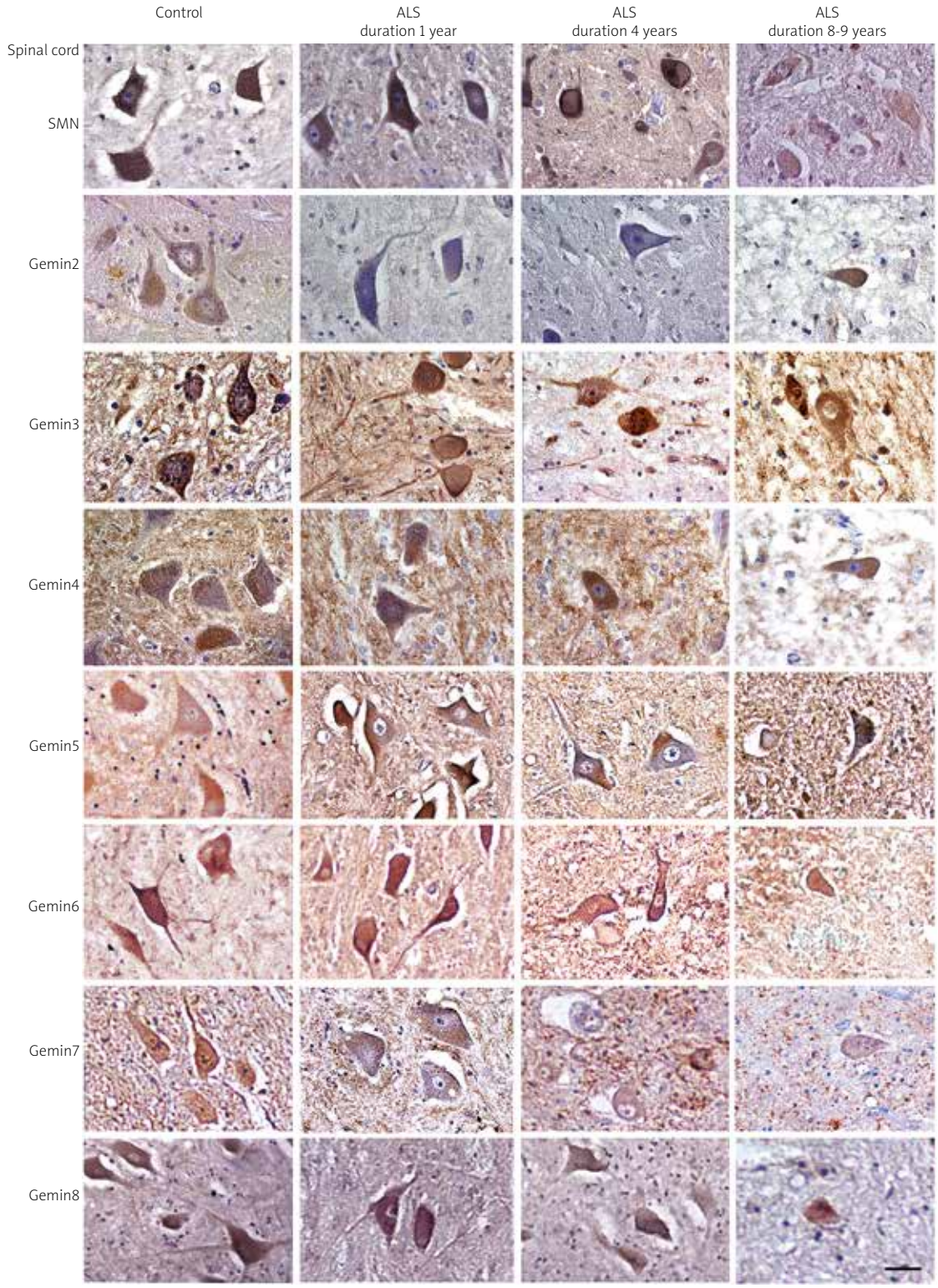

Fig. 1. Representative photomicrographs showing immunoexpression of survival of motor neuron protein (SMN) and Gemins 2-8 in motoneurons of cervical spinal cord anterior horns of controls (leftmost column) and donors with end-stage sporadic amyotrophic lateral sclerosis (ALS) of different duration; scale bar: 50 µm. Note uniformly poor (irrespective of disease duration) staining for Gemin2 contrasting with that for other studied subunits of the SMN-Gemins complex in ALS patient samples.

