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Genetic Mapping of a Mouse Modifier Gene That Can Prevent ALS Onset

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Mutations in the cytoplasmic Cu/Zn superoxide dismutase (*SOD1*) gene on human chromosome 21q22.1 cause 10–20% of familial amyotrophic lateral sclerosis (ALS) cases. The expression of the ALS phenotype in mice carrying the murine G86R *SOD1* mutation is highly dependent upon the mouse genetic background. This is similar to the phenotypic variation observed in ALS patients containing identical *SOD1* mutations. In the FVB/N background, mice expressing mG86R *SOD1* develop an ALS phenotype at ~100 days. However, when these mice were bred into a mixed background of C57Bl6/129Sv, the onset of the ALS phenotype was delayed (143 days to >2 years). Using 129 polymorphic autosomal markers in a whole genome scan, we have identified a major genetic modifier locus with a maximum lod score of 5.07 on mouse chromosome 13 between D13mit36 and D13mit76. This 5- to 8-cM interval contains the spinal muscular atrophy (SMA)-associated gene *Smn* (survival motor neuron) and seven copies of *Naip* (neuronal apoptosis inhibitory protein), suggesting a potential link between SMA and ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by the progressive degeneration of motor neurons in the spinal cord, brain stem, and pre- and postcentral neocortical gyri (Patterson *et al.*, 1994). Most ALS is sporadic with only 10–25% of cases being familial. The genetic etiology of ALS is heterogeneous and includes dominant, recessive, and X-linked forms. Mutations in the cytoplasmic Cu/Zn superoxide dismutase (*SOD1*) gene on human chromosome 21q22.1 cause 10–20% of familial cases and a small percentage of sporadic ALS cases (Rosen *et al.*, 1993; Jackson *et al.*, 1997). More than 60 different mutations, spread among each exon of the *SOD1* gene, have been identified. These include 54 point mutations in 40 different amino acid codons, 5 premature terminations, 1 single amino acid deletion, and a 3-amino-acid insertion (Andersen *et al.*, 1997). It is currently unknown how these disparate mutations in *SOD1* cause ALS. Other genes causing ALS remain to be identified.

Transgenic mice that develop ALS-like phenotypes have been constructed with five mutant forms of human *SOD1:* hG93A, hG85R, hG37R, hD90A, and truncated SOD1 (Gurney *et al.*, 1994; Dal Canto and Gurney, 1994; Bruijn *et al.*, 1997; Wong *et al.*, 1995; Deng *et al.*, 1999; Brannstrom *et al.*, 1998). Another model of ALS was generated using a mutated mouse gene with the G86R mutation that corresponds to the human G85R mutation (Ripps *et al.*, 1995). In each case, the mutant *SOD1* allele is expressed in a normal mouse background; moreover, the transgenic mice have normal or increased SOD1 activity levels but still develop motor neuron disease similar to that observed in human ALS patients. The increased levels of SOD1 protein and activity seen in the transgenic mice are unrelated to the ALS phenotype as several groups have demonstrated that mice overexpressing wildtype human SOD1 do not develop an ALS-like syndrome (Dal Canto and Gurney, 1994; Wong *et al.*, 1995). Mice with *SOD1* null alleles also do not develop symptoms of ALS (Reaume *et al.*, 1996). These results suggest that mutations in *SOD1* cause a novel gain-of-function that is lethal to motor neurons.

Transgenic mouse models of ALS have been widely used to test potential therapeutic treatments and the effects of the over- and underexpression of specific genes on the expression of the ALS phenotype. The mouse models of ALS vary in their age of onset, disease progression, and certain histopathologic features, mimicking the diversity of phenotypes observed in human ALS patients (Gurney *et al.*, 1994; Dal Canto and Gurney, 1994; Bruijn *et al.*, 1997; Wong *et al.*, 1995; Deng *et al.*, 1999; Brannstrom *et al.*, 1998; Ripps *et al.*, 1995). The best pharmacologic treatment observed in any of the ALS mouse models thus far was 2% creatine.

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Klivenyi *et al.* (1999) demonstrated that 2% creatine increased survival of the early onset (~143-day life span) G93A *SOD1* ALS mice by 26 days. The effect of creatine treatment was better than that observed previously with carboxyfullerenes, penicillamine, or riluzole (Klivenyi *et al.,* 1999).

