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Deficiency in the *ALS2* gene does not affect the motor neuron degeneration in SOD1^{G93A} transgenic mice

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Abstract

Dysfunction of the *ALS2* gene has been linked to one form of juvenile onset autosomal recessive amyotrophic lateral sclerosis (ALS). Previous *in vitro* studies suggest that over-expression of *ALS2* protects cells from mutant Cu/Zn superoxide dismutase (SOD1)-induced cytotoxicity. To test whether *ALS2* plays a protective role against mutant SOD1-mediated motor neuron degeneration *in vivo*, we examined the progression of motor neuron disease in SOD1^{G93A} mice on an *ALS2* null background. Our data suggest that deficiency in the *ALS2* gene does not affect the pathogenesis of SOD1^{G93A} mice.

Keywords

Amyotrophic lateral sclerosis (ALS); ALS2; Alsin; SOD1; SOD1^{G93A} mice

1. Introduction

Amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease, manifests as progressive muscle weakness and spastic paralysis, reflecting a selective loss of upper and lower motor neurons in the CNS [2]. Mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) cause motor neuron degeneration through a gain of toxic property [2]. Recently, mutations in a second ALS-related gene (*ALS2*) were identified that cause a rare recessive form of juvenile onset ALS [5,9]. Previously, we and others have generated *ALS2* knockout (*ALS2*$^{-/-}$) mice that failed to display any obvious motor neuron degeneration [1,4]. Since over-expression of *ALS2* protects cells from SOD1-mediated cytotoxicity and loss of *ALS2* predisposes neurons to paraquat-induced oxidative stress [1,7], *ALS2* may serve as a risk factor for motor neuron disease. In this study, we examined whether the deficiency in the *ALS2* gene affected the well-characterized motor neuron degeneration in SOD1^{G93A} transgenic mice [3].

2. Methods

The B6SJL-SOD1^{G93A} mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). The generation of *ALS2*$^{-/-}$ mice, rotarod test and histological analysis were conducted as previously described [1]. Survival data were analyzed using a log-rank test and statistical significant differences were at a minimal level of significance of $p < 0.05$.

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Conflicts of interest There is no conflict of interest involved in any part of this study.

3. Results

To investigate whether loss function of *ALS2* affects the motor neuron degeneration in the well-characterized SOD1^{G93A} transgenic mice [3], we first crossed SOD1^{G93A} mice with *ALS2*$^{-/-}$ mice to generate SOD1^{G93A}/*ALS2*$^{+/-}$ and *ALS2*$^{+/-}$ mice. These mice were then intercrossed to generate both SOD1^{G93A}/*ALS2*$^{+/+}$ and SOD1^{G93A}/*ALS2*$^{-/-}$ mice. Age-matched littermates were used in all experiments. Motor coordination was measured by a rotarod test starting from 8 weeks of age. We could not detect any significant differences between SOD1^{G93A}/*ALS2*$^{-/-}$ and SOD1^{G93A}/*ALS2*$^{+/+}$ mice in this motor test (Fig. 1A , $p = 0.54$). There was also no significant difference between SOD1^{G93A}/*ALS2*$^{-/-}$ and SOD1^{G93A}/*ALS2*$^{+/+}$ mice in the body weight (Fig. 1B, $p = 0.67$) or survival rate (Fig. 1C, $p = 0.11$). We counted the numbers of motor neurons per lumbar spinal cord section (10 [H9262]m thickness) at the end stage of the mouse (Fig. 1D), and failed to detect any significant differences between these two groups of mice (SOD1^{G93A}: 9.9 ± 0.9 versus SOD1^{G93A}; *ALS2*$^{-/-}$: 12.0 ± 1.3; $p = 0.20$). Together, our data indicate that the *ALS2*-deficiency does not affect the motor neuron degeneration in SOD1^{G93A} transgenic mice (Fig. 1).

4. Discussion

SOD1^{G93A} transgenic mice die within 4−5 months of age and display extensive degeneration of spinal motor neurons [3]. Based on previously reported *in vitro* data that *ALS2* plays a protective role against mutant SOD1-mediated toxicity [7], we hypothesized that SOD1^{G93A}/*ALS2*$^{-/-}$ mice would exhibit a shorter life span and display more severe motor neuron degeneration compared with SOD1^{G93A}/*ALS2*$^{+/+}$ mice. Surprisingly, we did not observe any obvious effects of the loss of *ALS2* gene on motor neuron degeneration or survival of SOD1^{G93A} transgenic mice (Fig. 1), suggesting that *ALS2* plays a very limited role in protecting spinal motor neurons from SOD1-mediated toxicity *in vivo*. The absence of obvious alteration in the pathogenesis of SOD1^{G93A} transgenic mice lacking *ALS2* gene could be related to gene redundancy, where genes with similar function can compensate for the loss of function of *ALS2*. Recently, an *ALS2*-related protein called ALS2CL has been characterized, which is highly homologous to the C-terminal half of *ALS2* [6]. Despite both *ALS2* and ALS2CL interact with the Rab5 GTPase, they appear to play different roles in the Rab5-mediated endosomal trafficking [6]. It is questionable whether ALS2CL can compensate for the loss of *ALS2*. Another caveat for this study is that the extremely rapid progression of motor neuron degeneration in this line of SOD1^{G93A} transgenic mice may potentially mask any further deteriorating effect on the motor neuron caused by the deficiency in the *ALS2* gene. However, although it is not statistically significant, the loss of *ALS2* gene seems to protect the motor neuron from degeneration in SOD1^{G93A} transgenic mice (Fig. 1), echoing a recent finding of SOD1^{G93A}/Loa double mutant mice in which a mutation in the dynein heavy chain partially rescues the axonal transport defect of spinal motor neurons in SOD1^{G93A} transgenic mice [8]. It remains a challenging task to define the pathogenic pathways mediated by either the missense mutations of *SOD1* or the loss of function mutations of *ALS2* gene.

