Characterization of the TDP-43 ΔNLS mice: behavior and biomarker analyses

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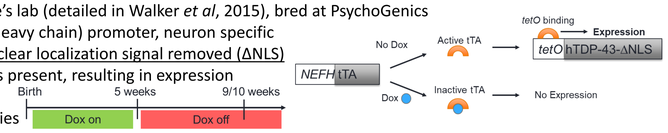
Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of upper and lower motor neurons producing muscle denervation, motor impairments and brain atrophy. Deposition of insoluble cytosolic inclusions of TAR-DNA binding protein (TDP-43) correlates with ALS-related pathology in affected tissues. To study the progression of ALS phenotypes and establish a model for testing therapeutic interventions, we characterized TDP-43ΔNLS mice (Walker et al, 2015). These inducible rNLS8 mice were generated by crossing transgenic mice expressing tTA under the control of the human neurofilament heavy chain (NEFH) promoter with tetO-hTDP-43ΔNLS mice containing a defective nuclear localization motif (ΔNLS). Administration of Dox suppresses expression of total and phosphorylated forms of hTDP-43ΔNLS, rescuing the disease phenotype. TDP-43ΔNLS mice showed dramatic loss of body weight following Dox cessation, increased tremors and hindlimb clasping, impaired gait and muscle strength, and decreased survival compared to tTA control mice. EMG assessment of muscle function 4 weeks after DOX removal showed an increase in the latency of muscular contraction and decreased response amplitudes of muscle contractions following motor nerve stimulation in TDP-43ΔNLS animals, impairments whose increasing severity correlated with the amount of time spent off Dox. Histological analysis revealed strong overexpression of TDP-43 in perinuclear cytoplasmic inclusions along with deposition of pTDP43 aggregates in multiple brain regions including hippocampus, cerebral cortex, dorsal striatum and cerebellum. This model recapitulates deregulated translocation of TDP-43 from nucleus to the cytoplasm, a major pathology seen in ALS patients. TDP-43 pathologies were accompanied by increased expression of inflammatory marker transcripts in cortex, astrogliosis, and microglial activation in affected brain regions. Similar pathologies were detected in spinal cord but at lower level of severity than in the brain. Dramatic elevations in neurofilament light chain (NF-L), a biomarker of neurodegeneration, were seen in plasma and CSF of TDP-43ΔNLS animals at 10 weeks of age, 5 weeks after Dox withdrawal. In summary, expression of human TDP-43ΔNLS in this mouse model of ALS resulted in the development of cytoplasmic inclusions in multiple brain regions and spinal cord, a loss of murine nuclear TDP-43, progressive loss of muscle strength and function, and motor impairments leading to death. These impairments were observed in both genders. This mouse model of ALS provides a rapid progressive phenotype for testing novel therapeutic strategies.

Methods

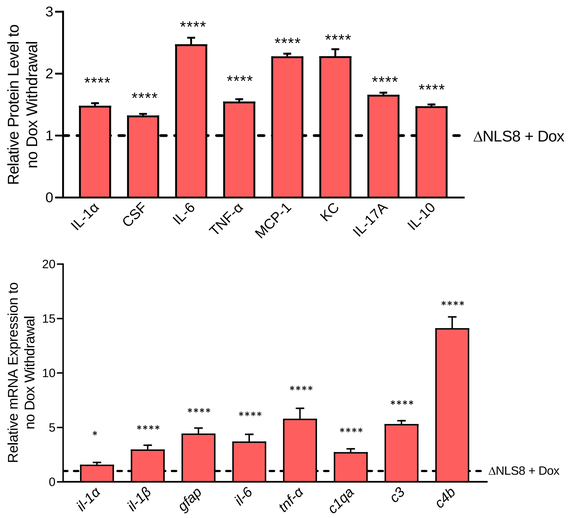
- Bigenic mouse model, generated in Virginia Lee's lab (detailed in Walker *et al*, 2015), bred at PsychoGenics
  - *NEFH*-tTA: tTA under NEFH (neurofilament heavy chain) promoter, neuron specific
  - *tetO*-hTDP-43ΔNLS: Human TDP-43 with nuclear localization signal removed (ΔNLS)
- tTA binds to tetO binding region when no dox is present, resulting in expression
- Dox diet inactivates tTA, no expression
- Dox-diet until five weeks of age for mouse studies



