Identification of quantitative trait loci for survival in the mutant dynactin p150Glue'd mouse model of motor neuron disease

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Abstract

Amyotrophic lateral sclerosis (ALS) is the most common degenerative motor neuron disorder. Although most cases of ALS are sporadic, 5–10% of cases are familial, with mutations associated with over 40 genes. There is variation of ALS symptoms within families carrying the same mutation; the disease may develop in one sibling and not in another despite the presence of the mutation in both. Although the cause of this phenotypic variation is unknown, it is likely related to genetic modifiers of disease expression. The identification of ALS causing genes has led to the development of transgenic mouse models of motor neuron disease. Similar to families with familial ALS, there are background-dependent differences in disease phenotype in transgenic mouse models of ALS suggesting that, as in human ALS, differences in phenotype may be ascribed to genetic modifiers. These genetic modifiers may not cause ALS rather their expression either exacerbates or ameliorates the effect of the mutant ALS causing genes. We have reported that in both the G93A-hSOD1 and G59S-hDCTN1 mouse models, SJL mice demonstrated a more severe phenotype than C57BL6 mice. From reciprocal intercrosses between G93A-hSOD1 transgenic mice on SJL and C57BL6 strains, we identified a major quantitative trait locus (QTL) on mouse chromosome 17 that results in a significant shift in lifespan. In this study we generated reciprocal intercrosses between transgenic G59S-hDCTN1 mice on SJL and C57BL6 strains, we identified survival QTLs on mouse chromosomes 17 and 18. The overlapping region contains eighty-seven genes with non-synonymous variations predicted to be deleterious and/or damaging. Two genes in this segment, NOTCH3 and Saft/SAFB1, have been associated with motor neuron disease. The identification of genetic modifiers of motor
neuron disease, especially those modifiers that are shared by SOD1 and dynactin-1 transgenic mice, may result in the identification of novel targets for therapies that can alter the course of this devastating illness.

**Introduction**

Amyotrophic lateral sclerosis (ALS) is a degenerative motor neuron disease (MND) resulting in progressive paralysis, muscle atrophy, and ultimately death, with a median survival of less than five years. However, there is variability in ALS severity, with 20% of patients living longer than five years and 10% of patients living 10 years or more. Although most cases of ALS are sporadic (SALS), approximately 5–10% of ALS cases are familial (FALS), with mutations associated with over 40 genes [1–4]. There is also wide variation of ALS symptoms even within families carrying the same familial mutation; the disease may develop in one sibling and not in another despite the presence of the same mutation in both. Although the cause of this phenotypic variation has not been determined, it is likely related to genetic modifiers of disease expression inherent in the genetic heterogeneity even within members of the same family.

Mutations in the Cu/Zn superoxide dismutase (SOD1) gene were the first identified in FALS [5, 6]. The identification of SOD1 mutations led to the development of a transgenic mouse model of ALS carrying the human G93A mutant allele [7]. These mice develop motor neuron pathology and clinical symptoms remarkably similar to those seen in ALS patients [8–11]. Similar to families with SOD1 mutations, there are background-dependent differences in disease phenotype in transgenic G93A-hSOD1 mice. We have previously reported that the G93A-hSOD1 transgene in ALR/LtJ, NOD.Rag1KO, SJL/J or C3H/HeJ backgrounds show a more severe phenotype whereas a milder phenotype is observed in C57BL6 (B6), C57BL/10ScSnJ (B10), BALB/cByJ and DBA/2J inbred strains [12, 13]. Utilizing these mouse strains, we identified a major quantitative trait locus (QTL) on mouse chromosome (Chr) 17 that results in a significant shift in lifespan [14], suggesting that, as in human ALS, the differences in phenotype can be ascribed to genetic modifiers of motor neuron disease.

The identification of other FALS associated mutations has led to additional animal models of motor neuron disease such as the mutant (G59S) human dynactin p150Glued transgenic mouse (G59S-hDCTN1). This model is based on a slowly progressive, autosomal dominant lower motor neuron variant of FALS in humans that is linked to a mutation in the p150Glued subunit of the dynactin complex [15]. Transgenic mice that carry the mutated human dynactin-1 transgene (G59S-hDCTN1) demonstrate pathologic changes and clinical features seen in SALS [16, 17]. These mice develop spontaneous tremors between five and six months of age. Once near end-stage of the disease, the mice start to lose weight, stop grooming, and eventually become paralyzed [16]. Similar to our finding in G93A-hSOD1 mice [12, 13], we observed a more severe phenotype with significant acceleration of disease onset and age of death in G59S-hDCTN1 mice bred onto the SJL background and a milder phenotype with delayed onset and extended lifespan when bred onto the B6 background [18].

In this study, we report the results of QTL analysis of an intercross between G59S-hDCTN1 transgenic mice on a resistant (C57BL/6j) and a susceptible (SJL/j) mouse strain.

**Materials and methods**

**Animals**

Mice from two inbred strains were used in this study: C57BL/6j (B6) and SJL/J (SJL). G59S-hDCTN1 mice were originally produced by Dr. Philip C. Wong at The Johns Hopkins...
University School of Medicine by injecting the transgene containing the G59S substitution into fertilized eggs from C57BL6/SJL F1 hybrid mice [17]. The mice were then maintained on a mixed B6xSJL/F1 background. From these mice, we developed two inbred strains (C57BL/6J and SJL/J) by backcrossing the original mixed B6/SJL mice to either pure B6 or SJL for seven generations [18]. An intercross was generated from short-(SJL), and long-(B6) lived strains for mapping of quantitative trait loci that modify the onset and survival of G59S-hDCTN1 transgenic mice. The F1 mice were derived from crosses in both directions [SJL ♀ x B6 ♂] and [B6 ♀ x SJL ♂] and in all but one litter, the transgene was carried in the male. From the F1 cross, 167 F2 mice were generated. The F2 generation is the first generation where both parental phenotypes occur for an autosomal gene. In an autosomal gene there are four possible genotypes B6xB6 (BB), B6xSJL or SJLxB6 (BS) and SJLxSJL (SS). Thus, in an F2 cross at any location of an autosomal gene, the genotype is such that at that location 25% of the mice are BB, 50% BS and 25% SS. This is not the case for the sex chromosomes. One of the X chromosomes in all females but not males carry the paternal grandmother (pgm) X chromosome (either all S or all B). At the other X chromosome, any region can be either B or S depending on cross over events. In males, the Y chromosome is always that of the paternal grandfather (either all S or all B).

