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ALS-linked TDP-43^{M337V} knock-in mice exhibit splicing deregulation without neurodegeneration

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Abstract

Abnormal accumulation of TAR DNA-binding protein 43 (TDP-43), a DNA/RNA binding protein, is a pathological signature of amyotrophic lateral sclerosis (ALS). Missense mutations in the *TARDBP* gene are also found in inherited and sporadic ALS, indicating that dysfunction in TDP-43 is causative for ALS. To model TDP-43-linked ALS in rodents, we generated TDP-43 knock-in mice with inherited ALS patient-derived TDP-43^{M337V} mutation. Homozygous TDP-43^{M337V} mice developed normally without exhibiting detectable motor dysfunction and neurodegeneration. However, splicing of mRNAs regulated by TDP-43 was deregulated in the spinal cords of TDP-43^{M337V} mice. Together with the recently reported TDP-43 knock-in mice with ALS-linked mutations, our finding indicates that ALS patient-derived mutations in the *TARDBP* gene at a carboxyl-terminal domain of TDP-43 may cause a gain of splicing function by TDP-43, however, were insufficient to induce robust neurodegeneration in mice.

Keywords: Amyotrophic lateral sclerosis (ALS), TDP-43, TDP-43 knock-in mice

Main text

Abnormal accumulation of TDP-43 has been identified as a pathological signature of amyotrophic lateral sclerosis (ALS), an adult neurodegenerative disease characterized by a selective loss of motor neurons, and a part of frontotemporal dementia (FTD) [1]. Cytoplasmic accumulation of TDP-43 with a loss of TDP-43 from nuclei, known as a TDP-43 pathology, is observed in almost all forms of ALS, including sporadic and familial ALS. To date, more than 50 mutations in the *TARDBP* gene, encoding TDP-43, have been identified in inherited and sporadic ALS, implicating TDP-43 dysfunction as a central component for ALS pathogenesis [2]. TDP-43 is a ubiquitously expressed DNA/RNA binding nuclear protein and plays multifunctional roles in RNA metabolism, including pre-mRNA splicing, translational control, and mRNA stability [3]. Of note, TDP-43 is known to control its own mRNA stability through binding to the 3′ UTR, indicating that the level of TDP-43 protein is tightly regulated [3]. Indeed, overexpression of wild-type TDP-43 in mice induces neurodegeneration, whereas elimination of TDP-43 leads to embryonic lethality [4, 5]. However, it is still unclear whether dysfunction in TDP-43 leads to neurodegeneration through a gain or loss of TDP-43 function. To model TDP-43-mediated neurodegeneration in mice, several lines of transgenic mice have been developed and reproduced some features of neurodegeneration observed in human ALS/FTD. However, the overexpression approach has a limitation in differentiating the role between wild-type and mutant TDP-43 in motor neuron health and disease in mice [4, 5].

Based on these backgrounds, we set out to create a knock-in mouse model carrying an ALS patient-derived mutation in the murine *Tardbp* gene. Of more than 50 known mutations, we chose TDP-43^{M337V} mutation for the following reasons: TDP-43^{M337V} protein has a long half-life in cells, the ALS patients with this mutation show earlier disease onset [6, 7], and an amino acid

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sequence of 241–414 including a methionine residue at position 337 is highly conserved among mouse and human. We engineered mice with n.1009 A > G (M337V) mutation in the murine *Tardbp* gene by utilizing CRISPR/Cas9 genome-editing technology (Additional file 1). Both homozygous and heterozygous mice carrying the allele of TDP-43^{M337V} developed normally as recently reported (Fig. 1a, Additional file 1: Figure S1, S2) [8].