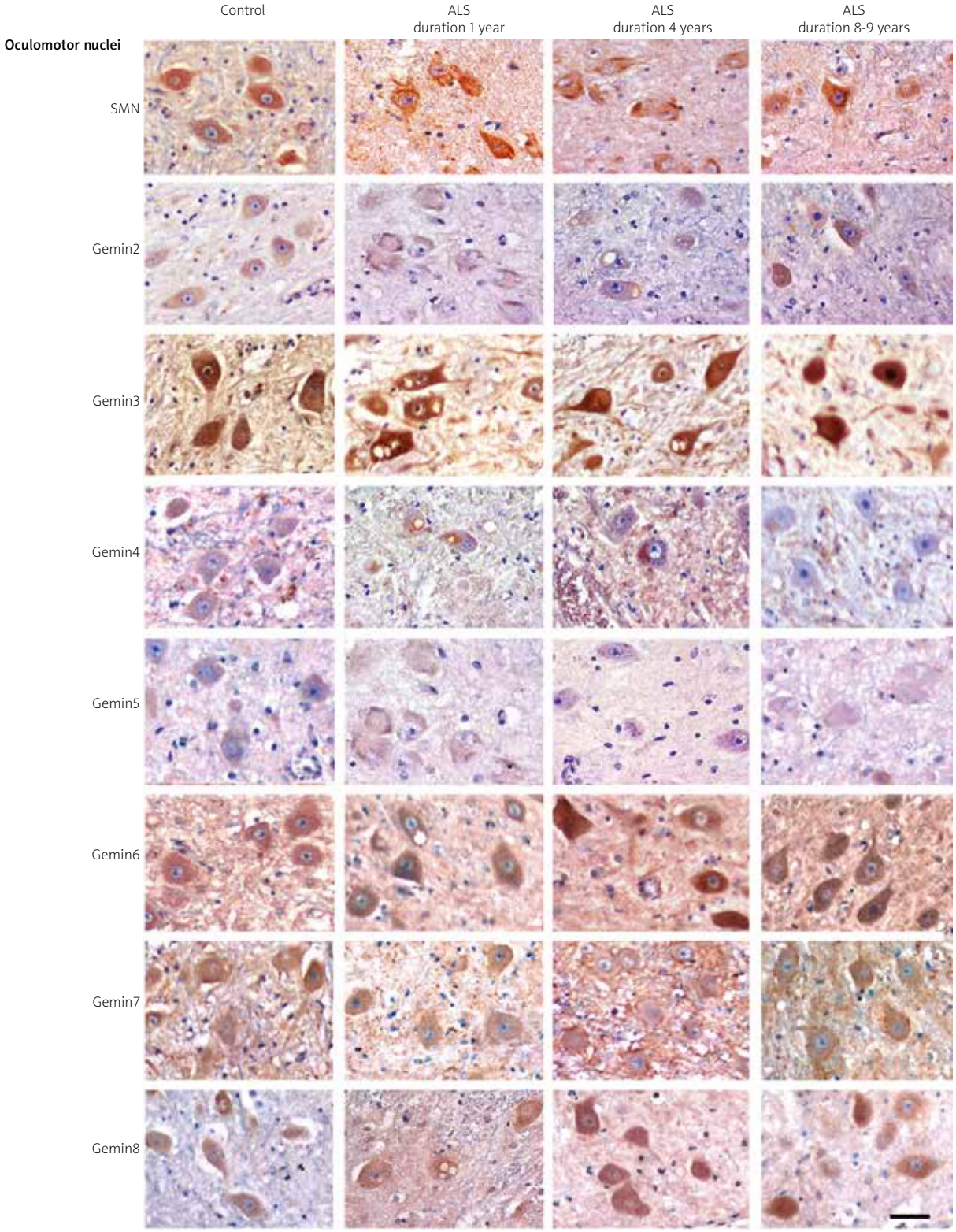

Fig. 2. Representative photomicrographs showing immunoexpression of survival of motor neuron protein (SMN) and Gemins 2-8 in oculomotor motoneurons of controls (leftmost column) and donors with end-stage sporadic amyotrophic lateral sclerosis (ALS) of different duration; scale bar: 50 µm. Note uniformly poor staining for Gemin2 in ALS patient samples.

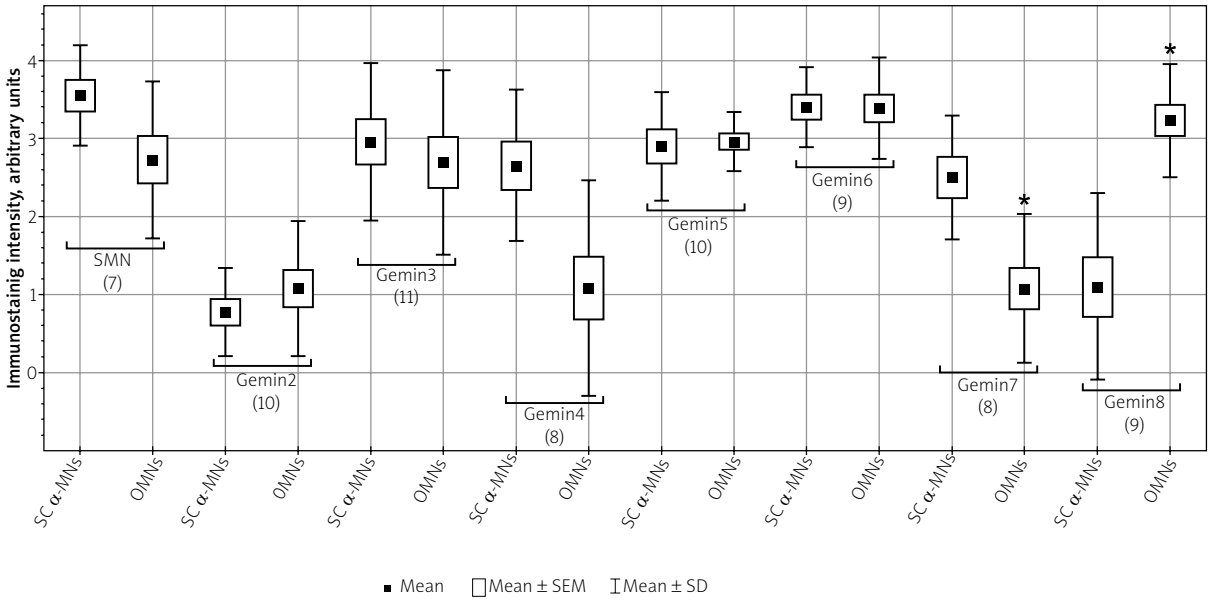

Fig. 3. Comparison of survival of motor neuron protein (SMN) and Gemins 2-8 immunoexpression in the α-motoneurons of cervical spinal cord (SC) anterior horns and the oculomotor motoneurons from the same end-stage sporadic amyotrophic lateral sclerosis patients. *$p < 0.05$ vs. the respective SC value (Wilcoxon signed rank test); the numbers of sample pairs are shown in parentheses.

patterns in the OMNs and the cervical SC α-MNs. In the latter, SMN and Gemin6 showed the highest expression that on average was only marginally higher than that of Gemins 3-5 and 7, while Gemins 2 and 8 showed a clearly lower expression (Fig. 4A). In the OMNs, Gemins 6 and 8 showed the highest expression that, however, was only slightly and non-significantly higher than that of SMN, Gemin3 and Gemin5, while the expression of Gemins 2, 4 and 7 was noticeably lower (Fig. 4B). Interestingly, while there was a statistically significant apparent deficit in the expression of Gemin2 and Gemin8 in relation to that of SMN in the spinal α-MNs, the expression of neither Gemin2, nor Gemin4, nor Gemin7 was significantly lower than that of SMN in the OMNs.