The mice were maintained in humidity- and temperature-controlled rooms and fed Pico-Lab rodent diet 20 ad libitum with free access to water. The mice were genotyped using DNA isolated from a 0.5 cm piece of mouse tail. Isolation was performed with the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD 20874). All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Temple University and were carried out in accordance with the National Institute of Health guide for the care and use of laboratory animals.

**Genotyping**

The presence of the G59S-hDCTN1 transgene was determined by PCR as previously described [17]. When breeding mice each carrying one copy of the G59S-hDCTN1 transgene, the transgene copy number of the offspring was determined by quantitative real-time PCR using mouse IL-2 as a reference gene as previously described [19]. Assays were performed, in duplicate, on a Chromo 4 Quantitative PCR System (Bio-Rad, Hercules, CA). For the QTL analysis, the F2 mice were genotyped with 136 SNPs across the genome that differentiate C57BL/6J from SJL/J mice (S1 Table).

**Life span**

Life span was defined as age of sacrifice for any reason. Mice were sacrificed when they demonstrated limb paralysis or were unable to right themselves in 10 seconds when placed on their side. In addition, mice were also euthanized if their health deteriorated to a degree such that the veterinary staff recommended euthanasia. We differentiated between these two phenotypes. Euthanasia due to limb paralysis or inability to right themselves was defined as “survival”, whereas euthanasia due to health deterioration was defined as “health sacrifice” (health_sac). Euthanasia was performed by an overdose of CO₂ followed by cervical dislocation. All efforts were taken to minimize pain and discomfort. Progressive deterioration of the animals’ health leading to death was not allowed. Natural death was not an end point in this study.
QTL analysis

QTL analysis was performed using R/QTL version 1.46.2 [20] running in R for Windows version 4.0.5. A book by Broman and Sen provides a review of statistical QTL mapping in experimental crosses and is an excellent guide to the use of the R/qtl software package [21]. For one-dimensional scans, pseudo-markers were generated at 2 centimorgan (cM) spacing for each Chr using the Carter–Falconer map function and whole genome scans were performed using 128 imputations [22]. One thousand permutations were performed to determine the thresholds for QTL detection [23]. Logarithm of the odds (LOD) scores were calculated with no covariates and with the inclusion of sex and the paternal grandmother (pgm) of the F2 cross as both additive and interactive covariates. Four thresholds 1%, 5%, 10% and 63% were calculated from the permutation results. QTL with LOD score above the 1% threshold were considered significant, while those above the 63% threshold were considered suggestive [24].

For two-dimensional scans, pair wise scans were performed using 2 cM spacing. All possible pairs of QTL locations on each Chr were tested for association with the life span. The likelihood from the full model (pseudo-marker pair and the interaction between them) and the null model (no genetic effect) was compared and LOD scores were calculated.

QTL and possible QTL’QTL interactions identified from single QTL scan and pair wise scan were fit into multiple regression models. By doing so, variations of the phenotype in the models were estimated. P values for terms in the multiple regression model were calculated. Terms were dropped sequentially until all the terms in the model were significant at 1% level (p<0.01) for main QTL effects and 0.1% (p<0.001) for the interaction effects. The position of the QTL was refined using the refine QTL command. This routine iteratively scans the QTL to identify the positions with the maximum LOD score.

Genomic variations

The SJL sequence was obtained in BAM format from the Sanger Institute web site (https://www.sanger.ac.uk/data/mouse-genomes-project/). Sequence variation (SNPs, insertions and deletions) were determined in the QTL regions with samtools and bcftools [25] using the genome sequence file for SJL and the C57BL6 reference genome as inputs. Variations in the coding regions were determined using PROVEAN [26] (http://provean.jcvi.org/index.php). PROVEAN was also used to determine variations that resulted in non-synonymous protein changes as well as changes predicted to be deleterious and/or damaging.

Statistics

Significance between groups was determined by analysis of variance (ANOVA) using the Tukey-Kramer post-hoc multiple comparison test. The data was considered significantly different if p<0.05 unless otherwise noted. Statistical calculations were accomplished with the aid of SYSTAT version 13 (SYSTAT Software Inc., Chicago, IL).

Results

Effect of genetic background on lifespan

We have previously shown that G59S-hDCTN1 mice demonstrate a shortened lifespan when bred on a SJL background (273.3 ± 7.1 days, N = 41) as compared to mice on the B6 background (444.4 ± 4.6 days, N = 49) [18]. There was no significant difference in lifespan (p>0.05) between male and female mice in either background [18]. The mean lifespan ± standard error in the 167 mice in the B6xSJL F2 cross in this study was 492.8 ± 9.4 days. The lifespan of the G59S-hDCTN1 transgenic mice in the F2 cross was significantly different from the B6 background (p<0.05).
different (p<0.05) from transgenic mice in either the B6 or the SJL background. The F2 B6xSJL mice were classified into two groups based on the reason for euthanasia. The first group of 125 mice were euthanized due to limb paralysis or failure to right themselves when placed on their side. This group was named "survival" and demonstrated a mean age at sacrifice of 499.9 ± 10.6 days. The second group of 42 mice were euthanized due to causes other than limb paralysis or failure to right themselves such as; enlarged abdomens, liver tumors, prolapsed rectum loss of greater than 30% of their peak body weight and eye ulcers. This group was named "health_sac" and demonstrated a mean age at sacrifice of 471.5 ± 19.8 days. There was no statistically significant difference in the age at sacrifice (p>0.05) between groups.

Distribution of the survival and health_sac phenotypes of the F2 mice
The survival phenotype distribution of the F2 mice in this study was very different from the survival phenotype of the G93A-hSOD1 F2 mice in our previous linkage study [14]. The survival distribution of the G93A-hSOD1 F2 was intermediate between the B6 and SJL distributions (Fig 1A). In contrast, 40% of the F2 mice in this study demonstrated greater survival than the longest-lived mouse in the B6 group (Fig 1B). This distribution also applied to the health_sac phenotype, where 38% of the mice lived longer than the longest-lived mouse in the B6 group, suggesting that combinations of B6 and SJL alleles result in phenotypes milder than the phenotype observed in B6 mice.

Effect of sex and F2 paternal grandmother on lifespan
There was a small but not significant (p = 0.1621) difference in the life span of the F2 mice by sex, however, male mice that required euthanasia due to limb paralysis or inability to right themselves (survival) lived significantly longer (p = 0.0315) than females. There was no difference in the time of sacrifice of the health_sac group by sex (Table 1).

All groups demonstrate a significant difference in life span depending on the direction of the F2 cross. Mice with the paternal grandmother (pgm) of the F2 cross in the B6 strain lived significantly longer (p<0.05) than mice with pgm in the SJL strain (Table 2).

