Interaction of Als2 gene deficiency with dietary neurotoxin leads to increased pathophysiological features

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Introduction

Autosomal recessive juvenile onset amyotrophic lateral sclerosis (ALS2) are reported to be caused by loss of function mutations of the ALS2 gene encoding alsin, a protein with suggested roles in endosomal transport and guanine nucleotide exchange activity1. To establish an animal model of ALS2, mice that are deficient in the Als2 gene are generated, and have previously been shown to exhibit significant but subtle neuropathological changes and behavioural alterations2. This raises the possibility that the ALS2 phenotype is a result of the interaction of genetic and environmental factors. Therefore, we studied the influence of dietary exposure to β‐stero1d β‐D‐glucoside (BSSG) on the motor performance of Als2 and wild‐type mice, hypothesizing that Als2 mice are possibly more susceptible to such neurotoxicity. BSSG is the largest fraction of steryl glucoside in cycad seeds and has been shown to induce amyotrophic lateral sclerosis‐parkinsonism dementia complex in mice, with a dominant phenotype resembling ALS5.

Methods

Mice were given their diet pellet after removing their regular diet of mouse chow from their cages at a fixed time of day and monitored weekly for weight changes. A comprehensive behavioral analysis was performed weekly, including ventral plane videography for gait analysis, open field testing, stepping reflex scoring, and measurements of rotarod and wire hang performance. Upon consuming the entire pellet, mouse cages were replenished with regular mouse chow ad libitum until the next feeding time. Mice were exposed daily to BSSG for 18 weeks and at the end of the experiment animals were perfused and processed for histology. Anterior horn cells were visualized with fast cresyl violet staining for Nissl substance, FITC choline acetyltransferase (ChAT) or GFAP.

Summary of Results

Mice deficient in Als2 gene show subtle differences in gait patterns when exposed to BSSG. These mice show shorter stance duration, stride distance and stride length than control‐fed KO mice (p < 0.05). In addition, these mice also exhibit increased stride frequency (p < 0.05). WT mice fed BSSG exhibit a greater stride frequency than their KO counterparts (p < 0.05). Exposure to BSSG results in a shorter stride and faster, more frequent steps during locomotion irrespective of genotype. Als2 deficient mice are less active, exhibiting slower and wider turns during meandering. The pathological features of anterior horn cells in these animals resembles clinical ALS.

Conclusion: BSSG effects gait dynamics while Als2 genotype exerts its effects on open field activity.

1. Gait Dynamics and Activity

Als2 mice exposed to BSSG exhibit shorter stride and faster, more frequent steps during locomotion irrespective of genotype. (A) Stride frequency, also underlined, is the number of times per second that a leg takes a complete stride. BSSG‐fed animals have a higher stride frequency in motion. (B) Stance duration is the time where the paw is in contact with the belt during motion, and is shorter in BSSG‐fed animals. (C) Stride duration is the time for one complete stride of one paw, and is shorter for BSSG‐fed animals. (D) Stride length is the distance between successive strides of the same paw, and is shorter for BSSG‐fed animals. A two‐way ANOVA of the entire data set for each parameter consistently showed diet p < 0.10 while both age and genotype were not significant.

Als2 mice are hypactive. KO mice were demonstrated to have a smaller meander, and they spend less time in motion and travel shorter distances than WT mice. In addition, KO mice spend less time in the centre of the arena and showed a preference to remain along the perimeter of the arena. More importantly, administration of BSSG to their diet does not reverse nor amplify this significance between groups. Two‐way ANOVA analysis of the entire data set for each parameter consistently showed genotype p < 0.01 while both age and diet were not significant.

References


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