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Research Report

# Evolution of gait abnormalities in SOD1 $^{G93A}$ transgenic mice

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the loss of upper and lower motoneurons. Clinically, it is manifested by weakness, muscle atrophy and progressive paralysis and ends up with patients' death 2–5 years after diagnosis. Although these symptoms lead in many cases to gait deficits in patients, an exhaustive locomotor profile of animal models mimicking the disease has not been assessed yet. In this work we evaluated the locomotor performance of the SOD1$^{G93A}$ mouse model of ALS using computerized treadmill gait analysis. SOD1$^{G93A}$ mice presented early (8 weeks of age) gait abnormalities, evidenced by an increase in the time of the propulsion phase of hindlimb stance. The alterations progressed during the disease until a complete disturbance of normal gait. This finding is meaningful to the field because the identification of a significant difference in a functional endpoint as early as 8 weeks might be a step forward resolving the debate about treatment of mice prior to the symptomatic phase in efficacy studies. These results also point out that digitizing analysis of treadmill locomotion may be useful to evaluate whether new therapeutic approaches are improving functional outcome of the animals.



## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common form of motoneuron disease, characterized by degeneration of both upper and lower motoneurons. Clinically, ALS manifests as weakness, muscle atrophy and progressive paralysis, and finishes with patients' death a few years after diagnosis (Wijesekera and Leigh, 2009; Worms, 2001), since there are no effective treatments available (Ludolph and Jesse, 2009). The development of transgenic animal models carrying genetic mutations described in familial ALS cases (Rosen, 1993) has facilitated the study of etiopathogenic factors and therapeutic strategies for the human disease. The most widely used is the SOD1$^{G93A}$ transgenic mouse, whose phenotype recapitulates the clinical and histopathological features of ALS (Miana-Mena et al., 2005; Ripps et al., 1995; Turner and Talbot, 2008). Although animal models carrying SOD1 mutations have been developed based on familial cases of ALS, it has been recently described that alterations of SOD1 protein are also related to sporadic ALS cases (Bosco et al., 2010), thus, increasing the applicability of studies based on these transgenic animals.

Detailed gait analysis applied to common laboratory species has provided valuable information regarding human and quadruped locomotion (Clarke and Still, 1999; Gillette and

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*Abbreviations:* ALS, amyotrophic lateral sclerosis; CMAP, compound muscle action potential; CV, coefficient of variation; PL, print length; SOD, superoxide dismutase; TS, toe spreading

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Angle, 2008; Varejão et al., 2001), and has shown sensitivity to detect disease- or injury-related changes. Until now, the most common behavioral locomotion techniques used in mice have been footprint analysis (mainly used to evaluate recovery from peripheral nerve lesions) (de Medinaceli et al., 1982; Varejão et al., 2001) and the rotarod test (mainly used in peripheral neuropathies and neurodegenerative models including SOD1 mice) (Kaplan and Murphy, 1972; Knippenberg et al., 2010; Miana-Mena et al., 2005; Verdu et al., 1999). However, they are limited by their low sensitivity and specificity.

Historically, the locomotor performance of SOD1$^{G93A}$ mice has been evaluated using the rotarod test (Gurney et al., 1994; Miana-Mena et al., 2005) as one of the primary measures of disease progression and its modification by therapeutical strategies. However, the rotarod test lacks sensitivity, since it only begins to detect motor deficits at 13 weeks of age. These deficits progress rapidly until complete hindlimb paralysis at 16–18 weeks, thus providing a relatively narrow time frame in which to detect possible changes. Thus, rotarod is not sufficiently sensitive to detect motor deficits prior to the onset of overt clinical symptoms, despite histological and electrophysiological evidences demonstrating earlier motor abnormalities in pre-symptomatic SOD1 mice (Azzouz et al., 1997; Fischer et al., 2004; Kennel et al., 1996; Mancuso et al., 2011). Moreover, the rotarod test is not always able to reveal functional improvement, even when the treatment applied improves the condition of the animals or improves the survival of motoneurons (Fischer et al., 2005). For these reasons, in the current study we sought to evaluate locomotor performance of SOD1$^{G93A}$ mice using a digital video system that captures paw placement during treadmill locomotion and calculates standardized gait parameters (DigiGait™, Mouse Specifics Inc.). Our goal was to determine whether locomotor abnormalities could be detected early in the disease, prior to onset of overt symptoms, as well as during symptomatic and end-stage disease. The capability of early detection would likely increase the possibility of demonstrating functional improvement, the primary objective during testing of potential therapeutic approaches.