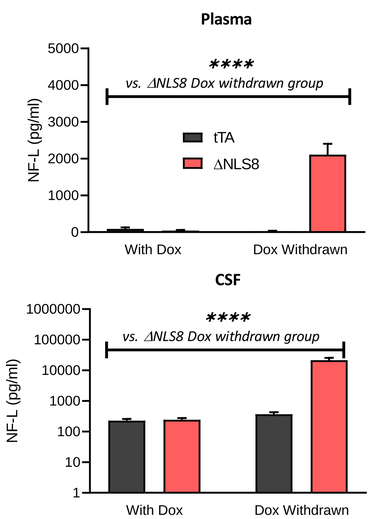
- Body weights were taken twice weekly and averaged; clasping and tremor were assessed weekly
- Kaplan-Meier curves were generated to facilitate survival analysis using Mantel-Cox test
- Gait analysis was performed using Psychogenics proprietary NeuroCube system, which uses computer vision to automatically capture and score changes in gait (geometry and dynamics), paw pressure, paw imaging, body positioning, and other measures in mice or rats.
- Compound muscle action potential (CMAP) responses were obtained from isoflurane-anesthetized animals using a Natus Neurology VikingQuest EMG system. Sub-dermal stimulus needle electrodes were placed at the sciatic notch, recording electrodes were placed at a predetermined distance in the gastrocnemius muscle. Maximal responses were generated and composite responses (average of five responses) were used for analysis.
- For IHC, brain tissues were collected and drop-fixed in 4% PFA and processed using standard immunohistochemical methods.
- For quantification of NF-L in plasma and CSF, samples were collected from animals at 10 weeks of age, 5 weeks following removal of dox diet. Measurement of NF-L was performed using the Quanterix system.
- Inflammatory protein markers were quantified using a multiplex Luminex assay
- Inflammatory mRNA transcripts were quantified by qPCR

Walker AK, Spiller KJ, Ge G, et al. Functional recovery in new mouse models of ALS/FTLD after clearance of pathological cytoplasmic TDP-43. Acta Neuropathol. 2015;130(5):643–660.