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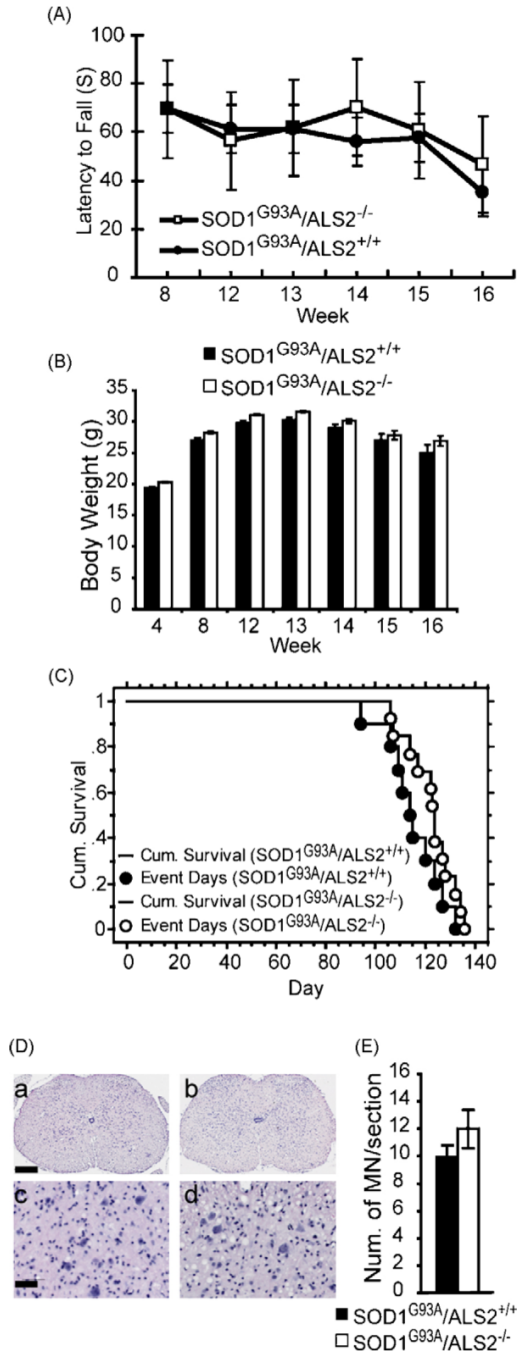

Fig. 1.

The pathogenesis of SOD1^{G93A}/*ALS2*$^{-/-}$ mice. (A) Male SOD1^{G93A}/*ALS2*$^{+/+}$ (*n* = 10) and SOD1^{G93A}/*ALS2*$^{-/-}$ (*n* = 13) mice were tested on an accelerated rotating rod at 8, 12, 13, 14, 15, and 16 weeks of age and the latency to fall was recorded. (B) The bodyweight of SOD1^{G93A}/*ALS2*$^{+/+}$ (*n* = 13, 8, and 7 at 4−13, 14−15, and 16 weeks of age, respectively) and SOD1^{G93A}/*ALS2*$^{-/-}$ (*n* = 14, 13, and 12 at 4−12, 13−14, and 15−16 weeks of age, respectively) mice were measured. (C) Kaplan–Meier plot of cumulative probability of survival of SOD1^{G93A}/*ALS2*$^{+/+}$ (*n* = 10) and SOD1^{G93A}/*ALS2*$^{-/-}$ (*n* = 13) mice. (D) HE staining revealed motor neurons in lumbar spinal cords of SOD1^{G93A}/*ALS2*$^{+/+}$ (a and c) and SOD1^{G93A}/*ALS2*$^{-/-}$ mice (b and d). Scale bar = 1000 [H9262]m (a) or 100 [H9262]m (c). (E) Quantification

of the numbers of motor neurons remained in the age-matched SOD1^{G93A}/*ALS2*$^{+/+}$ (n = 10) and SOD1^{G93A}/*ALS2*$^{-/-}$ (n = 10) mice. Error bars represent SEM.