TDP-43 plays a pivotal role in regulating alternative splicing as well as controlling the level of TDP-43 mRNA itself by a negative feedback mechanism. Therefore, we first examined whether ALS-linked TDP-43^{M337V} mutation affects the expression level of its own mRNA in mice. Analysis of TDP-43 mRNA levels in the brains of 700-days-old homozygous TDP-43^{M337V} mice (TDP-43$^{M337V/M337V}$) revealed that there was no difference in expression level between wild-type and TDP-43$^{M337V/M337V}$ mice (Fig. 1b). In addition, the mRNA levels of *Notch1* and *Nek1*, known as TDP-43 target genes, were unaffected by homozygous M337 V mutation (Additional file 1: Figure S3). We next examined whether TDP-43^{M337V} deregulates alternative splicing of mRNAs that are known as splicing targets of TDP-43. Among the several splicing targets examined, we found a 1.49-fold increase in inclusion of *Kcinp2* exon 2/3, a 0.85-fold decrease in exclusion of *Sort1* exon 17b, and a 0.63-fold decrease in exclusion of *Sema3f* exon 5 in the brain of TDP-43$^{M337V/M337V}$ mice (Fig. 1c). Although there were no significant changes in other splicing targets, *Poldip3* and *Eif4h* (Additional file 1: Figure S4), changes in splicing of *Kcinp2, Sort1, and Sema3f* in TDP-43$^{M337V/M337V}$ mice are consistent with a gain of TDP-43 function [9, 10].

Since the mislocalization of TDP-43 protein in cytoplasm is a pathological signature of ALS, we examined subcellular localization of TDP-43^{M337V} mutant protein in the affected tissue in TDP-43$^{M337V/M337V}$ mice. Both mutant and wild-type TDP-43 proteins expressed at the similar level, and were predominantly localized in nucleus of brain and spinal cords of 700-days-old TDP-43$^{M337V/M337V}$ and wild-type mice (Fig. 1d, e), suggesting that disease-causing missense mutation in TDP-43 alone did not alter the protein level itself and was insufficient to induce protein mislocalization in mice. Moreover, carboxyl-terminal (C-terminal) fragments of TDP-43, characteristic of TDP-43 pathology, were not detected in the brains and spinal cords of TDP-43$^{M337V/M337V}$ mice (Fig. 1d), and there was no detectable loss of motor neurons or reactive gliosis in TDP-43$^{M337V/M337V}$ mice (Fig. 1e-g, Additional file 1: Figure S5). Nuclear Gems, where SMN complex resides to control splicing, are known to be regulated by TDP-43 and FUS [11–13]. In ventral horn neurons of TDP-43$^{M337V/M337V}$ mice, the number of nuclear Gems was not altered (Additional file 1: Figure S6). We further examined whether TDP-43$^{M337V/M337V}$ mice show motor dysfunction with aging. Measurement of rotarod and clasping scores as well as body weights revealed no difference in those scores between TDP-43$^{M337V/M337V}$ and wild-type mice until 18 months old (Fig. 1h, i, Additional file 1: Figure S2).

The present study demonstrates that homozygous TDP-43^{M337V} mice generated by CRISPR/Cas9 show splicing deregulation of some TDP-43 target mRNAs without apparent deterioration in motor function and pathology until 20 months old. Recently, homozygous TDP-43^{Q331K} knock-in mice showed a reduced number of parvalbumin-positive interneurons and cognitive dysfunction with phenotypic heterogeneity [9]. Homozygous TDP-43^{G298S} or TDP-43^{M337V} knock-in mice showed very mild denervation of hindlimbs at 2.5 years of age [8]. Besides, heterozygous TDP-43^{M323K} mice, generated by *N*-ethyl-*N*-nitrosourea (ENU) random mutagenesis, showed modest neurodegenerative phenotype [10]. These mutant mice uniformly show very mild phenotypes, likely because the 2-years-life span of rodents may be insufficient to induce neurodegeneration derived from splicing deregulation caused by mutant TDP-43.

Our TDP-43$^{M337V/M337V}$ mice showed splicing deregulation of TDP-43 target mRNAs, *Kcinp2, Sort1, and Sema3f*, suggesting that M337 V mutation causes a gain of function in TDP-43. Gain of TDP-43 function is also suggested in TDP-43^{Q331K} and TDP-43^{M323K} mice [9, 10]. All three missense mutations discussed here are located in the low complexity region at the C-terminal of TDP-43, suggesting that ALS-causing TDP-43 mutations in the C-terminal region may cause gain of TDP-43 function, at least, at an initial disease stage. This point makes a good contrast with the role of N-terminal TDP-43 fragment in dominant-negative function in TDP-43 [14]. In our study, the mRNA and protein levels of TDP-43 were unchanged in TDP-43^{M337V} mice, while they were moderately upregulated in TDP-43^{Q331K} [9]. This difference may explain the more modest phenotype of our TDP-43^{M337V} mice.

