Characterization of SOD1-, TDP-43(TAR6/6)- and Induced TDP43 ALS Mouse Models Using NF-L and other Biomarkers

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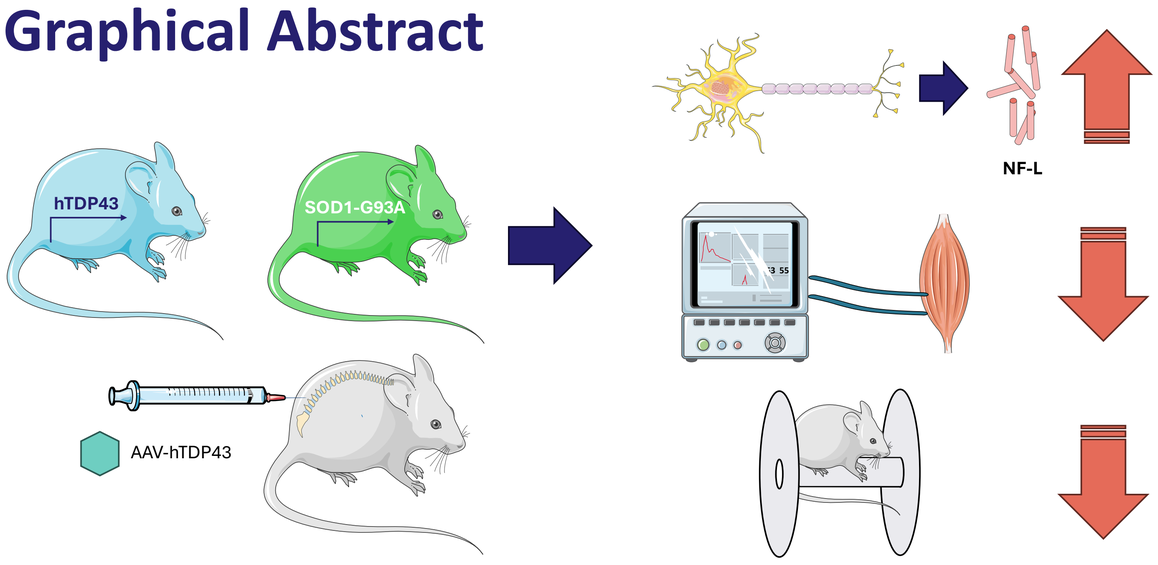
Background

Amyotrophic lateral sclerosis (ALS) is characterized by fatal progressive muscle weakness and wasting. These symptoms are caused by progressive neuronal loss in the motor cortex, the brain stem nuclei and the spinal cord (specifically, the anterior horn). To follow disease progression in animal models, apart from clinical representation, several key biomarkers can be employed. Neurofilament light chain (NF-L) is a sensitive marker for axonal damage and can be very useful to determine disease progression in various neurodegenerative diseases, including ALS. To enhance translational relevance and phenotypic robustness of several ALS mouse models, we characterized levels of NF-L in plasma and cerebrospinal fluid (CSF), alongside complementary ALS biomarkers to determine the robustness and translationality of the NF-L biomarker, and to compare the impact of the respective genetic alterations and other interventions on biomarker levels.

Methods

We employed homozygous TAR6/6 mice and SOD1-G93A expressing mice as genetic models for ALS. Furthermore, we used wild type mice that were administered AAV-TDP43 intrathecally. Finally, we used a second-hit approach where we administered AAV-TDP43 to hemizygous TAR6 mice. NF-L was quantified from CSF and plasma at various model-specific timepoints.

Graphical Abstract



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Results

Progressive Increase in NF-L in B6SJL.SOD1-G93A Transgenic Mice Coincides with Motor Neuron Impairment and Microgliosis

