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Background and gender effects on survival in the TgN(SOD1-G93A)1Gur mouse model of ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disorder. While most cases of ALS are sporadic, 10–15% are familial, and of these 15–20% possess a mutation in the gene that codes for the enzyme Cu/Zn superoxide dismutase (SOD1). In families of ALS patients with specific SOD1 mutations, affected members demonstrate significant heterogeneity of disease and a large variation in age of onset and severity, suggesting that there are genetic modifiers of disease expression.

Transgenic mice expressing mutant forms of SOD1 demonstrate symptoms similar to those seen in patients with ALS. We have observed in our colony of G93A SOD1 transgenic mice a milder phenotype in mice in a C57BL/6J background than the C57BL/6J × SJL/J hybrid background used by Jackson Laboratories to maintain their colony. To investigate the effect of genetic background on phenotype, we have constructed congenic lines on two genetic backgrounds, C57BL/6J (B6) and SJL/J (SJL). We report the influence of background and gender on the survival of these congenic lines compared to the hybrid C57BL/6J × SJL/J background.

The mean survival of G93A SOD1 mice in the hybrid B6/SJL background was 130 days, with females surviving significantly longer than males. When compared to the hybrid B6/SJL background, the survival of mice in the SJL background significantly decreased, and the gender difference in survival was maintained. On the other hand, mean survival in the B6 background significantly increased, and in contrast to the B6/SJL and SJL backgrounds, there was no difference in survival between males and females. Transgene copy numbers were verified in all animals to ensure that any phenotypic differences observed were not due to alterations in copy number. This is the first report of a shortened lifespan when the G93A SOD1 transgene is placed on the SJL/J background and an increased survival with the loss of gender influences when the transgene is placed on the C57BL/6J background.

Keywords: ALS; Genetic modifiers; SOD1; Mice; Background; Gender

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive disorder of the motor system leading to death within 2 to 5 years. Although most cases of ALS are sporadic in origin, 10–15% are inherited. Among the population with familial ALS (FALS), 15–20% possesses a mutation in the SOD1 gene that codes for the enzyme Cu/Zn superoxide dismutase [1,2]. There are more than 100 identified FALS-associated mutations in the SOD1 gene [3]. One paradox about human FALS is that some siblings of the affected proband can have inherited the same mutation at the SOD1 locus but do not show signs of the disease. Furthermore, there is significant heterogeneity of disease within families [4]. These puzzling findings have been seen in multiple studies of unrelated populations and represent a major gap in our knowledge of the role of the SOD1 gene in the pathogenesis of ALS. It has been suggested that there are protective

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modifiers in recessively inherited human SOD1 induced ALS [5]. Finally, the large variation in age of onset and severity in human ALS patients with specific SOD1 mutations lends further support to the likelihood that there are genetic modifiers of disease expression. Identification of these genes would increase insight into the pathogenesis of ALS and may provide therapeutic targets for treatment.

Transgenic mice and rats expressing mutant forms of SOD1 develop motor neuron pathology and clinical symptoms similar to those seen in patients with ALS, affording researchers with excellent animal models of this disease [6–11]. One of the most widely used animal models of ALS is mice expressing multiple copies of the (G93A) human mutant form of the SOD1 (hSOD1) gene [12,13]. Mice that carry 25±1.5 copies of the G93A hSOD1 gene [14] show weakness, tremors, dragging of the hind limbs, and a loss of coordination by 90 days of age. The disease progresses from a loss of motor neurons, and by 120–150 days of age, the mice demonstrate rear limb paralysis and inability to right themselves in 30 s when placed on their side. At this time, for humane reasons, the mice are sacrificed. These animals show considerable variance in the onset and duration of the clinical signs even among siblings. This suggests that there is genetic variability similar to human disease in this rodent model that could be examined to determine if there are disease modifying genes.