Discussion

It is well known that loss of SMN expression is irreconcilable with cell viability [8] and the same applies to the lack of any vertebrate Gemin expression tested thus far (Gemins 2-5); reviewed in [13], for a recent addition see [32]. There also is indirect evidence from a *Drosophila* study suggesting that Gemin8 is essential for survival and motor function, but Gemin6 and Gemin7 may be not [27]. A major deficit in any of the essential Gemins may thus be expected to exert a detrimental effect on MN viability and function.

A number of studies have shown that the canonical SMN-Gemins complex involved in UsnRNPs biosynthesis is made of few disparate subcomplexes that probably take part in its progressive construction. These modules include some SMN-containing structures that target and function in diverse cellular compartments, including neurite granules [48,51,53]. There are also few SMN-free structures, some of which as well as their single elements may have functions beyond the complete SMN-Gemins complex [5,10,11,16,21,23,34]. The exact *in vivo* stoichiometry of all these subcomplexes is unknown [43] and one cannot judge with certainty about relative deficit(s) of their individual components based on whole-cell-based assessments.

Immunohistochemistry revealed diverse expression patterns of single components of the SMN-Gemins complex in both the α-MNs of cervical SC anterior horns and the OMNs in our sALS material. Particularly striking was the difference in the expression of Gemin8 between the two locations, while the expression of Gemin2 in the same was similarly poor.