QTL analysis identifies regions that modify the survival and health_sac phenotypes
Linkage analysis revealed suggestive QTLs on Chrs 17 and 18 for the survival phenotype (Fig 2, S2 Table). In addition, two dimensional scans reveal suggestive interactions between the Chr 17 QTL and a region on Chr 4 as well as interactions between the X Chr and regions on Chrs 5, 9, 13 and 18 (S4 Table).

All putative QTLs identified by the one-dimensional scans as well as interactions between QTLs identified by the two-dimensional scans were fitted into a multiple regression model using the function fitqtl. The QTL locations were then refined using the function refineqtl. As stated in the methods, the multiple regression model require significance at the 1% level (p<0.01) for main QTL effects and a more stringent 0.1% (p<0.001) for interaction effects. For the survival phenotype, both the Chr 17 and 18 QTLs were significant at the 1% level (p<0.01). Following running refineqtl the maximum likelihood position for the Chr 17 QTL remained at 43.8 cM whereas the Chr 18 QTL was calculated to be at 25.9 cM. The effects of the QTLs differed; the BB genotype at the Chr17 QTL demonstrated longer survival than the SS (Fig 3), whereas at the Chr18 QTL the BB genotype demonstrated shorter survival than the SS (Fig 4). None of the QTL-QTL interactions identified in the two-dimensional scans were significant at the 0.1% level. The refined QTL locations and QTL intervals for the Chrs 17 and 18 QTLs of the survival phenotype are shown in Table 3.
Fig 1. Survival curves for SOD1 and dynactin-1 transgenic mice. Survival curves for transgenic C57BL6/J, SJL/J mice and their F2 cross with [A] the G93A human SOD1 transgene or [B] the G59S human dynactin-1 transgene.

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Linkage analysis also revealed suggestive QTLs on Chr 4, 6, 10, 13 and 15 for the health_sac phenotype (S3 Table) and two-dimensional scans also revealed interactions for the health_sac phenotype between regions on Chr 4 and 13, Chr 5 and 10, Chr 6 and 19 as well as Chr 13 and 19 (S4 Table).

For the health_sac phenotype, when fitted into multiple regression models, the QTLs on Chr 6 and 15 were significant at the 1% level ($p < 0.01$). Following running refineqtl the maximum likelihood position for the Chr 15 health_sac QTL remained at 16.3 cM whereas the Chr 6 QTL was calculated to be at 33.5 cM. The effects of the Chr 6 and 15 QTLs differed. Mice with the BB genotype at Chr 6 demonstrated longer life span due to health sacrifice than mice with the SS genotype, whereas at the Chr 15 QTL mice with the BS genotype demonstrated longer life span due to health sacrifice than mice with either the BB or SS genotype, (S1 and S2 Figs). None of the QTL interactions identified in the two-dimensional scans were significant at the 0.1% level. The refined QTL locations and QTL intervals for the Chr 6 and 15 QTLs of the health_sac phenotype is shown in (Table 4).

Discussion

We have reported a more severe phenotype with significant acceleration of disease onset and decreased life span in G59S-hDCTN1 mice bred onto the SJL background and a milder phenotype with delayed onset and increased life span when bred onto the B6 background [18]. The present study genetically mapped changes in life span through genome-wide analysis of an intercross between G59S-hDCTN1 transgenic mice on the mild phenotype (C57BL/6J) and the severe phenotype (SJL/J) mouse strains. The result of this study demonstrates that the causes of decreased life span in the G59S-hDCTN1 transgenic mice are complex. In contrast to the high copy G93A-hSOD1 transgenic mice, where euthanasia is precipitated by hind limb paralysis or inability to right itself, deteriorating health requiring euthanasia in the G59S-hDCTN1 transgenic mice resulted from multiple conditions. We split lifespan into two phenotypes: euthanasia due to limb paralysis or inability to right themselves as “survival”, whereas euthanasia due deteriorating health such that the veterinary staff recommended euthanasia as “health_sac”.

The effect of sex on lifespan differed depending on the phenotype. Male mice in the survival phenotype lived significantly longer ($p = 0.0315$) than females. This was not the case for the health_sac phenotype. However, both phenotypes demonstrated a significant difference in life

Table 1. Effect of sex on life span of F2 G59S-hDCTN1 mice.

| Phenotype    | Males          | Female         | t-Test  |
|--------------|----------------|----------------|---------|
|              | N   | Mean±se | N    | Mean±se | p value |
| Life span    | 75  | 507.3±15.5 | 92   | 480.9±11.3 | 0.1621  |
| Survival     | 49  | 528.1±17.7 | 76   | 481.7±12.8 | 0.0315  |
| Health_sac   | 26  | 468.0±28.8 | 16   | 477.1±23.7 | 0.8266  |

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Table 2. Effect of the paternal grandmother of the F2 cross on life span of G59S-hDCTN1 mice.

| Phenotype    | C57BL6/J pgm | SJL/J pgm | t-Test  |
|--------------|--------------|-----------|---------|
|              | N | Mean±se | N    | Mean±se | p value |
| Life span    | 99 | 525.2±12.6 | 68   | 445.6±11.8 | 0.00002 |
| Survival     | 67 | 536.2±16.1 | 58   | 458.0±10.9 | 0.00016 |
| Health_sac   | 32 | 502.1±19.2 | 10   | 373.7±44.9 | 0.00432 |

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span depending on the strain of the paternal grandmother (pgm). Mice with pgm in the SJL strain had reduced life span (p < 0.01) compared with mice with pgm in the B6 strain. Suggesting that genetic elements in the X chromosome that differ between strains modify phenotype, either directly, or in combination with autosomal genes.

![Whole genome LOD plot](https://doi.org/10.1371/journal.pone.0274615.g002)

Fig 2. Whole genome LOD plot for the survival phenotype of the G59S-hDCTN1 mice. LOD plot of a whole genome scan for the Survival phenotype with pgm as an additive covariate and sex as an interactive covariate. Chromosome locations are on the X axis, LOD score on the Y axis. The green line indicates suggestive QTL threshold at a LOD of 3.2.

![Effect plot of Survival](https://doi.org/10.1371/journal.pone.0274615.g003)

Fig 3. Effect plot of genotype on survival at the chromosome 17 QTL of G59S-hDCTN1 mice. Effect of the genotype at chromosome 17, 43.8 cM on survival. The survival in days is plotted as the mean ± SE. The genotypes are BB for homozygous B6, SS for homozygous SJL and BS for heterozygous B6/SJL.
This study identified QTL loci on mouse Chrs 17 and 18 for the survival phenotype and on Chrs 6 and 15 for the health sacrifice phenotype that when fitted into multiple regression models were significant at the 1% level ($p < 0.01$). The two phenotypes clearly differed. The survival phenotype demonstrated a significant ($p < 0.05$) difference with sex whereas the health_sacrifice phenotype did not. In addition, the genetic loci identified for each phenotype were located on different Chrs suggesting that different genetic elements modify the survival and health sacrifice phenotypes in the G59S-hDCTN1 mouse. Given that the principal aim of our study is to identify genetic modifiers of disease expression in models of motor neuron disease, we focused the discussion on the survival phenotype.