The hSOD1Tg mice were originally bred on a genetically heterogeneous background of two strains SJL and C57BL/6 (B6). We have maintained the transgene by breeding male hemizygotes to a C57BL/6 × SJL F1 control female. We refer to this background as B6/SJL. We have bred the transgene on the two homogeneous backgrounds (B6 and SJL) in order to develop congenic lines, which would be useful for studying modifying genes as well as developing a more homogeneous clinical paradigm for testing various treatments. Since the SJL mice carry a mutation in the dysferlin gene [15] which can cause a myopathy and confound the ALS phenotype, we examined an intercross generation bred from (SJL × B6.Tg)F1 or (B6 × SJL.Tg)F1 hybrids of the congenic lines for any linkage of survival to the SJL mutation in dysferlin.

The results of this study show that mice of the SJL congenic line demonstrate a more severe phenotype whereas mice of the B6 congenic line demonstrate a milder phenotype than mice of the hybrid B6/SJL background. We also demonstrated that there was no linkage of dysferlin alleles to animal survival, indicating that the more severe phenotype in the SJL congenics was not related to the dysferlin myopathy. Furthermore, in the SJL background, females demonstrated prolonged survival similar to that previously reported in the B6/SJL background [16], while on the B6 background, the gender difference in survival was absent.

2. Materials and methods

We have established a colony of transgenic mice expressing the mutant gene (G93A) for human SOD1 (hSOD1). This colony was started from mice obtained from The Jackson Laboratory (Bar Harbor, ME) and originally produced by Mark Gurney et al. [12]. Jackson Laboratories has maintained the transgene by breeding hemizygotes to an F1 with the two parental strains being SJL/J and C57BL/6J and its designation B6SJL-TgN(SOD1-G93A)1Gur. The colony was derived at Jackson Laboratories from G93A mutant mice with 25±1.5 copies of the human SOD1 transgene [14]. The colony is maintained by breeding male transgenic animals to naive B6SJLF1/J dams. From this mouse colony, we bred B6SJL-TgN(SOD1-G93A)1Gur males to either naive non-transgenic SJL/J dams, or to B6 dams. We have then continued to breed the offspring to non-transgenic SJL/J dams, or to B6 dams. Both of these congenic lines are currently at the eighth generation (N8). An intercross was created in both parental directions from either (SJL × B6.Tg)F1 or (B6 × SJL.Tg)F1 hybrids; F2 mice were assessed for latency and survival, and genotyped using an allele-specific PCR specific for the dysferlin mutation to determine dysferlin genotypes in the F2 (see below). A *t*-test was used to assess non-random segregation of latency or survival times to the SJL-derived alleles of the dysferlin marker.

The mice in our colony were genotyped using DNA isolated from a 0.5 cm piece of mouse tail. Isolation was performed with the GenElute™ mammalian genomic DNA miniprep kit (Sigma, St. Louis MO). Quantitative real-time PCR was used to determine whether a mouse was transgene positive and to estimate the number of transgene copies in its genome [17]. The estimation of transgene copy numbers was performed by determining the difference in threshold cycle (ΔCT) between the transgene (human SOD1) and a reference gene (mouse IL-2). The ΔCT is a direct index of transgene copy number. In transgenic mice that carry multiple copies of a mutant gene, such as the G93A SOD1 mouse model of ALS, the clinical phenotype is stable only as long as the number of transgene copies remains constant [14,18,19]. In any of the transgenic strains, as long as the ΔCT remains constant, the number of copies remains unchanged.

The presence of a mutation in the dysferlin gene found in SJL mice [15] was analyzed by amplification of tail DNA. The primers used to identify the SJL-Dysferlin mutation were 5′-CTCTGTGGGTGGGATGTTTT-3′ (forward) and 5′-GCCCCTAGTGCTGAGAATCA-3′ (reverse). These primers flank a 141 bp DNA segment that is absent in mice carrying the dysferlin mutation [20] and amplify a 634 base pair product in normal mice and a 493 base pair product in dysferlin mutant mice. For the amplification reaction, 10 μl of DNA (1 ug/ml) was added to 15 μl of master mix containing (final concentrations) 10× PCR buffer (50 mM KCl, 10 mM Tris–HCl, 1.5mM $Mg(OAc)_2$, Eppendorf,