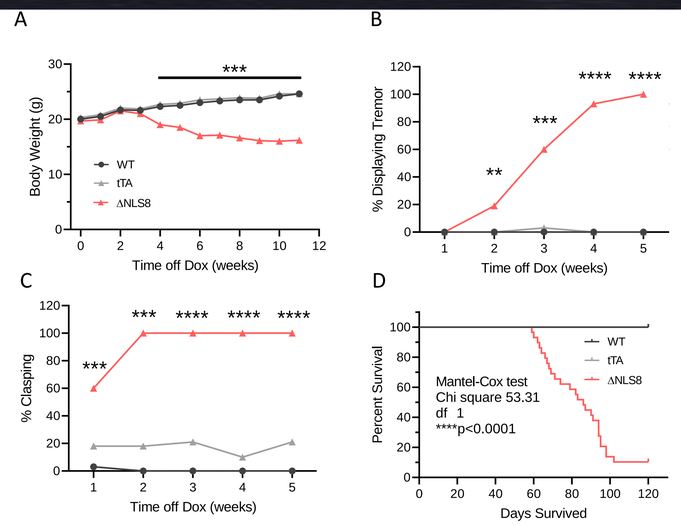
Results



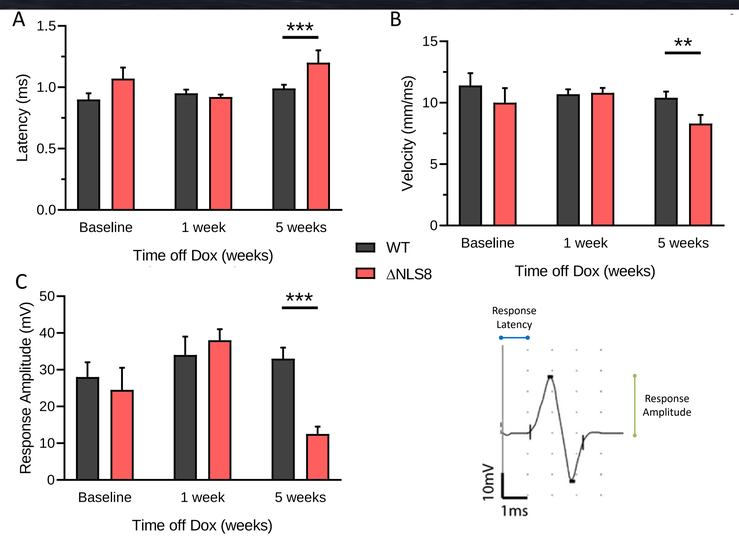
Inflammatory Marker Proteins and mRNA in Cortex of 10 Weeks Old ΔNLS8 (5 Weeks dox Removal). **A**. Inflammatory marker proteins IL-1α, CSF, IL-6, TNF-α, MCP-1, KC, and IL-17A, IL-10, levels were significantly elevated in cortex of ΔNLS8 dox-withdrawn mice as compared to those of dox-on mice. Proteins were measured by multiplex Luminex assay and presented normalized to the input protein. **B**. Transcript expression levels for inflammatory markers il-1α, il-1β, gfap, il-6, tnf-α, c1qa, c3 and c4b were significantly elevated in cortical lysates of ΔNLS8 dox-withdrawn group compared to those of dox-on group. mRNA expression levels were measured by RT-qPCR.



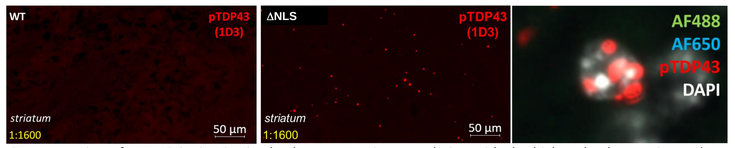
Plasma and CSF NF-L concentrations were significantly increased in 10-week old ΔNLS8 mice that were maintained off doxycycline for 5 weeks, as compared to those maintained on doxycycline treatment for the same time. NF-L levels were measured by IMOA Quanterix technology. Dox withdrawal had no statistically significant effect on tTA control mice (n=8).



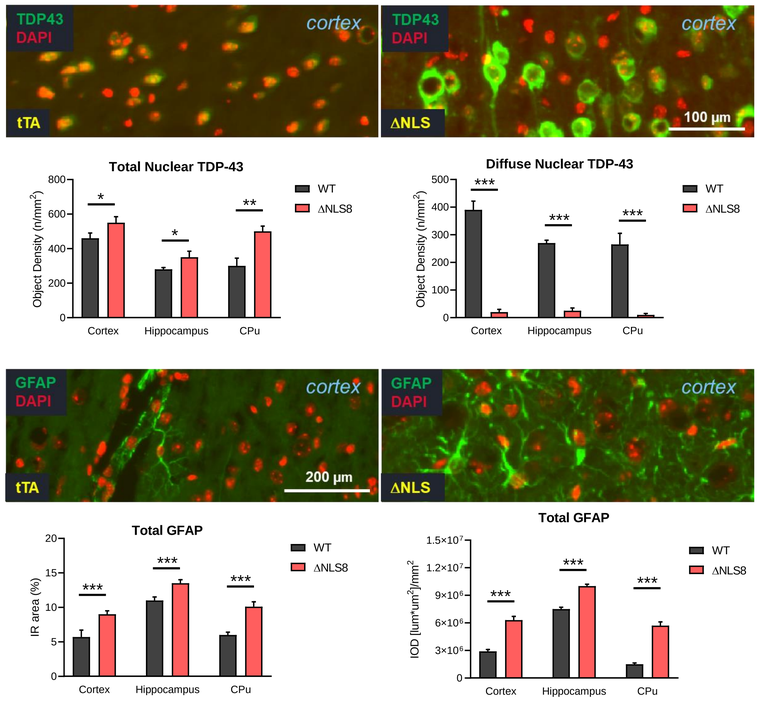
Broad health and behavioral outcomes in combined male and female WT, tTA and ΔNLS mice, following removal of dox-containing chow. **A.** Body weight over time by group. **B.** Onset of tremor phenotype in ΔNLS mice is observed approximately 2 weeks following removal of dox diet. **C**. Clasping behavior is observed in all ΔNLS mice at 2 weeks following removal of dox diet. **D**. Kaplan-Meier survival curves, showing survival over time. ΔNLS animals have an median lifespan of 87.5 days, whereas no mortality was observed out to 120 days in either WT or tTA animals.