Several investigators tested the effects of altered neurofilament expression on ALS. Williamson *et al.* (1998) demonstrated that the absence of *NF-L* delayed ALS onset and increased survival by 45 days in the hG85R *SOD1* mice that normally have a life span of 388 days. Couillard-Despres *et al.* (1998) demonstrated that the overexpression of either of two isoforms of human *NF-H* delayed the onset of ALS in the hG37R *SOD1* mice that have a mean life expectancy of 9.5 months. *NF-H* with 43 KSP (Lys-Ser-Pro phosphorylation sites) repeats was more protective than *NF-H* with 44 KSP repeats, delaying ALS onset by ~6 and ~2 months, respectively. In each of these experimental models, axonal neurofilament levels are decreased and perikaryal levels of NF-H are increased. The mechanism by which increased levels of NF-H protect mice from the toxicity of mutant SOD1 is unknown.

In similar experiments, Kostic *et al.* (1997) demonstrated that the overexpression of human *Bcl-2,* a potent inhibitor of apoptotic cell death, delayed ALS onset and death in the hG93A *SOD1* mice. There are two lines of the hG93A *SOD1* mice with different copy numbers of the transgene and different ages of ALS onset. This protective effect of *Bcl-2* was observed in both the early (~146-day life span) and the late (~240-day life span) onset hG93A *SOD1* mice. In these mice, *Bcl-2* overexpression delayed ALS onset and death by 15 and 33 days, respectively.

These experiments suggest that the ALS pathology induced by *SOD1* mutations can be influenced by a number of other genetic factors. While they may provide insights into the mechanism of mutant *SOD1*-induced ALS, they leave open the question of what naturally occurring genetic variations affect the penetrance of the ALS phenotype due to specific *SOD1* mutations. Indeed, the large variation in age of onset and severity in human ALS patients with specific *SOD1* mutations (Andersen *et al.,* 1997) suggests the presence of such genetic modifiers.

We sought to test the effect of antioxidant accumulation on the development of ALS. In humans, uric acid is thought to account for the majority of the antioxidant capacity of human serum; however, in mice uric acid is converted to allantoin by the uricase enzyme prior to excretion. Therefore, to mimic effectively the human situation, mice lacking the uricase enzyme were required. Uricase knockout mice that accumulate uric acid were produced by Wu *et al.* (1994). We chose to examine the effect of the uricase null background on the ALS phenotype in mice expressing mG86R *SOD1.* The mG86R *SOD1* mice in the FVB/NJ background typically develop hind-limb paralysis between 90 and 120 days (Ripps *et al.,* 1995; Figs. 1 and 2) with death occurring 3 to 4 days after paralysis onset. By breeding an mG86R *SOD1* mouse to a uricase knockout mouse, two mice (II-1 and II-2) in the first generation (Fig. 1), which were heterozygous for the uricase null allele, lived for more than 1 year, an extension of 240 days, prior to developing symptoms of ALS. Because mice that are heterozygous for the uricase null allele do not accumulate uric acid (Wu *et al.,* 1994), uric acid accumulation was not the cause of the delayed ALS onset in these mice. The uricase null mice are maintained in a mixed C57Bl6/129Sv background through brother–sister mating. We hypothesized that the genetic differences between the mouse strains most likely accounted for the delayed development of ALS in these mice. The genetic loci responsible for these protective effects were sought through further breeding and genetic mapping experiments.

MATERIALS AND METHODS

Animals. Inbred FVB/NJ mice were obtained from The Jackson Laboratory. Mice were housed in microisolater cages under specific pathogen-free conditions and bred at the Center for Laboratory Animal Care at the University of Colorado Health Sciences Center (UCHSC). All mouse experiments were approved by the USHSC Institutional Animal Care and Use Committee. The uricase null mice (Wu *et al.,* 1994) were a generous gift from Dr. C. Thomas Caskey. Both the mG86R *SOD1* mice (Ripps *et al.,* 1995) and the uricase null mice (Wu *et al.,* 1994) were rederived after their arrival at the UCHSC. The uricase null allele was genotyped using the following primers: URA1, 5′-GACCTTTGCAATGAACATCTG; URA2, 5′-TGTGGATGAATGCATGGACG; and URA4, 5′-ACCACCAAGCGAAACATCGC. This PCR results in a 250-bp product for wildtype mice, 250- and 500-bp products for heterozygotes, and 500- and 1400-bp products for uricase null mice. The mG86R *SOD1* mutation was detected by digesting the 133-bp PCR product generated by EH128 (5′-ATCCACCTGATGCTGTTTTA) and EH129 (5′-CCAATGATGGAATGCTCTCC) with *Fsp*I (New England Biolabs), which detects the presence of the mutation.

