Characterization of an Inducible Intrathecal TDP43-AAV Amyotrophic Lateral Sclerosis Model

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Background

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease characterized by degeneration of motor neurons in the brain and spinal cord. Dysfunction of the transactive response (TAR) DNA-binding protein 43 (TDP-43), involved in RNA metabolism and gene regulation, has been described as hallmark of familial and sporadic ALS, as well as frontotemporal dementia, and is characterized by mislocalization, aggregation, and hyperphosphorylation. It further correlates with cytoplasmic stress granule formation, which in turn affects RNA translation during cellular stress. Accordingly, transgenic mouse models overexpressing human (h)TDP-43 display neurodegeneration associated with cognitive and motor deficits. However, these models often show a rapid disease progression and high mortality, limiting their use in preclinical studies. Thus, we aimed to establish an inducible ALS mouse model with a milder disease progression.

Methods

Adult hemizygous TDP-43 overexpressing mice as well as wild type (WT) littermates were intrathecally (IT) injected with an adeno-associated virus 9 (AAV9) carrying human TDP-43 and evaluated for behavioral deficits. At baseline, as well as at 6 and 12 weeks after IT injection, animals were longitudinally analyzed for motor deficits using wire suspension test, beam walk test and open field test. Additionally, Neurofilament-Light Chain (NF-L) levels were analyzed in *in vivo* plasma samples at baseline, 6 and 12 weeks of age as well as in terminal cerebrospinal fluid (CSF) samples at 6 and 12 weeks of age.

Results

Behavior

General activity, anxiety as well as rearing behavior was tested in the open field test. Minor impact of IT hAAV9-hTDP43 injection was detected in distance traversed reflecting general activity, only reaching significance in hemizygous TDP-43 mice at 6 weeks post injection. No impact on anxiety levels measured via thigmotactic behavior could be observed throughout the entire testing period (data not shown). Motor impairment could be detected in terms of rearing behavior in both hemizygous TDP-43 and WT mice at 6 and 12 weeks post injection.

To measure changes in motor coordination beam walk test was performed. Similar to rearing behavior in the open field test a significant and progressing impairment could be detected in both hemizygous TDP-43 and WT mice at 6 and 12 weeks post injection.

Neuromuscular abnormalities were assessed in the wire hanging test with a 300 seconds cut-off time. Compared to empty vector-treated controls, both hemizygous TDP-43 and WT mice injected wit hAAV9-hTDP-43 showed a reduced performance already 3 weeks after injection, and the latency to fall was further reduced until 12 weeks post-injection onwards, indicating progressing reduced motor strength.

Open Field Test

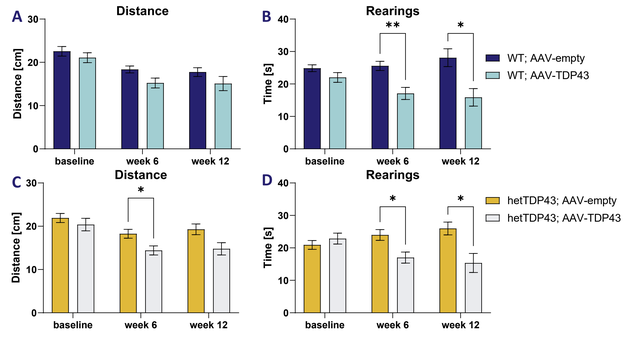

Figure 1: Longitudinal analysis of general activity and motor function in IT AAV9-hTDP-43-injected mice. Distance traversed (**A, C**) and number of rearings (**B, D**) in the open field test was evaluated at baseline, 6 and 12 weeks post-injection. Mean ± SEM. n = 12-24 per group. Two-way ANOVA followed by Bonferroni's *post hoc* test. *p <0.05, **p <0.01, ***p <0.001.