Our previous linkage study on G93A-hSOD1 transgenic mice identified QTLs on Chrs 17 and 7 for the survival phenotype. At both QTL locations, mice with BB alleles demonstrated longer survival than mice with SS alleles. The allele order for the survival phenotype at the QTL locations was BB > BS > SS. In this study, the QTL on chromosome 17 followed a slightly different pattern, with an allele order for survival of BB = BS > SS (Fig 3). In addition, the QTL on Chr 18, demonstrates a completely different pattern with an allele order for survival of BS > SS > BB (Fig 4). In other words, mice that are heterozygous or homozygous for SJL alleles at the Chr 18 QTL location demonstrated longer survival than mice with B6 alleles. On average, autosomal chromosome locations in the F2 mice are 25% BB, 50% BS and 25% SS. The survival of mice with a BS genotype at the Chr 17 QTL is no different than mice with a BB genotype, whereas BS mice at the Chr 18 QTL have longer survival than both SS and BB. The fact that half of the F2 mice have BS genotype at the QTL locations may explain why many B6/SJL F2 mice demonstrate longer survival than mice on a pure B6 background (Fig 1B).

Table 3. Multiple regression model for the survival phenotype in G59S-hDCTN1 mice.

| Source | Additive covar | Interactive covar | Location (cM) | Interval (cM) | Percent variance | F value | p value |
|--------|----------------|------------------|---------------|--------------|-----------------|---------|---------|
| Chr 17 | Sex            |                  | 43.8          | 27.8–57.8    | 8.651           | 3.596   | 0.00840 |
| Chr 17 | Sex            |                  | 43.8          | 27.8–57.8    | 6.303           | 5.241   | 0.00662 |
| Chr 18 | Pgm            | Sex              | 25.9          | 13.9–37.9    | 6.235           | 5.184   | 0.00697 |
| Pgm    |                 |                  | 10.397        | 17.29        | 6.18x10^{-5}    |         |         |
| Sex    |                 |                  | 8.435         | 4.636        | 6.18x10^{-5}    |         |         |

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The location of the Chr 17 survival QTL on the G93A-hSOD1 mouse partly overlaps with the Chr 17 survival QTL in the G59S-hDCTN1 mouse (Fig 5). The survival QTL in SOD1 transgenic mice demonstrates the highest LOD scores between 3 and 17 cM (Fig 5A). In contrast, the survival QTL in dynactin-1 transgenic mice demonstrate the highest LOD scores between 28 and 58 cM (Fig 5B). However, there is an area between 17 and 40 cM were the QTL maps overlap (Fig 5), suggesting that genetic elements in this region may modify survival in both models of motor neuron disease.

![Fig 5](https://doi.org/10.1371/journal.pone.0274615.g005)

**Fig 5.** LOD plots of chromosome 17 QTLs of SOD1 and dynactin-1 transgenic mice. Chromosome 17 LOD plots. [A] Chromosome 17 Survival QTL in F2 B6xSJL G93A-hSOD1 mice. [B] Chromosome 17 Survival QTL in F2 B6xSJL G59S-hDCTN1 mice.

Table 4. Multiple regression model for the health sac phenotype in G59S-hDCTN1 mice.

| Source | Additive covar | Interactive covar | Location (cM) | Interval (cM) | Percent variance | F value | p value |
|--------|----------------|-------------------|---------------|--------------|------------------|---------|---------|
| Chr 6  | Sex            |                   | 33.5          | 1.5–61.5     | 14.89            | 6.861   | 0.00341 |
| Chr 15 | Pgm            |                   | 16.3          | 10.3–50.0    | 18.38            | 4.234   | 0.00753 |
| Chr 15 | Pgm            |                   | 16.3          | 10.3–50.0    | 14.78            | 6.808   | 0.00354 |
| Pgm    |                |                   | 30.76         | 9.446        | 9.446            | 1.38x10^-4 |       |
| Sex    |                |                   | 18.64         | 5.724        | 5.724            | 0.00308 |         |

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The QTL region on Chr 17 contains 251 genes where SJL mice demonstrate non-synonymous variations (S5 Table). Ninety-six of those genes have PROVEAN or SIFT scores that predict the variations to be deleterious and/or damaging to protein function (Table 5).

The distal segment of the QTL that does not overlap with the survival QTL in SOD1 transgenic mice contains 29 genes with non-synonymous variations, nine of them demonstrated variations predicted to be deleterious and/or damaging with LRPPRC as the most likely candidate genetic modifier of ALS. The leucine-rich pentatricopeptide repeat containing (LRPPRC) protein regulates mitochondrial mRNA stability and an amino-acid substitution of this protein causes the French-Canadian type of Leigh syndrome (LSFC), a progressive neurodegenerative disorder characterized by mitochondrial complex IV deficiency [27, 28].

The proximal segment that overlaps with the survival QTL in SOD1 transgenic mice contains 222 genes with non-synonymous variations, eighty-seven of them demonstrated variations predicted to be deleterious and/or damaging (Table 5). Two genes in this segment, NOTCH3 and Safb/SAFB1, have been associated with motor neuron disease. Mutations in NOTCH3 have been shown to cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common form of hereditary stroke [29]. NOTCH3 mutations have also been associated with a number of neurodegenerative diseases such as; Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, ALS and fronto-temporal lobar degeneration [29–31]. SAFB1 is an RNA binding protein implicated in the regulation of transcription, stress response, DNA repair and RNA processing [32]. A recent study suggests that SAFB1 tethers FUS to the chromatin compartment thorough N-terminal DNA-binding motif, indicating that SAFB1 could be a FUS’s functional platform in the chromatin compartment regulating RNA splicing and ligand-dependent transcription. This interaction may shed light on the etiological significance of nuclear matrix-associated proteins in ALS pathogenesis [33].

This region also contains the mouse major histocompatibility complex (H2) [34, 35]. Twenty-two genes within H2 demonstrated non-synonymous variations deemed to be deleterious and/or damaging, with some regions such as H2-K1, H2-d1, H2-Q2 and H2-Q4 having 20 or more variations each (Table 5). The Major Histocompatibility Complex has been shown to have a protective role in ALS [36, 37].