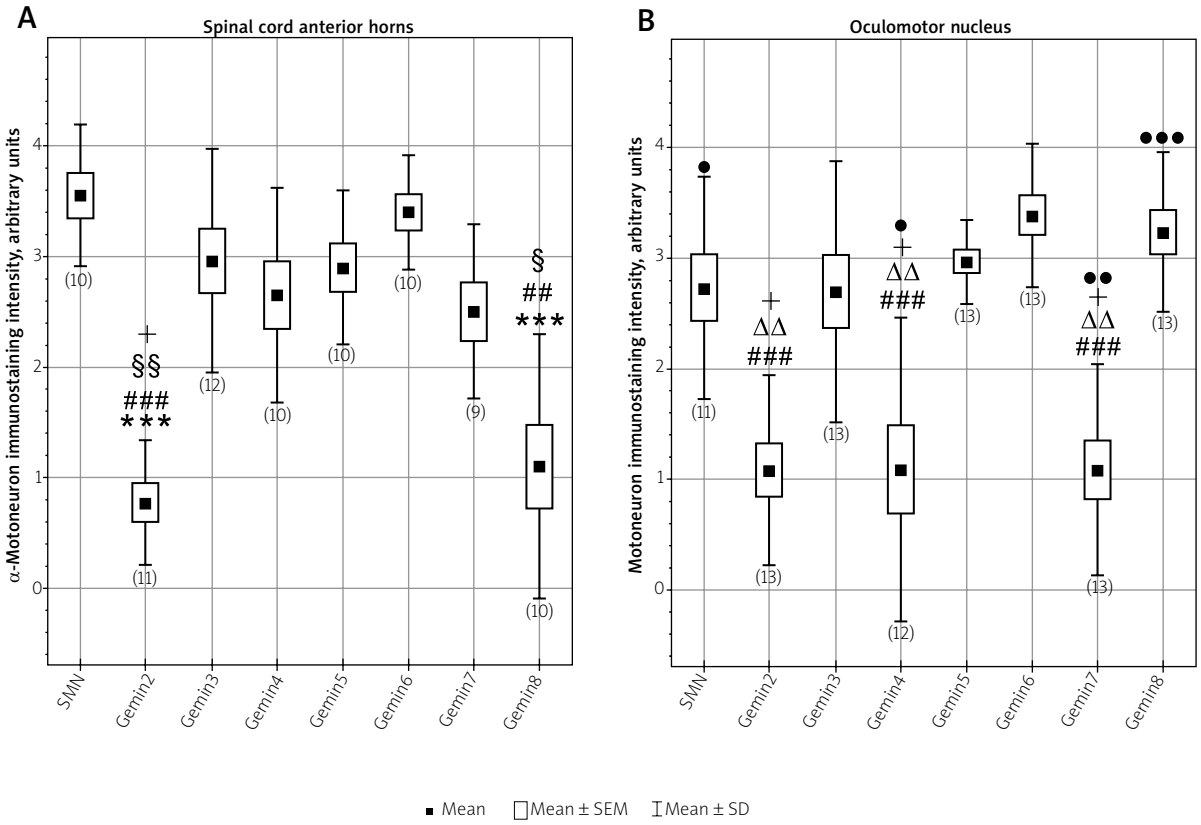

Fig. 4. Comparison of Gemin immunoexpression patterns in cervical spinal cord (SC) anterior horn α-motoneurons **(A)** and oculomotor motoneurons **(B)** of end-stage sporadic amyotrophic lateral sclerosis patients. ***$p < 0.001$ vs. survival of motor neuron protein (SMN); $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ vs. Gemin3; $^{+}p < 0.05$ vs. Gemin5; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Gemin6; $^{\Delta\Delta}p < 0.01$ vs. Gemin8 (Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test). •$p < 0.05$, ••$p < 0.01$, •••$p < 0.001$ vs. the respective data for the SC anterior horn α-motoneurons (the Mann-Whitney *U* test). The numbers of samples are shown in parentheses.

Also, while SMN content was generally high in both the spinal α-MNs and the OMNs, it was significantly lower in the latter in both study groups, which difference was in apparent contrast to the respective ALS vulnerability of these MN subsets. However, SMN may be less important for OMNs survival because of their relative enrichment in other proteins fostering the resistance and/or paucity of proteins that promote their vulnerability (see Introduction).

In metazoans, the amount of SMN and Gemin2 was found to be usually far larger than that of any of the other then-known Gemins 2-6, hence the core of the SMN-Gemins complex was guessed to comprise only SMN and Gemin2; actually, the core of proteins interacting directly with SMN includes also Gemin3 and Gemin8 [11,15,40]. While SMN is needed to link up the different proteins and hence plays a key role in the design of the complete SMN-Gemins complex, the main task of Gemin2 is to stabilize interactions between the various components and hence to stabilize the activity of the complex [37,38,60]. Gemin8 is essential for the complex structure and activity as well, due to its forming a subcomplex by direct interaction with the Gemin6-Gemin7 heterodimer that also binds Unrip via Gemin7 [39]. A complex comprising only SMN, Gemin2 and Gemin8 was shown to be necessary and enough to accept Sm proteins in the assembly of UsnRNPs [14], but Gemin7 is required for efficient UsnRNPs assembly [37,38]. Gemin5 provides the recognition of UsnRNA component for the assembly [4] and binds to Gemin2 in the cytoplasm, but not in the nucleus, while Gemin4 binds to both Gemin8 and Gemin3 and likely serves as a cofactor of the latter that is the DEAD box helicase [23]. Gemin4 is also the sole member of the canonical SMN-Gemins complex which carries

a classical nuclear localization signal motif and is likely important for nuclear import of the SMN-Gemins complex or its subunits [32].

In an earlier study, we speculated that a relative Gemin2 deficit may result in lowering the stability of the SMN-Gemins complex, hence a large disparity in the expression of SMN and Gemin2 may be a risk factor for MN degeneration and death, and thus for ALS [42]. Remarkably, Gemin2 expression in the OMNs did not significantly exceed that in the spinal α-MNs in sALS patients in the present study (p = 0.33). However, while the expression of Gemin2 was significantly lower than that of SMN in the spinal α-MNs, the respective disparity in the OMNs did not reach significance. This may be related, at least in part, to anatomical differences between the two MN types. Spinal α-MNs compared to OMNs have considerably larger soma but noticeably thicker and many times longer axon, and their axon volume comprises much larger fraction of the total cell volume. Notably, SMN has functions in MN axons which do not involve some or all Gemins, including Gemin2 [12,30,51]. One may thus guess that the difference in SMN expression between the two MN subsets is related to a higher demand for it, but not necessarily for the other elements of the SMN-Gemins complex, in the extremely long axons of the spinal α-MNs. By the same token, the Gemin2-unbound SMN would likely represent a larger fraction of the total SMN content in the spinal α-MNs and would contribute this way to the seeming major Gemin2 deficit.