All knock-in mice carrying ALS-linked missense mutations in TDP-43 do not show robust TDP-43 pathology even in homozygous mutant mice. Perhaps, additional conformational change of TDP-43 protein may be needed to develop TDP-43 pathology. Finally, our results from TDP-43^{M337V} knock-in mice further strengthen the findings that mutations at the C-terminal region of TDP-43 likely cause a gain of TDP-43 splicing function at an initial stage of the disease, which may be followed by the loss of TDP-43 function due to a loss of TDP-43 proteins from nuclei.

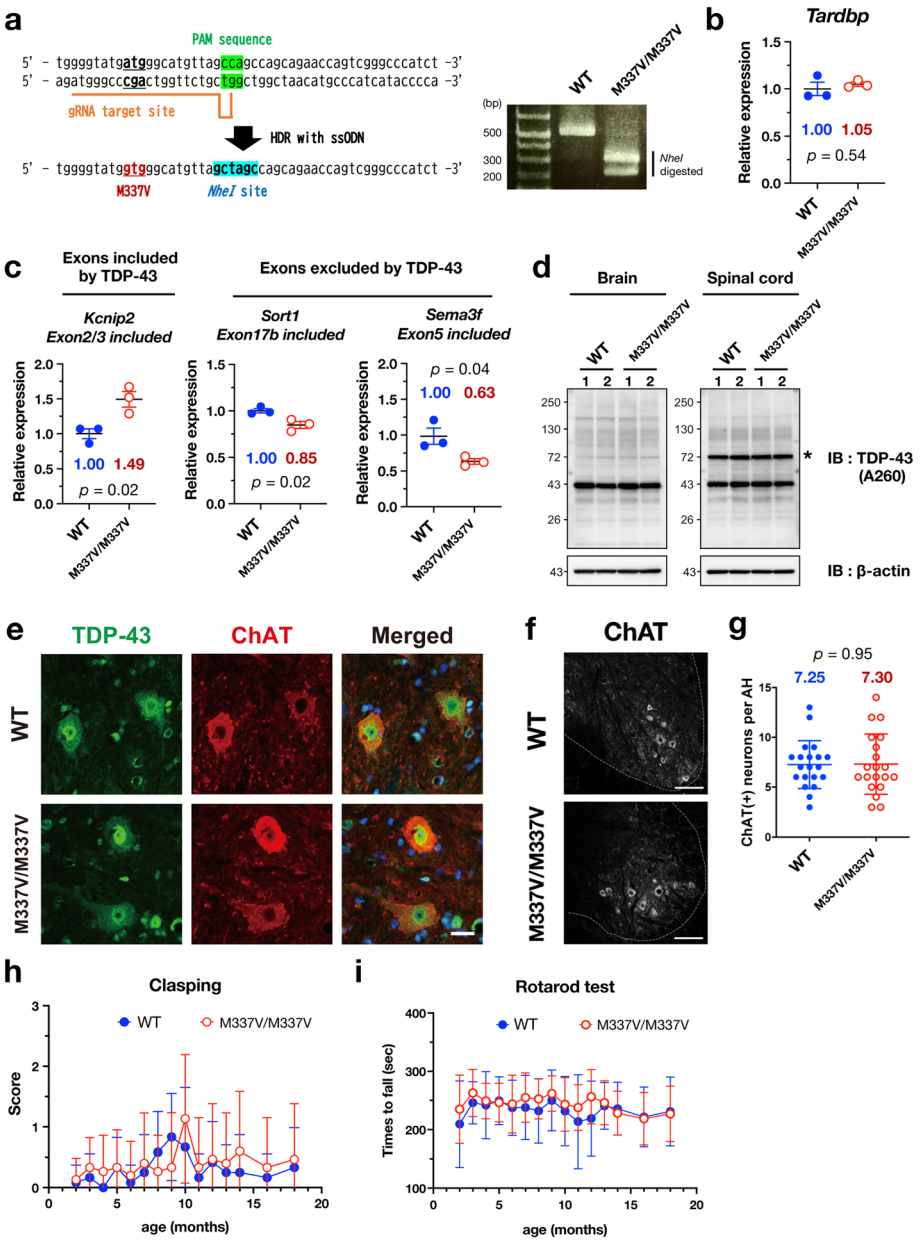

Fig. 1 Characterization of TDP-43^{M337V} knock-in mice. **a** Schematic illustration of introducing TDP-43^{M337V} mutation into an endogenous murine *Tardbp* exon 6 (left panel). The representative genotyping result is also shown (right panel). *Nhe I* restriction site is introduced in the mutant allele, resulting in no change of the amino acid at *Nhe I* site. **b** The expression level of *Tardbp* mRNA was not altered in the brains of 700-days-old TDP-43$^{M337V/M337V}$ (M337 V/M337 V) mice and wild-type (WT) littermates. **c** Alternation in splicing of genes regulated by TDP-43. The level of mRNA containing exons included by TDP-43 (*Kcnip2* exon 2 and 3) was increased, while the levels of mRNA containing exons excluded by TDP-43 (*Sort1* exon 17b and *Sema3f* exon 5) were reduced, suggesting a gain-of-function mechanism in TDP-43$^{M337V/M337V}$ mice. Relative expression levels of mRNA normalized to the WT control are plotted with SD ($n = 3$ each (**b**, **c**)) and were analyzed by unpaired *t*-tests. **d** Representative immunoblots of TDP-43 and β-actin in the brains and spinal cords of 700-days-old TDP-43$^{M337V/M337V}$ mice and WT littermates. Asterisk denotes a non-specific band. **e** and **f** Representative immunofluorescence images of the anterior horn in lumbar spinal cords of 700-days-old TDP-43$^{M337V/M337V}$ mice and WT littermates stained with anti-TDP-43 (3H8, green) and anti-ChAT (red) antibodies along with the merged images. TDP-43 was not mislocalized in motor neurons of TDP-43$^{M337V/M337V}$ mice (**e**). Low magnification images stained with anti-ChAT antibody (**f**). Scale bars: 20 μm (**e**), 100 μm (**f**). **g** Quantification