

Lack of *Socs2* expression reduces lifespan in high-growth mice

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Abstract The high-growth (HG) phenotype in mice is characterized by a 30–50% postweaning overgrowth with a substantial increase in plasma insulin-like growth factor I (IGF1) levels, which is directly related to a deletion (*hg*) on chromosome 10 that includes the suppressor of cytokine signaling 2 (*Socs2*) gene. Reduced plasma IGF1 levels have been associated with extended lifespan in mice, although the aging-related effects of abnormally high IGF1 levels without elevated growth hormone levels have never been assessed in mammals. Within this context, the *hg* deletion was introgressed into C57BL/6J (B6) and FVB backgrounds, and a survival analysis was performed on the longevity records of 200 B6 (91 wild-type and 109 homozygous *hg* mutants) and 69 FVB (32 wild-type and 37 *hg* mutants) mice. Longevity was examined using a piecewise Weibull proportional hazards model solved through a Bayesian perspective and Markov chain Monte Carlo sampling. Lifespan was significantly reduced in both strains in homozygous *hg* mice, with a death risk between 3.689 (B6) and

4.347 (FVB) times higher than in wild-type mice (non-overlapped highest posterior density regions at 95%). These results highlight the effects of the *Socs2* gene on aging regulation, likely related with variations described in plasma IGF1 levels. This result is consistent with previous research in dwarf mutant mice and other species, and characterizes the HG mutant mice as a unique and interesting animal model for accelerated aging research.

Keywords Cytokine signaling 2 · Growth hormone · Insulin-like growth factor I · Lifespan · Mice · Survival analysis

Introduction

The high-growth (HG) phenotype in mice is characterized by a 30–50% postweaning overgrowth without increasing adiposity (Bradford and Famula 1984; Corva and Medrano 2000) and is due to a 500 kb deletion on chromosome 10 that includes the *suppressor of cytokine signaling 2* (*Socs2*) gene (Horvat and Medrano 2001; Wong et al. 2002). This mutation deregulates the growth hormone (GH)/Insulin-like growth factor I (IGF1) system (Medrano et al. 1991), a biological pathway shown to be related to lifespan in mice (Coschigano et al. 2000; Bartke 2005; de Magalhães et al. 2005). Although GH/IGF1 overexpression has been related with shortened lifespan in mice (Cecim et al. 1994), the increase of

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plasma IGF1 with reduced plasma and pituitary GH levels of the HG mice (Medrano et al. 1991) highlights HG mice as a unique and relevant animal model for lifespan analyses. However, the effect of the HG mutation on lifespan has not yet been evaluated.

Materials and methods

The HG mutation was introgressed into C57BL/6J (B6^{+/+}) and FVB (FVB^{+/+}) genetic backgrounds by nine backcrosses to create the congenic strains B6^{hg/hg} and FVB^{hg/hg}, respectively. For this study, 91 B6^{+/+} (12 week weight: males, 38.1±0.8 g; females, 34.6±1.1 g; $P<0.10$), 109 B6^{hg/hg} (males, 48.8±0.8 g; females, 46.7±0.8 g; $P>0.10$), 32 FVB^{+/+} (males, 46.0±1.9 g; females, 39.0±1.7 g; $P<0.05$) and 37 FVB^{hg/hg} (males, 59.5±4.3 g; females, 60.8±1.6 g; $P>0.10$) mice were randomly picked after the breeding period (ages between 100 and 300 days) and kept under standard management and specific pathogen-free conditions to register their lifespan (Table 1). Although death causes were not determined for all mice, a histopathological study was done in a random sample of 16 B6^{+/+} and B6^{hg/hg} 2.5-year-old males. There were no differences between genotypes in histopathology (results not shown) or tumor incidence (40% and 43%, respectively; $P>0.10$). A survival analysis was performed with a piecewise Weibull proportional hazards model (Casellas 2007), where the effect of the mouse strain (B6 and FVB),

genotype (+/+ and hg/hg), sex/parturitions (male, female with zero, one or more than one parturitions) and their interactions were evaluated. The proportional hazard hypothesis was checked with the log test of Kalbfleisch and Prentice (1980). After comparing alternative models by the deviance information criterion (Spiegelhalter et al. 2002), the operational model included sex/parturitions (SP_i) effect and strain × genotype interaction ($S_j \times G_k$) as follows, $h(t|SP_i, S_j \times G_k) = h_0(t) \exp(SP_i + S_j \times G_k)$, where $h(t|SP_i, S_j \times G_k)$ was the hazard function at time t and $h_0(t)$ was the piecewise Weibull baseline hazard function (Cox 1972). The Bayesian analysis was performed by launching a single Monte Carlo Markov chain with 100,000 elements, after discarding the first 25,000 as burn-in (Raftery and Lewis 1992).