Genotyping and expression analysis. The Gentra systems DNA preparation kit was used to purify genomic DNA from 2-mm tail biopsies per the manufacturer's instructions. All polymorphic primer sets obtained from Research Genetics were used as recommended. The *Naip2* (neuronal apoptosis inhibitory protein) primers were synthesized as follows: primer 1, 5′-ACATACATTCACCCACTC-3′; and primer 2, 5′-CCAGTTTTCCTGGCAAGACA. This primer set lies within *Naip-rs6* approximately 60 kb telomeric to the *Smn* (survival motor neuron) gene (Endrizzi *et al.,* 1999). Some primer sets required the addition of 5% DMSO and/or annealing at 55°C. PCR products were resolved on either ethidium bromide-stained 4% agarose or 8 to 10% polyacrylamide gels.

RNA was purified from mouse spinal cord and brain by the method of Chomczynski and Sacchi (1987). RT-PCR was performed as described previously (Kunst *et al.,* 1997) using 1 μg of total RNA as starting material. The wildtype and mG86R SOD1 cDNA was amplified using primers EH129 and E3S (Ripps *et al.,* 1995), which span intron 3 of the mouse *SOD1* gene. The resulting PCR product was digested with *Fsp*I to identify the mG86R *SOD1* mutation.

Statistical analysis. Linkage was determined using both the FASTLINK (Cottingham *et al.,* 1993) and the GENEHUNTER (Kruglyak *et al.,* 1996) programs. Fewer female mice were protected from developing ALS than male mice. In generation III, 2 of 3 (66.6%) female mice were protected while 4 of 5 (80%) female mice had delayed ALS onset in generation IV (Fig. 1). All of the male mice in generations III and IV were protected. Therefore, for all markers, male penetrance was set to 99.9%, and female penetrance was set to