In addition, there are several genes in this segment whose function, when altered, can affect motor neuron survival such as; Marchf2, Ndufa7, Vps52, Tap1, Nelfe, Ehmt2, Vars2, Mdc1, Adgrf1, Aars2, Hsp90ab1, Abcc10, Cul9/Parc, Usp49, Foxp4, Trem3, Trem11 and Ticam1/TRIF.

MARCHF2 (MARCH-II) is a likely regulator of trafficking between the trans-Golgi network and endosomes [38] and may also function as a molecular bridge with ubiquitin ligase activity [39]. NDUFA7 is a subunit of mitochondrial complex I [40]. Genetic variants of Complex I genes may influence the nature of tissue response to inflammation in the central nervous system [41]. Vps52 is a subunit of the GARP and EARP complexes that has been shown to be involved in the regulation of neurite outgrowth [42]. The transporter associated with antigen processing 1 (TAP1) gene is involved in the pumping of degraded cytosolic peptides across the endoplasmic reticulum into the membrane-bound compartment where class I molecules assemble. The TAP1 and TAP2 proteins form the heterodimer transporter associated with antigen processing (TAP) complex Loss of TAP function leads to a loss of cell surface expression of MHC class I molecules [43]. NELFE is an essential component of the negative elongation factor (NELF) complex, which allows cells to coordinate and appropriately respond to signals by modulating the rate of transcriptional pause release [44]. Euchromatic histone-lysine N-methyltransferase 2 (EHMT2) along with EHMT1 comprises a histone
| Mouse Gene Symbol | Mouse Gene ID | Human Gene Symbol | Mouse Gene Name | Chr 17 Location | Del/Dam # Vars |
|-------------------|---------------|-------------------|-----------------|-----------------|---------------|
| Rrp1b             | 72462         | RRP1B             | ribosomal RNA processing 1B | GRCm39          | 2             |
| Notch3            | 18131         | NOTCH3            | notch 3         | 32339794–32385826 | 1             |
| Zip799            | 240064        | ZNF799            | zinc finger protein 799 | 33034423–33049235 | 1             |
| Cyp4f13           | 170716        | CYP4F3            | cytochrome P450, family 4, subfamily f, polypeptide 13 | 33143662–33166376 | 1             |
| Phb8-ps           | 74042         | PHD finger protein 8, pseudogene | 3283117–3286999 | 2             |
| Morc2b            | 240069        | MORC2             | microchondria 2B | 33332325–33358657 | 1             |
| Zip555a           | 77652         | zinc finger protein 555A | 33458692–33474119 | 1             |
| Zip555b           | 10004368      | zinc finger protein 555B | 33508518–33526215 | 2             |
| Marchf2           | 224703        | MARCHF2           | membrane associated ring-CH-type finger 2 | 33904666–33937644 | 2             |
| Ndufa7            | 66416         | NDUF A7           | NADH: ubiquinone oxidoreductase subunit A7 | 34043546–34057291 | 1             |
| Vps52             | 224705        | VPS52             | VPS52 GARP complex subunit | 34174786–34186009 | 10            |
| H2-K1             | 14972         | HLA-A             | histocompatibility 2, K1, K region | 34214991–34219321 | 24            |
| Rxrb              | 20182         | RXRB              | retinoid X receptor beta | 34250786–34257373 | 1             |
| Tap1              | 21354         | TAP1              | transporter 1, ATP-binding cassette, sub-family B (MDR/ TAP) | 34046527–34141699 | 3             |
| Gm15821           | 100502931     | predicted gene 15821 | 34430286–34433433 | 1             |
| H2-Ob             | 15002         | HLA-DOB           | histocompatibility 2, O region beta locus | 34457877–34473388 | 3             |
| H2-Ab1            | 14961         | HLA-DQA1          | histocompatibility 2, class II antigen A, beta 1 | 34476663–34488393 | 4             |
| H2-Aa             | 14960         | HLA-DQA1          | histocompatibility 2, class II antigen A, alpha | 34501718–34506797 | 3             |
| H2-Eb1            | 14969         | HLA-DRB5          | histocompatibility 2, class II antigen E beta | 34524841–34535648 | 3             |
| H2-Eb2            | 381091        | HLA-DRB2          | histocompatibility 2, class II antigen E beta2 | 34544639–34560386 | 1             |
| BC051142          | 407788        | cDNA sequence BC051142 | 34617794–34679708 | 4             |
| Btn4              | 632126        | butyrophilin-like 4 | 34685536–34696292 | 11            |
| Btn6              | 626481        | butyrophilin-like 6 | 34726778–34736326 | 9             |
| Notch4            | 18132         | NOTCH4            | notch 4         | 34783242–34807477 | 1             |
| Tnxb              | 81877         | TNXB              | tenasin XB      | 34879431–34938789 | 4             |
| Nelfe             | 27632         | NELFE             | negative elongation factor complex member E, Rdhp | 35069367–35075348 | 3             |
| Ehmt2             | 110147        | EHMT2             | euchromatic histone lysine N-methyltransferase 2 | 35117445–35133028 | 1             |
| D17H6S6E-5        | 110956        | D6S6E 5           | DNA segment, Chr 17, human D6S6E 5 | 35215654–35219722 | 1             |
| Ly6g6f            | 433099        | LY6G6F            | lymphocyte antigen 6 complex, locus G6F | 35299456–35304586 | 1             |
| H2-D1             | 14964         | HLA-A             | histocompatibility 2, D region locus 1 | 35481706–35486475 | 20            |
| H2-Q1             | 15006         | HLA-A             | histocompatibility 2, Q region locus 1 | 35539381–35544075 | 2             |
| H2-Q2             | 15013         | HLA-A             | histocompatibility 2, Q region locus 2 | 35561218–35565738 | 25            |
| H2-Q4             | 15015         | HLA-A             | histocompatibility 2, Q region locus 4 | 35598393–35604266 | 33            |
| H2-Q6             | 110557        | HLA-A             | histocompatibility 2, Q region locus 6 | 35648326–35649031 | 2             |
| Muc21             | 672682        | MUC21             | mucin 21        | 35928815–35937529 | 11            |
| Muc3d             | 268949        | MUC3D             | mucin like 3    | 35946644–35954587 | 2             |
| Vars2             | 68915         | VARS2             | valyl-tRNA synthetase 2, mitochondrial | 35966526–35978484 | 2             |
| Gtf2h4            | 14885         | GTF2H4            | general transcription factor II H, polypeptide 4 | 35978622–35984631 | 1             |
| Mdc1              | 240087        | MDC1              | mediator of DNA damage checkpoint 1 | 36152407–36170562 | 1             |
| Ppp1r18           | 76448         | PPP1R18           | protein phosphatase 1, regulatory subunit 18 | 36176485–36186488 | 1             |
| H2-T24            | 15042         | histocompatibility 2, T region locus 24 | 36316587–36331452 | 1             |