Compound muscle action potential (CMAP) responses in gastrocnemius muscle as recorded via EMG in combined male and female WT, and ΔNLS mice, following removal of dox-containing chow. Note deficits in measured parameters appear by 5 weeks following removal of dox diet. **A**. CMAP response latency **B**. Neuromuscular conduction velocity, a measure incorporating both nerve conduction velocity and latency at neuromuscular junction. **C**. CMAP response amplitude.

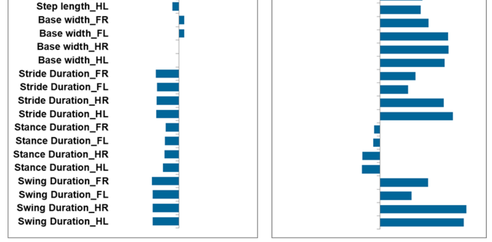


Overexpression of TDP-43 in ΔNLS mice leads to pTDP-43 accumulation with the highest loads seen in caudate putamen and cerebral cortex. While endogenous TDP-43 is located within nuclei of WT neurons, pTDP-43 aggregates accumulate around the nucleus. AF = autofluorescence at wavelengths indicated.



TOP: Overall nuclear object density is greater in ΔNLS compared to WT. and cerebral cortex. Diffuse signals at lower intensity as seen in WT are nearly absent in ΔNLS, as TDP-43 has localized to the cytoplasm. **BOTTOM**: Astrogliosis is significant in ΔNLS cerebral cortex, hippocampus, and dorsal striatum. GFAP staining in cortical tissue shown.

Time off Dox:	1 week	3 weeks
Discrimination vs WT:	96%*	90%*
Discrimination vs tTA:	76%*	94%*



NeuroCube gait analysis identifying salient differences in gait of ΔNLS animals compared to age-matched WT animals. Based on the decorrelated features significant discrimination between WT or tTA and ΔNLS animals is possible as early as 1 week following removal of dox diet. These plots show feature-specific differences in gait of ΔNLS animals (change relative to WT) at 1 and 3 weeks following removal of dox diet.

Summary

- To study the progression of ALS phenotypes and establish a model for testing therapeutic interventions, PsychoGenics has characterized the TDP-43ΔNLS mouse model of ALS.
- TDP-43ΔNLS mice showed dramatic loss of body weight following dox cessation, increased tremors and hindlimb clasping, impaired gait and muscle strength and decreased survival compared to WT and tTA mice.
- EMG assessment of muscle function in ΔNLS mice showed increased latency and decreased response amplitudes, with increasing severity correlated with the amount of time spent off dox diet.
- Histological analysis revealed strong overexpression of TDP-43 in perinuclear cytoplasmic inclusions along with deposition of pTDP43 aggregates.
- TDP-43 pathologies were accompanied by increased expression of inflammatory marker proteins and transcripts, astrogliosis, and dramatic elevations in neurofilament light chain in plasma and CSF of ΔNLS animals.

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