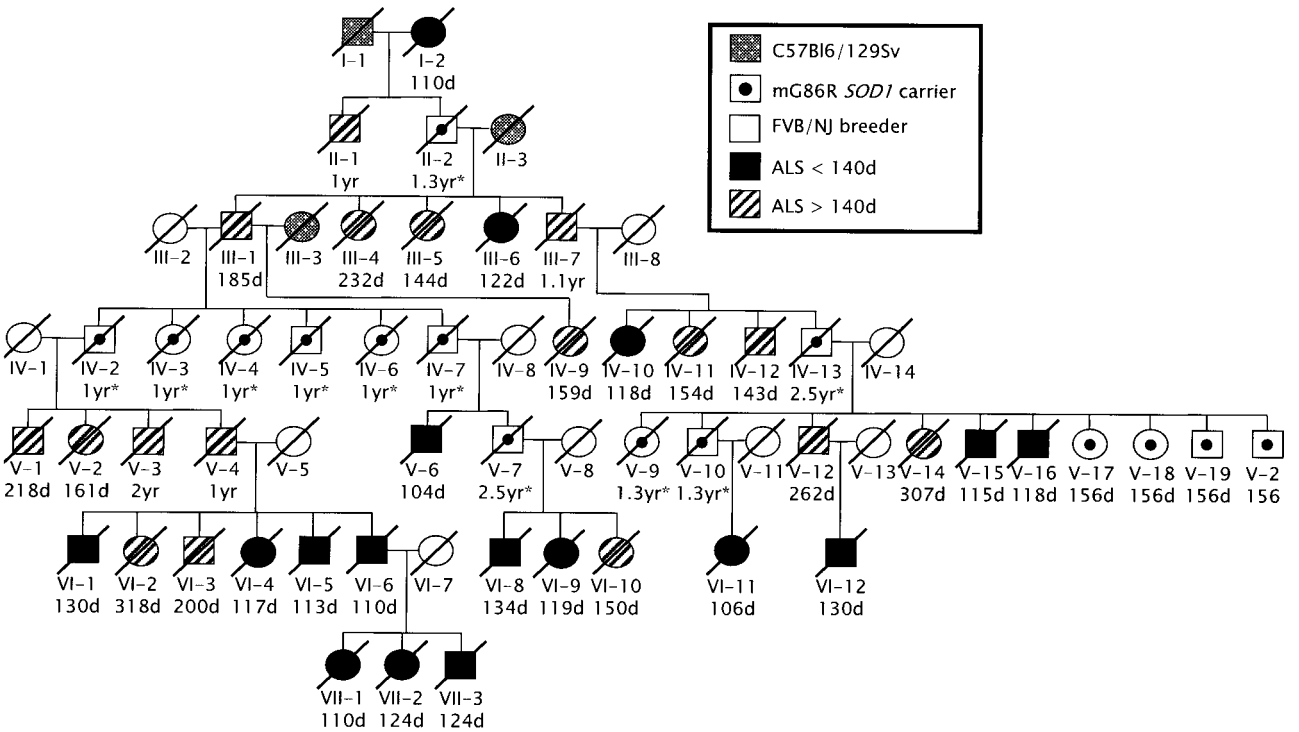

FIG. 1. Protection of mG86R *SOD1* mice. Symbols are as shown in the inset. Only offspring carrying the G86R *SOD1* mutation are shown. The asterisk denotes individual mice that were euthanized for other experiments prior to the development of hind-limb paralysis.

75%. The male phenocopy rate was estimated to be 5%, and the female phenocopy rate was estimated to be 25%. Due to pedigree size, the single mouse pedigree was split into three subpedigrees for the GENEHUNTER analysis. These three subpedigrees branched at individuals IV-2, IV-7, and III-7 (Fig. 1). Pedigrees and subpedigrees were analyzed both intact and with female offspring removed. Marker order and marker distances were obtained from the Research Genetics (http://www.resgen.com) and Whitehead databases (http://carbon.wi.mit.edu:8000/cgi-bin/mouse/index). Theoretical allele frequencies were calculated based upon the breeding scheme and the number of alleles observed for a given marker in the mice. The allele frequency of the modifier locus in the entire pedigree was predicted to be 21% based upon the averaged allele frequencies. An analysis, scoring phenotypes of only the mice with delayed ALS onset was also conducted using the FASTLINK program for all loci tested in the hopes of identifying additional modifier loci. No new linked regions were identified with this method.

RESULTS

Mice expressing murine G86R *SOD1* in the FVB/NJ background typically develop hind-limb paralysis between 90 and 120 days (Ripps *et al.*, 1995; Figs. 1 and 2). When the phenotype is allowed to progress in these mice, death typically occurs 3 to 4 days after paralysis onset. We bred an FVB/NJ mouse carrying the mG86R *SOD1* mutation to a uricase null mouse in a mixed C57Bl6/129Sv background. Two mice (II-1 and II-2) were born that were heterozygous for the uricase null allele and carried the mG86R *SOD1* mutation but lived for more than 1 year prior to developing symptoms of ALS (Fig. 1). One of these mice (II-2) was bred to a uricase null mouse, resulting in five offspring that carried the mG86R *SOD1* mutation. Only one of these mice, III-6 (Fig. 1), developed symptoms of ALS at the normal time for the FVB/NJ background. In the later generations, male mice carrying the mG86R *SOD1* mutation were bred to wildtype FVB/NJ female mice. Once the mice were >95% FVB/NJ (generations VII–XI), they consistently developed symptoms of ALS at the normal time (data not shown). A variety of phenotypes were segregating in our pedigree of mG86R *SOD1* mice with mixed genetic backgrounds. Two mice (IV-13 and V-7) appear to have completely escaped the onset of the ALS phenotype, living 2.5 years prior to euthanasia. Other mice had either normal (90–120 days) or delayed (143 days to >1 year) disease onset. The degree of protection is variable. Mouse III-1 developed symptoms of ALS at 185 days yet none of his offspring developed an ALS phenotype prior to euthanasia at 1 year. Because we euthanize all of our mice at the first sign of hind-limb paralysis, we do not know whether these protective genes also influence the progression of the ALS phenotype.

