THE NOGO-A PROTEIN IS NOT A BIOMARKER IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by a progressive degeneration of the upper and lower motor neurons. There is an increasing interest for biological markers to prompt an early diagnosis as well as to predict prognosis of the disease.

According to compelling evidence, skeletal muscle may represent an interesting source of biomarkers for ALS.

Expression of Nogo-A, a negative regulator of the axonal growth, was found to be strongly increased in denervated fibers of ALS muscle and to correlate with disease severity.

However, other studies detected elevated levels of Nogo-A also in regenerating fibers of many kind of myopathies as well as in not ALS-related denervated muscles.

To further assess the diagnostic/prognostic value of Nogo-A as ALS biomarkers, we analyzed Nogo-A protein levels in skeletal muscle of patients with ALS, neuromuscular diseases, and healthy subjects.

METHODS

A collection of 16 ALS muscle biopsies from the Tissue Biobank of the Neuromuscular Center, Department of Neurosciences, University of Padova, according to the following patients' clinical features:

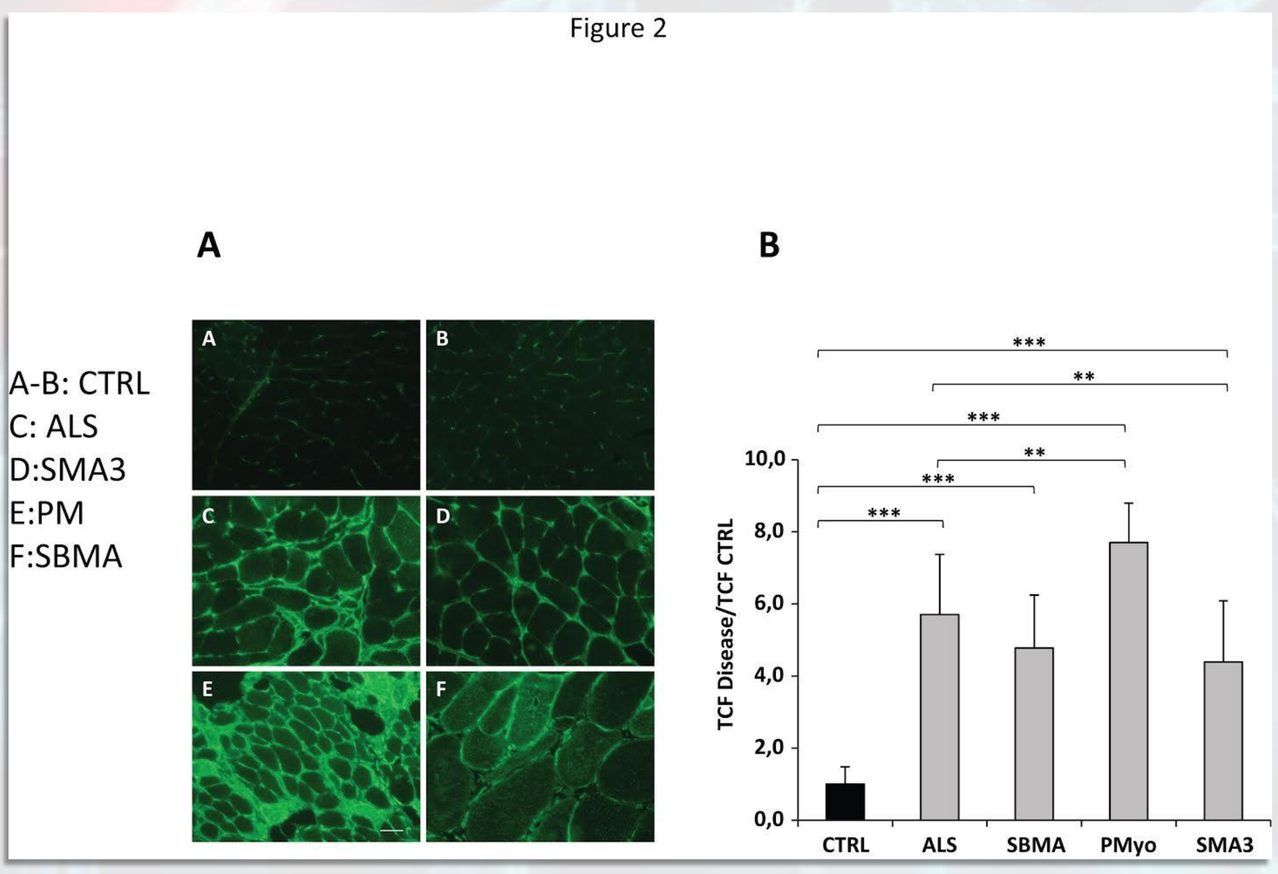
1. diagnosis of clinically probable or definite ALS;
2. lower limb onset without bulbar or respiratory involvement at biopsy;
3. time interval between disease onset and muscle biopsy between 11 and 13 months;
4. clinically affected vastus lateralis as site of biopsy;
5. availability of survival data.

The ALSFRSr (ref) score at the time of biopsy was also recorded.

Muscle samples from 8 patients affected with a denervative disease (4 SMA type 3, 3 SBMA, and 1 SPG11), 3 patients with polymyositis, 3 patients with dermatomyositis and 8 healthy age-matched control subjects were also analyzed.

Western blotting analysis and immunofluorescence analysis and quantification have been performed.