In the sALS material, disparities in the expression of other single components of the SMN-Gemins complex occurred both in the spinal α-MNs and in the OMNs, but their patterns differed. In contrast to the OMNs that showed a low expression of the essential Gemin4 and the (possibly) non-essential Gemin7, the spinal α-MNs showed a very low expression of the essential Gemin8. The latter deficit may greatly reduce formation of both the canonical SMN-Gemins complex and other functional complexes involving Gemin8 (see above) and hence interfere with a number of vital cellular functions. Notably, in contrast to the apparent deficits in Gemin2 and Gemin8 expression in the spinal α-MNs, none of the deficits found in OMNs resulted in the respective Gemin expression significantly below that of SMN. It may also be that some of the alleged deficits in the pattern of Gemins expression in the OMNs represent normal characteristics of these cells that are related to their specific excitability and functionality and the uniqueness of the respective motor units (for review see [35]), or are just epiphenomena of ALS.

A crucial question in the studies on motor neuron diseases is the cause and mechanism of selective MN death. Spinal α-MNs are particularly prone to degeneration because of vast length of their axons and their reliance on the cytoskeleton for mechanical stability, axonal transport, and signaling [36]. MNs of the same genetic background can highly differ by their SMN levels, which diversity was found in both controls and ALS patients; notably, SMN-poor MNs are at higher risk of death [46]. One reason is that SMN deficit hinders transport of mRNAs vital for neurite extension and stability, thus contributing to axon degeneration and MN death [19]. It is thus possible that the surviving spinal α-MNs in our sALS material showed a high SMN expression because those with a low SMN expression perished earlier in the disease course. It should be said here that a marked decrease (–50%) in the level of full-length SMN was reported in post-mortem SC tissue from sALS patients compared to that from people who died with no neurologic disease [55]. However, those data were obtained by Western blot analysis of lumbar samples of the entire SC and thus showed an averaged SMN level across all cell types present in the samples, of which MNs were a minority. Hence the reported drop in SMN level may have represented mostly the decrease in the number of surviving MNs. Major disparities in the immunoexpression of the various components of the SMN-Gemins complex obviously were not a critical obstacle to MNs' viability in either the cervical SC anterior horns or the ON.

It has been postulated that the constitutive characteristics of essential biology of MNs are the presence of unfolded (*i.e.*, damaged) proteins and inherent endoplasmic reticulum stress [26]. Notably, MNs are more vulnerable to the stress compared to other cell types, and their basal ER/unfolded protein stress level correlates positively with their size. An addition to the pre-existing stress may mess up a delicate balance between their endoplasmic reticulum stress and neuronal excitability and exceed the threshold level these cells can endure without triggering apoptosis [26].

In summary, our present results do not support the hypothesis that the difference in ALS vulnerability of spinal α-MNs and OMNs is related to their

expression patterns of SMN and Gemins 2-8, and particularly to the difference in Gemin2 expression. This is because the identified marked differences between the respective patterns of the immunoexpression of individual Gemins provide no clue about the actual role of the canonical SMN-Gemins complex or its individual constituents in OMNs' resistance to the pathologic process in ALS. One may still speculate that it is the deficit of Gemin8 which is of importance for spinal α-MNs' vulnerability in sALS. It may also be that some of the alleged deficits in the pattern of SMN and Gemins expression in the OMNs represent epiphenomena of the disease superposed on normal characteristics of this particular MN subset. These questions cannot be answered without more studies.

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Disclosure

The authors report no conflict of interest.

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