## 2. Results

### 2.1. *Rotarod test*

The performance of the SOD1 mice in the rotarod test started to decline between 12 and 13 weeks of age, and continued decreasing until the end stage of the disease, when most animals (75%) were paralyzed and completely unable to hold by themselves in the rod (Fig. 1). In contrast, wild type animals maintained performance during the cut-off 180 s time in the rotarod during all the follow up.

### 2.2. *Footprint analysis*

The analysis of the footprints recorded in the walking track test did not show significant differences in the print length and in the toe spread between SOD1 and wild type mice (Fig. 2; data not shown). Although other authors have considered the stride length as another parameter for the study of mouse locomotion (Knippenberg et al., 2010), we did not find it as a reliable measurement in this test because mice do not usually perform a regular voluntary walk across the walkway.

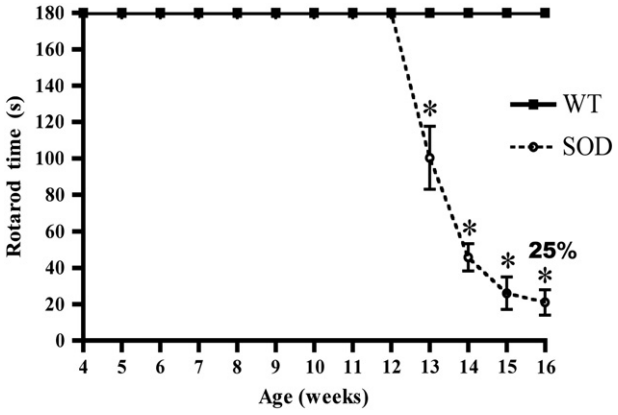


**Fig. 1 – Time on the rotarod in SOD1 (n=12) and wild type (n=10) mice: the time declined after 12 weeks of age in transgenic SOD1 mice, whereas wild type mice maintained permanence in the rotarod during 180 s during all the follow up. Values are mean ± SEM; * $p<0.05$ vs. wild type mice. Only 25% of SOD1 mice were able to maintain in the rotarod during at least 5 s to allow for measurement at 16 weeks.**

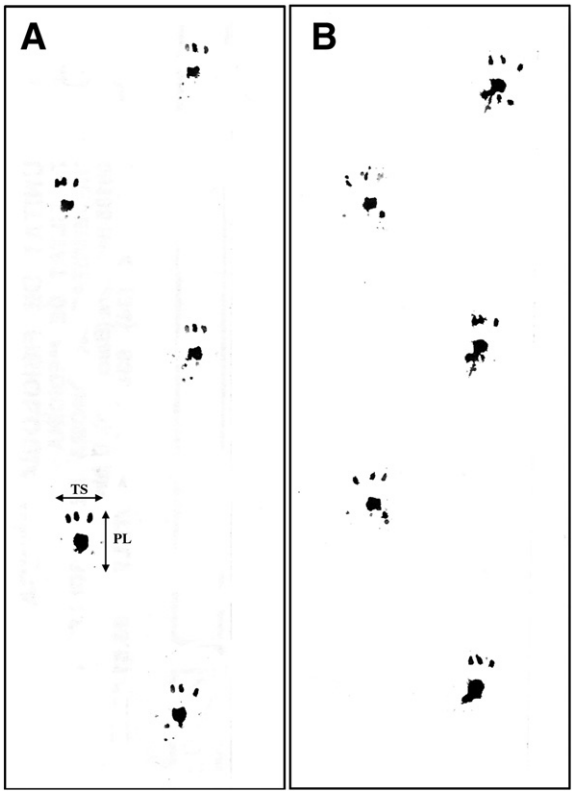


**Fig. 2 – Representative images of footprint analysis of (A) wild type and (B) SOD1 mice at 16 weeks of age. The analysis of the footprints did not show any differences in the print length and in the toe spreading between wild type (n=10) and transgenic SOD1 (n=12) animals. TS, toe spread; PL, print length.**