Beam Walk Test

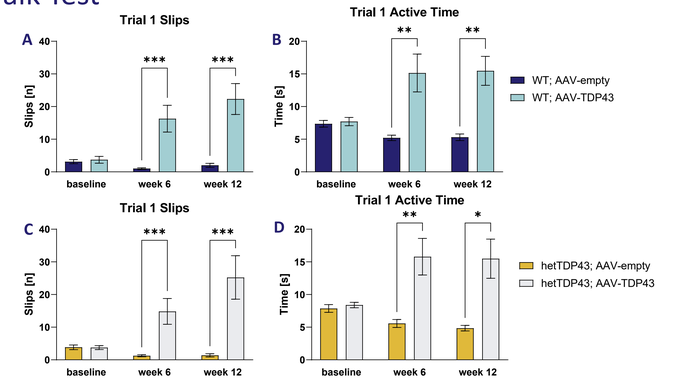

Figure 2: Longitudinal analysis of motor coordination in IT AAV9-hTDP-43-injected mice. Number of slips off a 13mm square beam (**A, C**) and time to traverse the beam (**B, D**) in the beam walk test was evaluated at baseline, 6 and 12 weeks post-injection. Mean ± SEM. n = 12-24 per group. Two-way ANOVA followed by Bonferroni's *post hoc* test. *p <0.05, **p <0.01, ***p <0.001.

Wire Hanging Test and Clasping

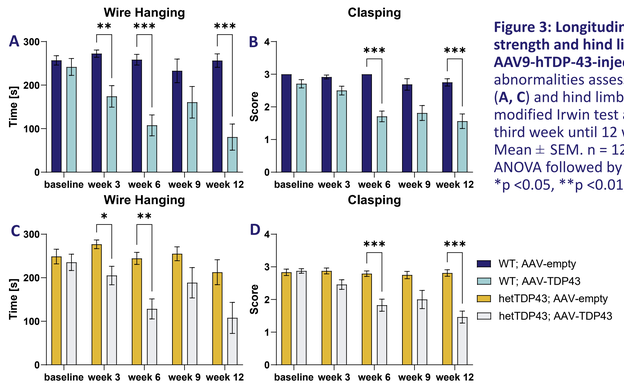

Figure 3: Longitudinal analysis of motor strength and hind limb clasping behavior in IT AAV9-hTDP-43-injected mice. Neuromuscular abnormalities assessed in wire hanging test (**A, C**) and hind limb clasping (**B, D**) in the modified Irwin test at baseline and every third week until 12 weeks post injection. Mean ± SEM. n = 12-24 per group. Two-way ANOVA followed by Bonferroni's *post hoc* test. *p <0.05, **p <0.01, ***p <0.001.

Biochemistry

NF-L levels were quantified by ELISA in *in vivo* plasma collected at baseline, 6 and 12 weeks post-injection, as well as in terminal CSF samples collected at 6 and 12 weeks post-injection in both WT and hemizygous TDP-43 mice. Compared to controls, the AAV9-hTDP-43 injected animals showed significantly increased plasma and CSF NF-L levels both at 6 weeks and 12 weeks post-injection with a peak at 6 weeks, indicating the highest rate of neurodegeneration at this time point.

Neurofilament-Light Chain

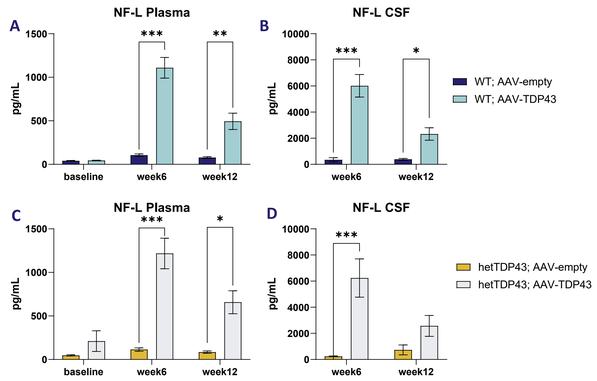

Figure 4: Analysis of neurofilament-light chain levels in plasma and terminal CSF samples in IT AAV9-hTDP-43 injected mice. NF-L levels were quantified by ELISA in *in vivo* plasma collected at baseline, 6 and 12 weeks post-injection (**A, C**) and in terminal CSF samples collected at 6 and 12 weeks 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

The observed behavioral deficits and increased levels of NF-L in IT TDP-43-AAV mice suggest that animals develop a robust and progressing phenotype in both heterozygous TDP-43 transgenic and WT mice. IT injected TDP-43-AAV mice are thus a valuable model for studying progressive pathological processes and the development of TDP-43-related therapies against ALS and frontotemporal dementia.

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