| H2-T23            | 15040         | HLA-E             | histocompatibility 2, T region locus 23 | 36340665–36343747 | 11            |
| H2-T22            | 15039         | histocompatibility 2, T region locus 22 | 36348020–36353639 | 17            |
| Gm11127           | 100529082     | HLA-F             | predicted gene 11127 (H2-K1) | 36366708–36369263 | 3             |
| Gm7030            | 630294        | HLA-F             | predicted gene 7030 (H2-9) | 36420611–36440317 | 1             |
| 2410017I17Rik      | 675325        | RIKEN cDNA 2410017I17 gene | 36455910–36474068 | 35            |
| Mouse Gene Symbol | Mouse Gene ID | Human Gene Symbol | Mouse Gene Name | Chr 17 Location | Del/Dam # Vars |
|-------------------|--------------|-------------------|----------------|----------------|----------------|
| Gm8909            | 667977       | HLA-A             | predicted gene 8909 (H2-gs17) | 36475335–36479429 | 21             |
| H2-T3             | 15043        |                   | histocompatibility 2, T region locus 3 | 36496464–36501179 | 13             |
| H2-M1.10.1        | 14985        |                   | histocompatibility 2, M region locus 10.1 | 36633750–36637047 | 1              |
| H2-M11            | 224754       |                   | histocompatibility 2, M region locus 11 | 36857967–36860142 | 1              |
| H2-M1             | 224756       |                   | histocompatibility 2, M region locus 1 | 36890900–36893111 | 1              |
| H2-M10.5          | 224761       |                   | histocompatibility 2, M region locus 10.5 | 37083802–37087126 | 1              |
| Polr1Has          | 76416        |                   | RNA polymerase I subunit H, antisense | 37269844–37276517 | 3              |
| Olfr90            | 258469       | OR2H2             | olfactory receptor 90 | 37394763–37399391 | 1              |
| Olfr92            | 258448       |                   | olfactory receptor 92 | 37421404–37430975 | 2              |
| Olfr93            | 258051       |                   | olfactory receptor 93 | 37451309–37472385 | 1              |
| Adgfr1            | 77596        | ADGFR1            | adhesion G protein-coupled receptor F1 | 43581220–43635628 | 1              |
| Supt3             | 109115       | SUPT3H            | SPT3, SAGA and STAGA complex component | 45088039–45430177 | 3              |
| Aars2             | 224805       | AARS2             | alanyl-tRNA synthetase 2, mitochondrial | 45817767–45831769 | 2              |
| Hsp90ab1          | 15516        | HSP90AB1          | heat shock protein 90 alpha (cytosolic), class B member 1 | 45878701–45884197 | 12             |
| 160014C23Rik      | 77240        | RIKEN cDNA 160014C23 gene | 46043790–46044770 | 2              |
| Abcc10            | 224814       | ABCC10            | ATP-binding cassette, sub-family C (CFTR/MPR), member 10 | 46614147–46639278 | 1              |
| Gm5093            | 328825       |                   | predicted gene 5093 | 46750504–46751023 | 1              |
| Cull9             | 78309        | CUL9/PARC        | cullin 9 | 46811498–46857314 | 5              |
| Pex6              | 224824       | PEX6              | peroxisomal biogenesis factor 6 | 47023839–47036467 | 2              |
| Cnpy3             | 72029        | CNPY3             | canopy FGF signaling regulator 3 | 47046631–47063140 | 1              |
| Rpl7l1            | 66229        | RPL7L1            | ribosomal protein L7-like 1 | 47084833–47093598 | 1              |
| Gm16494           | 10524635     |                   | non-histone chromosomal protein HMG-17 pseudogene | 47327623–47327881 | 2              |
| Gm4945            | 240110       |                   | ribosomal protein L29 pseudogene | 47353513–47353965 | 3              |
| Al661453          | 224833       |                   | expressed sequence Al661453 | 47747540–47781563 | 3              |
| Ccnd3             | 12445        | CCND3             | cyclin D3 | 47815976–47910616 | 1              |
| Usp49             | 228836       | USP49             | ubiquitin specific peptidase 49 | 47941615–47997663 | 1              |
| Prickle4          | 381104       | PRICKLE4          | prickle planar cell polarity protein 4 | 47999942–48005661 | 2              |
| Frs3              | 107971       | FRS3              | fibroblast growth factor receptor substrate 3 | 47999955–48015211 | 1              |
| Foxp4             | 74123        | FOXP4             | forhead box P4 | 48178058–48235570 | 1              |
| 9830107B12Rik     | 328829       |                   | RIKEN cDNA 9830107B12 gene | 48436215–48453439 | 7              |
| A530064D06Rik     | 328830       |                   | RIKEN cDNA A530064D06 gene | 48456296–48474443 | 3              |
| Trem3             | 58218        |                   | triggering receptor expressed on myeloid cells 3 | 48554085–48565869 | 1              |
| Trem1             | 71326        | TREML1            | triggering receptor expressed on myeloid cells-like 1 | 48669948–48674204 | 1              |
| Pp2d1             | 110332       | PP2D1             | protein phosphatase 2C-like domain containing 1 | 53814488–53846479 | 1              |
| Sult1c2           | 69083        | SULT1C2           | sulfotransferase family, cytosolic, 1C, member 2 | 54136665–54153367 | 1              |
| Vmn2r118          | 383258       |                   | vomeronasal 2, receptor 118 | 55897370–55932192 | 4              |
| Fsd1              | 240121       | FSD1              | fibronectin type 3 and SPRY domain-containing protein | 56293509–56303881 | 1              |
| Tiam1             | 106759       | TICAM1            | toll-like receptor adaptor molecule 1 | 56576319–56583786 | 2              |
| Safb              | 224903       | SAFB              | scaffold attachment factor B | 56891825–56913294 | 1              |
| Catsperd          | 106757       | CATSPERD          | cation channel sperm associated auxiliary subunit delta | 56935143–56971456 | 1              |
| Arhgap28          | 268970       | ARHGAP28          | Rho GTPase activating protein 28 | 68149708–68311115 | 3              |
| Gm16519           | 546695       |                   | ribosomal protein L12 pseudogene | 71236053–71236541 | 2              |
| Alk               | 11682        | ALK               | anaplastic lymphoma kinase | 72175967–72911622 | 1              |
| Vit               | 74199        | VIT               | Vitrin | 78815493–78934837 | 1              |
| Ndufa7            | 73694        | NDUFAF7           | NADH: ubiquinone oxidoreductase complex assembly factor 7 | 79244565–79255481 | 1              |
methyltransferase complex (GLP/G9a) that methylates lysine residues of histone H3. This methylation is associated with gene silencing in euchromatin [45].