of the numbers of ChAT-positive motor neurons per each anterior horn (AH) in the lumbar spinal cords of 700-days-old TDP-43$^{M337V/M337V}$ mice and WT littermates. For quantification, 20 AHs in three animals per each genotype were counted, and data are plotted as mean ± SD, and were analyzed by an unpaired *t*-test. **h** and **i** TDP-43$^{M337V/M337V}$ mice did not show any motor dysfunction phenotypes in the measurement of clasping score (**h**) and rotarod test (**i**). Data are plotted as mean ± SD, and were analyzed with two-way ANOVA. $n = 15$ for WT and 14 for M337 V/M337 V

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13041-020-0550-4.

Additional file 1. Material and methods. **Figure S1.** Direct sequencing of *Tardbp* gene exon 6 in heterozygous TDP-43^{M337V} knock-in mice. **Figure S2.** Body weights were not affected in TDP-43^{M337V} knock-in mice. **Figure S3.** Relative expression levels of *Notch1* and *Nek1* mRNAs were not altered in the brain of aged homozygous TDP-43^{M337V} mice. **Figure S4.** Splicing was not altered in *Eif4h* or *Poldip3*, which are also regulated by TDP-43, in the brain of aged homozygous TDP-43^{M337V} mouse brains. **Figure S5.** Gliosis was not observed in ventral horn of aged (700 days-old) homozygous TDP-43^{M337V} mice. **Figure S6.** The number of Gems was not affected in ventral horn neurons of aged (700 days-old) homozygous TDP-43^{M337V} mice.

Abbreviations

ALS: Amyotrophic Lateral Sclerosis; ChAT: Choline acetyltransferase; FTD: Frontotemporal dementia; SMN: Survival of motor neurons; TDP-43: TAR DNA-binding protein 43

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Authors' contributions

SW and KY designed the study. SW, KO, and YM performed the experiments with support from OK, AS, and FE under the supervision of KY. ET and SW established the TDP-43^{M337V} mice. SW, KO, and KY interpreted the data and wrote the manuscript. All authors approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All experiments using genetically modified mice were approved by the Animal Care and Use Committee and the recombinant DNA experiment committee of Nagoya University and RIKEN.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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