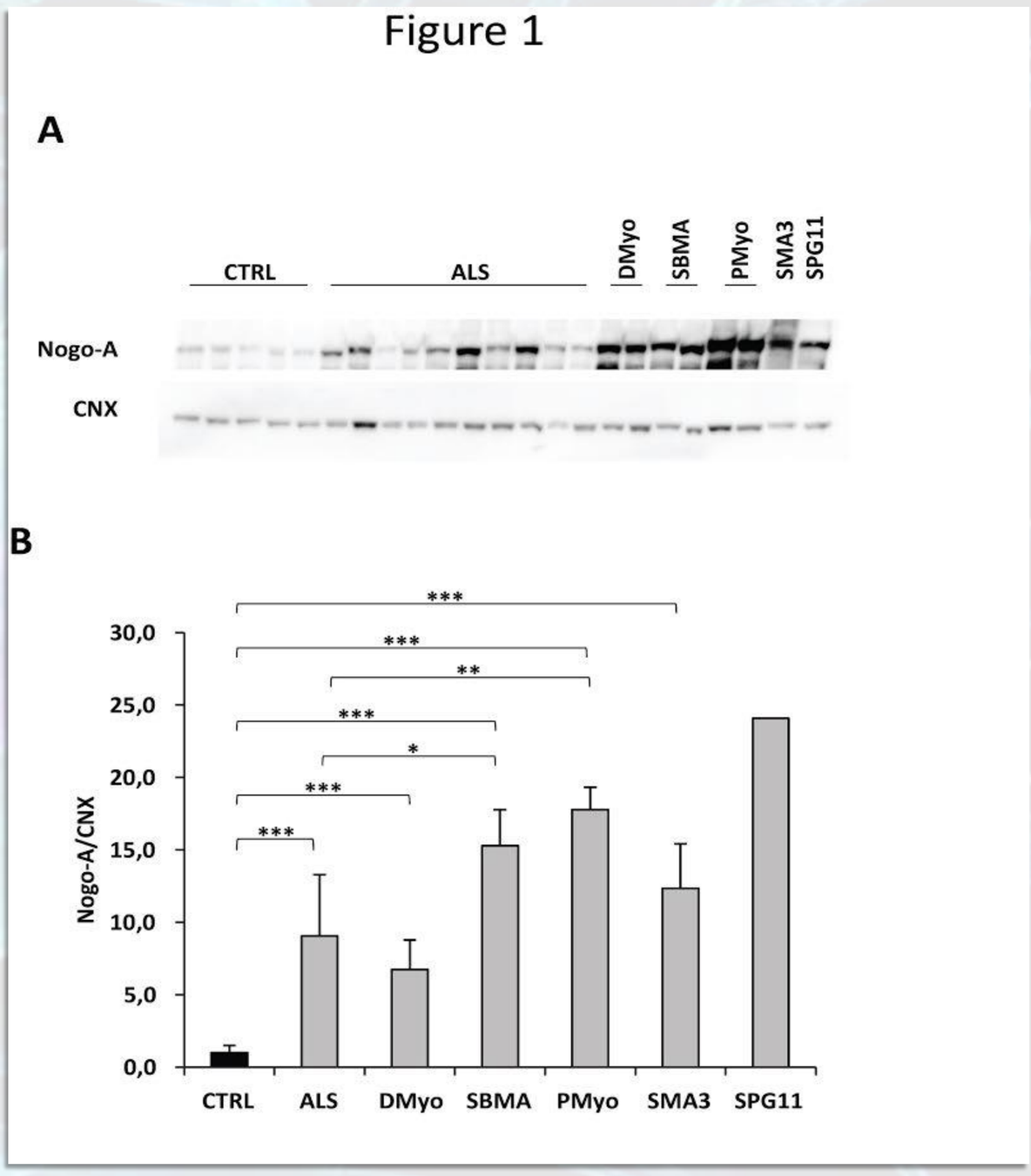
Figure 2



RESULTS

- Nogo-A protein levels were 9 times higher in ALS patients compared to healthy controls ($p<0.001$) (Figure 1).
- A significant increase of Nogo-A was also observed in muscle biopsies from SMA 3 ($p<0.001$ vs controls), SBMA ($p<0.001$ vs controls), polymiositis ($p<0.001$ vs controls), and dermatomyositis ($p<0.001$ vs controls) cases. SPG-11 Nogo-A values were even 24 times higher than in controls.
- Western blotting data were also confirmed by immunofluorescence analysis (Figure 2).
- No effect of Nogo-A muscle levels on ALS patient survival ($p= 0,40$).
- No difference in survival time ($p= 0,69$).
- No relation between Nogo-A and ALSFRSr scores was observed.

Figure 1



CONCLUSIONS

- Muscle Nogo-A levels were initially found increased in ALS patients but not in control groups, including peripheral neuropathies or myopathies.
- However, as previously noted by other groups, we have shown an unspecific overexpression of Nogo-A in muscle of a range of neuromuscular disorders, in addition to ALS.
- Moreover, our findings seem to dismantle a potential prognostic role of the protein, since we ruled out any relation of muscle Nogo-A levels with disease severity.
- The adoption of strict patients' selection criteria have allowed us to minimize the effect of the well known phenotypic variability of the disease on disease severity.

- We failed to confirm these muscle-derived molecules as biomarkers of ALS and rather we suggest these protein's abnormalities more properly represent signs of a not specific degeneration process.

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