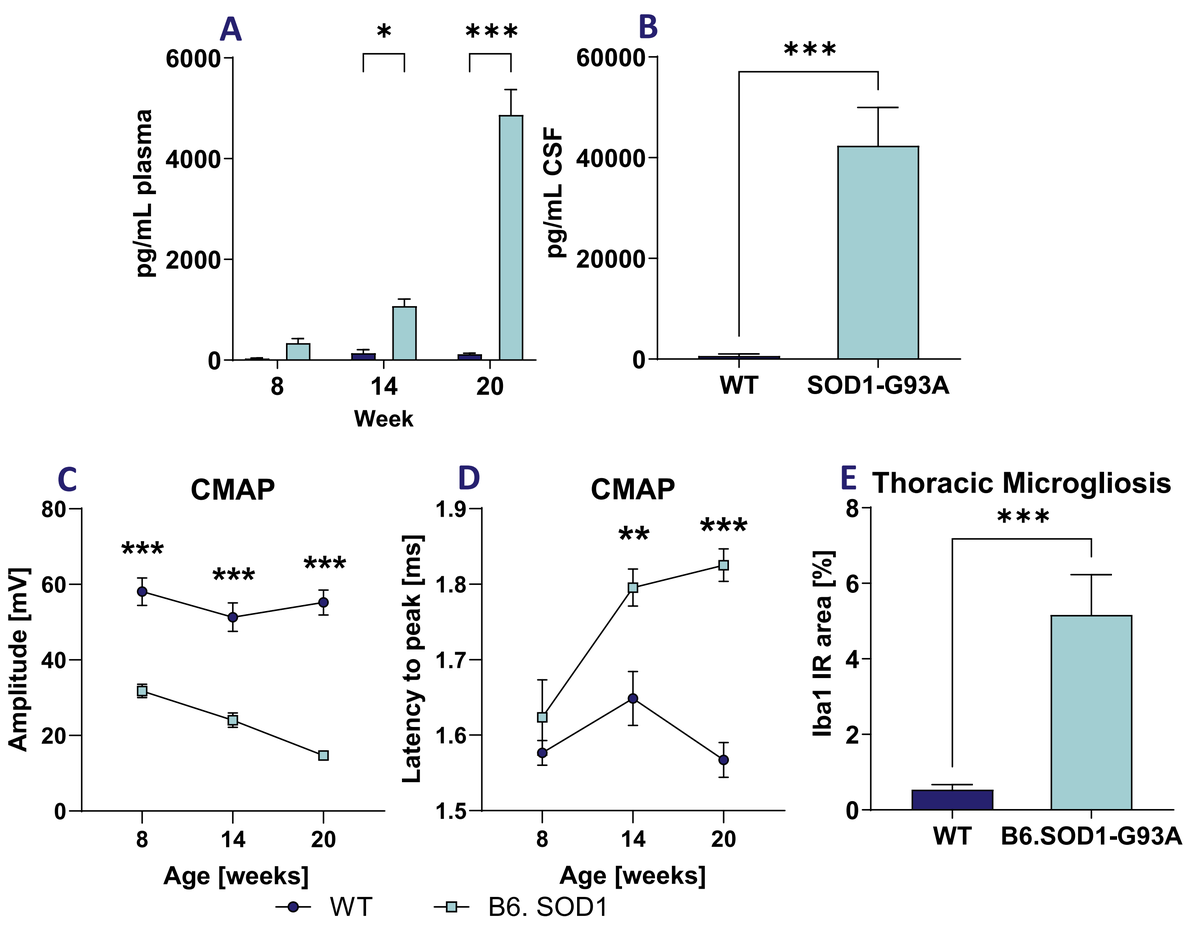

Figure 1: Analysis of NF-L, EMG and microgliosis in B6SJL.SOD1-G93A mice. A, B: Analysis of neurofilament light chain (NF-L) levels in plasma (longitudinal) and CSF (terminal). Displayed are NF-L levels in (**A**) plasma and (**B**) CSF samples collected at different ages, as determined by ELISA. Plasma: Two-way ANOVA with genotype as main factor, followed by Bonferroni's *post hoc* test. CSF: Mann-Whitney test. Mean ± SEM. *p <0.05, ***p <0.001. n = 8 per group. **C, D:** CMAP amplitude (**C**) or latency to peak (**D**) of electromyographic measurements in the gastrocnemius muscle. Two-way ANOVA followed by Bonferroni's *post hoc* test. Mean ± SEM; ***p<0.001. n = 16 per group. **E:** Quantification of microgliosis in B6.SOD1-G93A mice and wild type (WT) littermates. Iba1 immunoreactive (IR) area in the thoracic spinal cord. Mean + SEM; n = 8 per group; t-test or Mann-Whitney test; ***p <0.001.

Results

Substantial Increase in NF-L in Homozygous TDP43 Transgenic Mice Accompanied by Motor Disturbances