Results and discussion

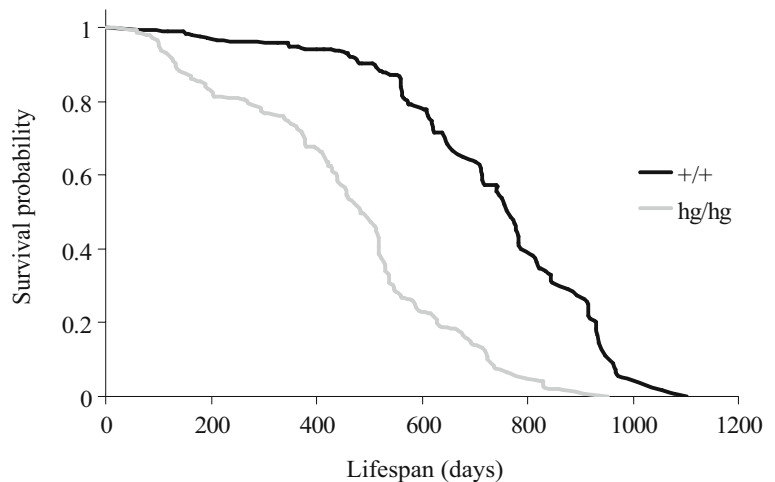
Non-parametric Kaplan-Meier survival curves (Kaplan and Meier 1958) between wild-type and HG mice, and between B6 and FVB strains showed significant differences ($P<0.001$ and $P<0.01$, respectively) when compared by log-rank test (Fig. 1), whereas there were no significant differences ($P>0.05$) when comparisons were made across sex/parturitions levels (see below for details about this effect). As is suggested in Fig. 1, mutant mice began dying at a young age (e.g., first 10% of deaths occurred before 132 days and 360 days of age in B6^{hg/hg} and B6^{+/+} mice, respectively, or before 105 days and 349 days of age in

Table 1 Descriptive statistics of the lifespan data set

	n	Censoring (%)	Complete records	
			Mean ± SE	Max ^a
Genotype				
+/+	123	45.53	693.54±27.31	1,103
hg/hg	146	10.27	448.31±18.39	953
Strain				
B6	200	30.50	556.62±21.05	1,103
FVB	59	16.95	471.63±24.48	894
Sex/parturitions				
Male	163	28.22	564.67±25.09	1,103
Female, 0 parturitions	65	12.31	470.42±42.92	894
Female, 1 parturition	23	56.52	494.50±290.25	822
Female, >1 parturitions	18	22.22	526.50±194.59	783

^aMaximum lifespan (days)

Fig. 1 Kaplan-Meier survival curves for wild-type (+/+) and high growth mutant mice (*hg/hg*) averaged across sexes and genetic backgrounds (FVB and B6)



FVB^{*hg/hg*} and FVB^{*+/+*} mice, respectively), and survival curves showed a similar trend, with survival differences persisting throughout the lifespan of wild type and mutant mice. Note that our dataset does not allow evaluation of mortality patterns at very early stages because mice were picked after the first breeding period.

The hazard ratio (HR) determines the quotient between the death probability intrinsic to two different levels of a given effect or interaction, and can be easily calculated as the exponential of the difference between the regression coefficient of both levels (Allison 1995). The death probability increased with

adult mouse size as suggested by HR (Table 2). Death risk in *hg/hg* mice was 3.689 (B6) and 4.347 (FVB) times higher than in their *+/+* counterparts (Fig. 1). Similarly, the FVB strain reached a heavier adult weight and exhibited a death risk between 1.74 (*+/+*) and 2.05 (*hg/hg*) times greater than B6 mice (Table 2). Note that differences between all $L_j \times G_k$ levels were statistically significant because all the highest posterior density regions at 95% did not overlap (Table 2). These HR implied large differences in predicted lifespan, as shown by modal estimates for males, where FVB^{*hg/hg*} and B6^{*hg/hg*} mice had a shorter lifespan (416.8 days and 525.3 days, respectively) in