EHMT2 has been shown to be an epigenetic regulator of VEGFA alternative splicing [46]. VEGF has been shown to be a modifier of motoneuron degeneration both in human ALS and the G93A-hSOD1 mouse model of ALS [47]. The VARS2 nuclear gene encodes mitochondrial valyl-tRNA (Val-tRNA) synthetase. Mutations in this gene cause combined oxidative phosphorylation deficiency-20 [48] and are also associated with early-onset mitochondrial encephalopathies [49]. The MDC1 protein is part of the DNA damage response (DDR) pathway. MDC1 plays an early and important role in the DDR [50, 51]. ADGRF1 (GPR110) has been shown to regulate immune function in both brain and periphery [52]. The mitochondrial aminolevulinic acid synthetase 2 (AARS2) has been linked to leukoencephalopathy [53]. The heat shock protein (Hsp) HSP90 is involved in protein folding, refolding, transport as well as protein degradation [54]. ABCC10 is a member of the superfamily of ATP-binding cassette (ABC) transporters, expressed in the blood brain barrier, whose function is to transport molecules across extra- and intra-cellular membranes [55]. PARC regulates p53 subcellular localization and apoptosis [56] and mediates the degradation of cytochrome c promoting neuronal survival [57]. USP49 is a histone H2B-specific deubiquitinase and has a critical role for H2B deubiquitination in cotranscriptional pre-mRNA processing events [58]. FOXP transcription factors are a protein subfamily known to coordinate the development of several organs including the Central Nervous System [59, 60]. These transcription factors, including FOXP4, are associated with neurodevelopmental disorders [61].

Variants within the TREM gene cluster are associated with the risk of developing Alzheimer’s disease, making them a therapeutic target for the treatment of neurodegenerative diseases [62, 63]. TICAM1/TRIF encodes an adaptor protein containing a Toll/interleukin-1 receptor (TIR) homology domain. TIR-domain-containing adapter-inducing interferon-β (TRIF) is an adapter in responding to activation of toll-like receptors. Mutations in this gene are associated with acute infection-induced encephalopathies. TRIF is dynamically modified by ubiquitination and deubiquitination, which plays a critical role in regulating its activity. Toll/IL-1R domain-containing adaptor-inducing IFN-β (TRIF)-dependent signaling is required for TLR-mediated production of type-I IFN and several other proinflammatory mediators. The TRIF pathway contributes to control of both viral and bacterial pathogens through promotion of inflammatory mediators and activation of antimicrobial responses [64].

The QTL on Chr 18 contains 85 genes were SJL mice demonstrate non-synonymous variations (S6 Table). Thirty of those genes have PROVEAN or SIFT scores that predict the variations to be deleterious and/or damaging to protein function (Table 6).

MATR3 and the protocadherin family of genes are the most likely ALS genetic modifying candidates in this QTL. MATR3 is the one gene in the Chr 18 QTL that has been directly linked to ALS [65, 66]. Mutations in the RNA/DNA-binding protein MATR3, which is known to interacts with TDP-43, has been shown to cause ALS [67].
Table 6. Protein coding genes in the Chr18 QTL of G59S-hDC TN1 mice demonstrating non-synonymous variations deemed to be deleterious and/or damaging.

| Mouse Gene Symbol | Mouse Gene ID | Human Gene Symbol | Mouse Gene Name | Chr 18 Location | Del/Dam # Vars |
|-------------------|---------------|-------------------|----------------|----------------|----------------|
| Gm10036           | 100040551     | ribosomal protein L11 pseudogene | Gm10036 | 15965851–15966384 | 1 |
| Gm10549           | 433171        | predicted gene 10549 | Gm10549 | 33597216–33607763 | 1 |
| Brd8              | 78656         | bromodomain containing 8 | Brd8 | 34731668–34757654 | 1 |
| Kdm3b             | 277250        | KDM3B lysine (K)-specific demethylase 3B | Kdm3b | 34910100–34971713 | 1 |
| Egr1              | 13653         | early growth response 1 | Egr1 | 34992876–34998037 | 1 |
| Hspa9             | 15526         | heat shock protein 9 | Hspa9 | 35070467–35087410 | 1 |
| Matr3             | 17184         | matrin 3 | Matr3 | 35695191–35726888 | 5 |
| Wdr55             | 67936         | WD repeat domain 55 | Wdr55 | 36893273–36896863 | 1 |
| Pcdha1            | 116731        | protocadherin alpha 1 | Pcdha1 | 37063237–37320714 | 2 |
| Pcdhb2            | 93873         | protocadherin beta 2 | Pcdhb2 | 37427865–37430677 | 3 |
| Pcdhb3            | 93874         | protocadherin beta 3 | Pcdhb3 | 37433852–37473638 | 4 |
| Pcdhb4            | 93875         | protocadherin beta 4 | Pcdhb4 | 37440508–37444225 | 5 |
| Pcdhb5            | 93876         | protocadherin beta 5 | Pcdhb5 | 37453434–37456968 | 7 |
| Pcdhb6            | 93877         | protocadherin beta 6 | Pcdhb6 | 37466974–37470727 | 3 |
| Pcdhb8            | 93879         | protocadherin beta 8 | Pcdhb8 | 37481874–37491657 | 9 |
| Pcdhb9            | 93880         | protocadherin beta 9 | Pcdhb9 | 37533908–37536962 | 12 |
| Diaph1            | 13367         | diaphanous related formin 1 | Diaph1 | 37976654–38068529 | 1 |
| Pcdh12            | 53601         | protocadherin 12 | Pcdh12 | 37568674–37571707 | 1 |
| Spink14           | 433178        | serine peptidase inhibitor, Kazal type 14 | Spink14 | 44160936–44165484 | 1 |
| Dtwd2             | 68857         | DTWD2 | Dtwd2 | 49852912–49886868 | 3 |
| Srfbp1            | 67222         | serum response factor binding protein 1 | Srfbp1 | 52598765–52625003 | 1 |
| Fbn2              | 14119         | fibrillin 2 | Fbn2 | 58141695–58343559 | 1 |
| Cdx1              | 12590         | caudal type homeobox 1 | Cdx1 | 61151934–61169271 | 1 |
| Arhgef37          | 328967        | Rho guanine nucleotide exchange factor (GEF) 37 | Arhgef37 | 61624728–61669665 | 1 |
| Gm9949            | 225609        | predicted gene 9949 | Gm9949 | 62313197–62317476 | 3 |
| Alpk2             | 225638        | alpha-kinase 2 | Alpk2 | 65398600–65527137 | 2 |
| Oacyl             | 319888        | O-acyltransferase like | Oacyl | 65831339–65884672 | 1 |
| Ldlrad4           | 52662         | low density lipoprotein receptor class A domain containing 4 | Ldlrad4 | 68066328–68401701 | 1 |
| Rnmt              | 67897         | RNA (guanine-7-) methyltransferase | Rnmt | 68433426–68457923 | 2 |
| Poli              | 26447         | polymerase (DNA directed), iota | Poli | 70641751–70663691 | 1 |