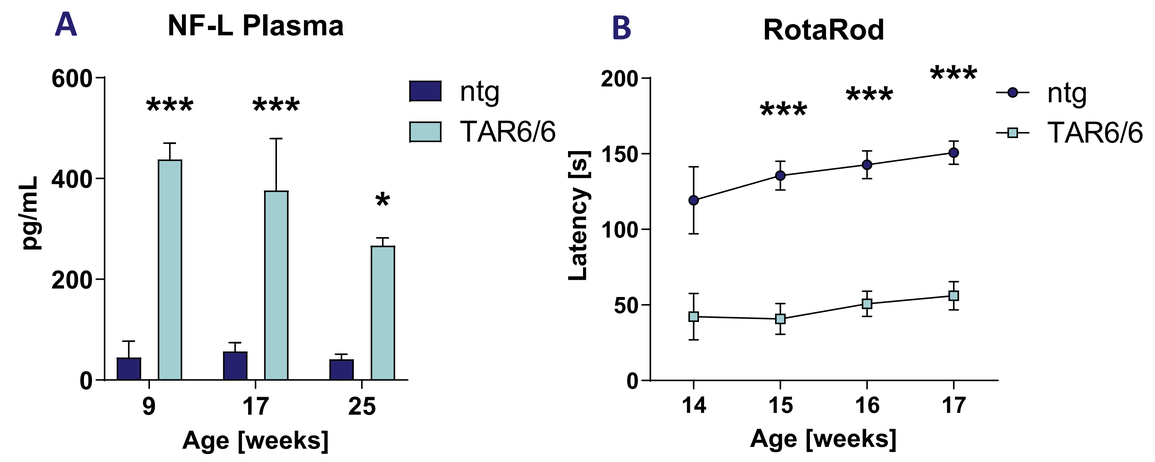

Figure 2: Analysis of NF-L and motor performance of TDP43 transgenic TAR6/6 mice. A: Quantification of NF-L in the plasma of TAR6/6 mice of mixed sex. NF-L levels in pg/ml in the plasma of 9 to 25 week old TAR6/6 mice compared to non-transgenic littermates (ntg). Two-way ANOVA with Tukey's multiple comparison test. Mean + SEM. n = 6. *p <0.05; ***p <0.001. **B:** Latency to fall from the RotaRod in 14 to 17 week old TAR6/6 mice compared to non-transgenic littermates (ntg). Mean ± SEM. TAR6/6 n = 12-7; ntg n = 16-7. Two-way ANOVA with Bonferroni's *post hoc* test. ***p <0.001.

NF-L Levels Peak Six Weeks after Intrathecal TDP43-AAV Injection in Wild Type and Hemizygous TDP43 transgenic Mice

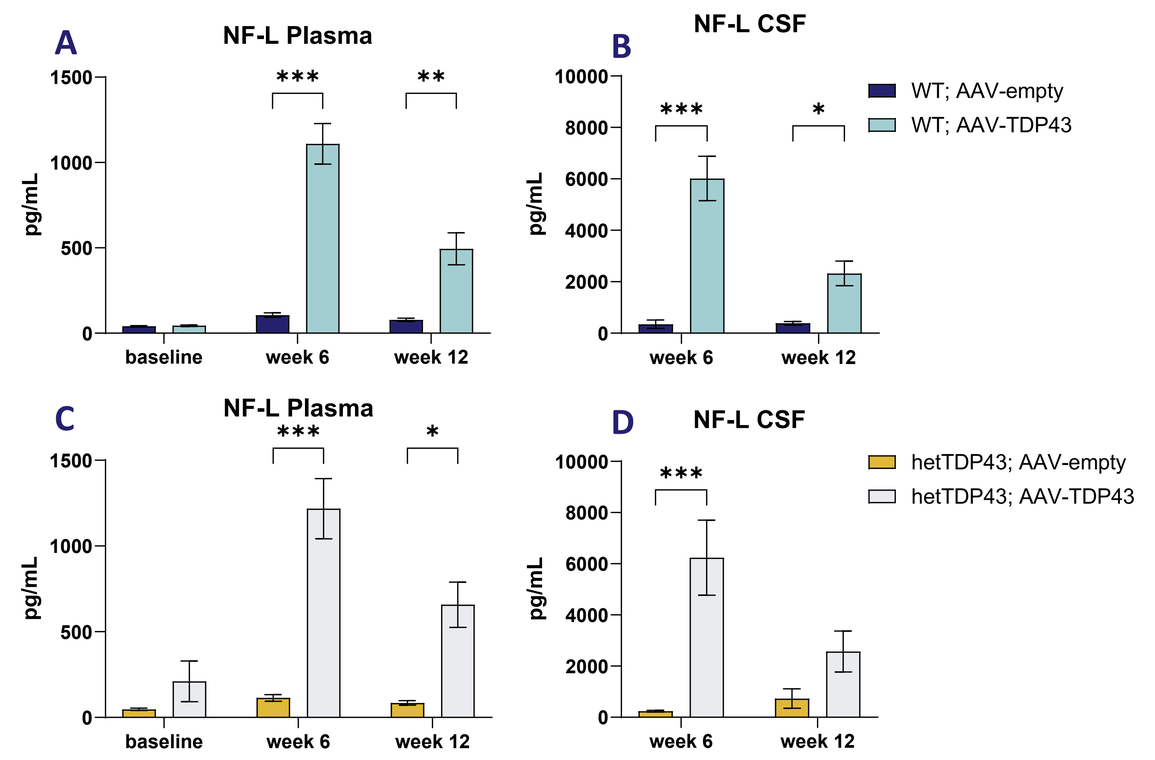

Figure 3: Analysis of NF-L in plasma and terminal CSF samples in IT AAV9-hTDP-43 injected mice. NF-L levels were quantified by ELISA in *in vivo* plasma collected at baseline, 6 and 12 weeks post-injection (**A, C**) and in terminal CSF samples collected at 6 and 12 weeks post injection (**B, D**). Mean ± SEM. n = 6-16 per group for plasma analysis, n = 6-8 per group for CSF analysis. Two-way ANOVA followed by Bonferroni's *post hoc* test. *p <0.05, **p <0.01, ***p <0.001.

Summary

We have found that NF-L was significantly increased to different levels across the ALS mouse models, which is in line with the clinical representation of these mice. Further we could show that a second hit approach of AAV-based TDP43 expression in heterozygous TAR6 mice exacerbates levels of NF-L. This comparative approach underscores the value of a wide range of different types of ALS mouse models in preclinical research and highlights NF-L as a cross-disease biomarker for neurodegeneration.

For more information about the models please visit: www.scantox.com

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