Table 2 Regression coefficients and hazard ratios for strain \times genotype and sex/parturitions effects on mice survival probability

Parameter	Regression coefficients ^f		Hazard ratio ^g
	Mode	Highest posterior density region at 95%	
Strain \times Genotype			
B6 \times <i>+/+</i>	-2.023 a	-2.294 to -1.760	0.132
B6 \times <i>hg/hg</i>	-0.719 b	-0.963 to -0.485	0.487
FVB \times <i>+/+</i>	-1.470 c	-1.700 to -1.247	0.230
FVB \times <i>hg/hg</i>	0 d		1.000
Sex/parturitions			
Male	0 a		1.000
Female, 0 parturitions	0.412 b	0.174 to 0.648	1.510
Female, 1 parturition	0.326 b	0.109 to 0.544	1.385
Female, >1 parturitions	0.158 a,b	-0.149 to 0.454	1.171

^f Within a Bayesian context, the posterior distribution of each regression coefficient (β) was characterized with its modal estimate and the higher posterior density region at 95%. Modal estimates followed by the same letter did not differ substantially (overlapped highest posterior density regions at 95%)

^g The hazard ratio is calculated as $\exp(\beta)$, and describes the ratio between the death probability of each level and the level of reference (“FVB \times *hg/hg*” and “Female, >1 parturitions”, respectively)

contrast to wild-type individuals (668.2 days and 804.9 days, respectively). Within this context, smaller adult body sizes appear to benefit lifespan (Roberts 1961; Eklund and Bradford 1977; Miller et al. 2002).

SOCS2 is a protein closely related to growth in mammals through the GH/IGF1 axis, an important physiological mechanism modulating aging from yeast to humans (for a detailed review, see Barbieri et al. 2003). The SOCS2 protein interacts with IGF1 and GH receptors (Dey et al. 1998) as a negative regulator of GH signaling (Favre et al. 1999; Greenhalgh et al. 2005). The lack of SOCS2 expression in the HG mouse increases plasma IGF1, and reduces plasma and pituitary GH levels (Medrano et al. 1991). These higher IGF1 plasma levels, combined with the reduced lifespan of the *hg/hg* mouse, suggests a key role for IGF1 on mice aging as recently suggested by several authors (Coschigano et al. 2000; Flurkey et al. 2001; Holzenberger et al. 2003). Although the putative IGF1-related mechanism modulating lifespan remains unclear, it seems associated with insulin release and sensitivity (Barbieri et al. 2003), and low IGF1 levels probably involving a greater resistance to oxidative stress (Holzenberger et al. 2003). Nevertheless, additional SOCS2-related mechanisms could also modulate mice lifespan (Rico-Bautista et al. 2006).

As reported in previous studies (Yunis et al. 1984), males showed the greatest survivability, whereas females did not reveal significant differences (Table 2). As the number of parturitions increased, the survival probability of females had a suggested increase. The relation between aging and obesity is well established (Barzilai and Gupta 1999) and the differential fat deposition between male and females (Prasetyo and Elsen 1989) could account for survival differences shown in Table 2, although further studies are required to confirm this hypothesis.

Although there are some mutant mouse strains, such as the Snell and Ames Dwarf mice (Brown-Borg et al. 1996; Flurkey et al. 2001), with increased lifespan attributable to GH/IGF1 deficiency, the HG mutant is the only mouse model on the opposite end of the spectrum, where a deletion of the *Socs2* gene that abnormally increases IGF1 without elevated GH levels increases adult body weight and significantly reduces lifespan. Controversy exists around mutations decreasing lifespan and their usefulness in aging research (Hasty and Vijg 2004; Miller 2004). The decreased lifespan of HG mice provides an interesting

model of premature senescence given that the GH/IGF1 pathway has an established role in longevity determination and aging. Nevertheless, its potential contribution to the understanding of normal aging processes remains unclear. Accelerated aging models offer unique opportunities to study specific aging phenotypes and the development of interventions to postpone or prevent them (Hasty and Vijg 2004).

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