There are 14 genes in the protocadherin family demonstrating non-synonymous variations in SJL/J mice within the Chr 18 QTL. Protocadherins are the largest mammalian subgroup of the cadherin superfamily [68]. Preventing the interaction of axons and dendrites from the same neuron during development, is mediated through the stochastic single-neuron expression of clustered protocadherin protein isoforms [69]. Most of the protocadherin proteins demonstrating variations belong to the alpha and beta clusters. The one gene in the gamma cluster (Pcdhcg3) demonstrated one non-synonymous SNP resulting in a single amino acid change (S651N) that was predicted by PROVEAN to be neutral, tolerated. Nine genes in the protocadherin family; Pcdha1, Pcdhb2, Pcdhb3, Pcdhb4, Pcdhb5, Pcdhb6, Pcdhb8, Pcdhb9 and Pcdh12 demonstrated non-synonymous variations with PROVEAN or SIFT scores that predict the variations to be deleterious and/or damaging to protein function. Protocadherins within the alpha cluster have been shown to contribute to neural circuit development [70]. Protocadherins in the beta cluster contribute to specifying the identity and diversity of...
individual neurons [71] and members of this cluster have been reported to control axon growth in zebrafish motor neurons [72].

In addition, there are several genes in this QTL demonstrating variations predicted to be deleterious and/or damaging, whose function, when altered, can affect motor neuron survival such as; Brd8, Kdm3b, Egr1, Hsp9 and Diaph1. BRD8 (p120) has been linked with DNA repair [73–75]. KDM3B has been identified as an antioxidant gene [76]. EGR1 is a transcription factor also known as nerve growth factor-induced protein A (NGFIA). EGR1 transcriptional regulation is complex and involves; transcription factor binding, cofactors recruitment, chromatin dynamics including histone methylation, acetylation and phosphorylation, as well as nucleosome positioning [77]. HSPA9 (Mortalin) is a mitochondrial chaperone of the heat shock protein 70 family [78, 79]. Mutations in HSPA9 may contribute to the risk of developing Parkinson’s disease [80]. Mutations in DIAPH1 results in mitochondrial dysfunction and immunodeficiency which can lead to seizures, cortical blindness, hearing loss and microcephaly syndrome [81, 82].

Our results shows that mice with SJL genotype at the Chr17 QTL demonstrated shorter survival than mice with C57BL6 genotype, whereas at the Chr18 QTL mice with the SJL genotype demonstrated longer survival than mice with the C57BL6 genotype. The phenotypic changes can result from alterations in protein coding genes within the QTLs, however they could also arise from altered non-coding RNAs such as microRNAs or long non-coding RNAs.

In addition, altered survival does not necessarily mean that all SJL-derived protein coding genes and/or non-coding RNAs at the Chr 17 QTL are deleterious or all beneficial at the Chr 18 QTL. It means that the total contribution of genetic elements that differ in SJL mice at the Chr 17 QTL results in reduced survival, whereas the total contribution of those at the Chr 18 QTL promotes increased survival. We hypothesize that the variations in survival demonstrated by mice with SJL alleles within the Chr 17 and 18 QTLs are due to either allelic non-synonymous genetic polymorphism(s) of protein coding genes and/or non-coding RNAs or their differential expression.

We further hypothesize that some of the genetic modifiers located in the Chr 17 region where the SOD1 and dynactin-1 QTLs overlap, will be shared by these two MND models despite the fact that motor neuron degeneration is caused by mutations in different proteins. We are currently undertaking studies using interval specific congenic (ISC) mice on a C57BL6/J background with Chr 17 SJL intervals in the region where the SOD1 and dynactin-1 QTLs overlap. We have bred the SOD1 and dynactin-1 transgenes into these ISC lines in order to determine which regions modify the survival of these mice. Our goal is to generate ISC mice with 1–3 Mb intervals that preserve the altered phenotype. These mice can then be used for the identification of genetic modifiers of motor neuron disease. The identification of genetic modifiers of motor neuron disease, especially those modifiers that are shared by SOD1 and dynactin-1 transgenic mice, may help to develop biomarkers predictive of disease progression. In addition, they will identify pathways that are important in motor neuron degeneration and lead to novel targets for therapies that can alter the course of this devastating illness.

Supporting information

S1 Dataset. Data dynactin F2 mice phenotype and genotype.
(XLSX)

S1 Fig. Effect plot of genotype on health sacrifice at the chromosome 6 QTL. Effect of genotype at chromosome 6, 33.5 cM on health sacrifice. The health sacrifice in days is plotted as the
mean + SE. The genotypes are BB for homozygous B6, SS for homozygous SJL and BS for heterozygous B6/SJL.

(TIF)

S2 Fig. Effect plot of genotype on health sacrifice at the chromosome 15 QTL. Effect of genotype at chromosome 15, 16.3 cM on health sacrifice. The health sacrifice in days is plotted as the mean + SE. The genotypes are BB for homozygous B6, SS for homozygous SJL and BS for heterozygous B6/SJL.

(TIF)

S1 Table. Single nucleotide polymorphisms across the mouse genome used to differentiate C57BL/6J from SJL/J mice.

(XLSX)

S2 Table. One-dimensional scans for the survival phenotype with pval less than or equal to 0.63.

(XLSX)

S3 Table. One-dimensional scans for the health sacrifice phenotype with pval less than or equal to 0.63.

(XLSX)

S4 Table. Two-dimensional scans for the survival and health sacrifice phenotypes with pvals less than or equal to 0.63.

(XLSX)

S5 Table. Gene locations in the chromosome 17 QTL where SJL mice demonstrate non-synonymous variations.

(XLSX)

S6 Table. Gene locations in the chromosome 18 QTL where SJL mice demonstrate non-synonymous variations.

(XLSX)

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