Age at ALS onset was used to plot the survival of these mice (Fig. 2). Mice containing the mG86R *SOD1* mutation that were 95 to 100% FVB/NJ were compared to mice that were 12.5 to 90% FVB/NJ. The mice that inherited genes from the uricase background (C57Bl6/129Sv) were dramatically protected from developing ALS due to the mG86R SOD1 mutation. All of the predominantly (95 to 100%) FVB/NJ mice developed symptoms of ALS prior to 130 days. At 130 days, 71% of the mixed strain mice were asymptomatic. At 1 year, 39% of the mixed strain mice were still alive. Interestingly, male mice were somewhat more protected than female mice by the genetic modifiers (Fig. 2). We may

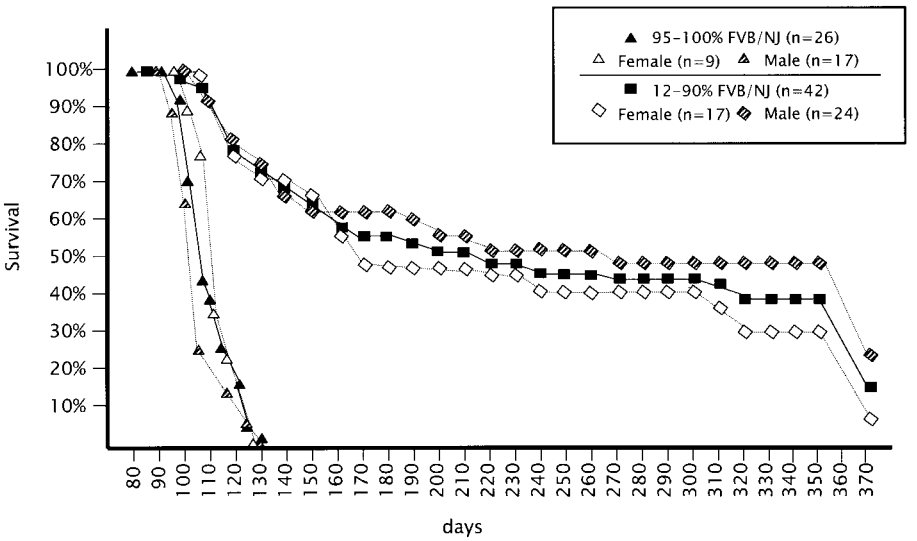

FIG. 2. Effect of genetic background on the survival of the G86R *SOD1* mice. In the FVB/NJ mouse strain, all of the mice develop ALS prior to 130 days (filled triangles). In a mixed genetic background (filled squares), ALS onset is significantly delayed. In the mixed genetic background, ~5% of the mG86R *SOD1* mice were still alive at 2 years of age with no symptoms of ALS (not shown). Female mice (open diamonds) are somewhat less protected than male mice (hatched diamonds). These sex-specific differences in ALS onset were not observed in the mG86R *SOD1* mice in the FVB/NJ background (open and hatched triangles).

have preferentially selected for genes that would protect male mice by exclusively breeding male mice with delayed ALS onset to wildtype FVB/NJ female mice. Indeed, mouse III-6, which should be heterozygous or homozygous for the protective alleles, died at 122 days, suggesting that the penetrance of the modifier genes is reduced in female mice. For this reason, male penetrance was set to 99.9% and female penetrance was set to 75% for all parametric linkage calculations as described under Materials and Methods. Sex-specific modifier genes are common in both mice and human (Towne *et al.*, 1997; O'Donnell *et al.*, 1998; Agulnik *et al.*, 1998; McIndoe *et al.*, 1999; Mogil *et al.*, 1997). No sex-specific differences in the age of ALS onset were observed in the mG86R *SOD1* mice in the FVB/NJ background. Thus natural genetic background effects prevent the development of ALS in this mouse model better than any of the other genes (*NF-L, NF-H,* and *Bcl-2*) previously tested by breeding experiments using the other ALS mouse models.

One way that the mG86R *SOD1* mice might have been protected from developing ALS is by a decreased or absent expression of the mutant transgene. We used RT-PCR to determine whether a mouse with delayed ALS onset (V-9; Fig. 1) expressed a similar proportion of mutant to wildtype *SOD1* as a mouse who developed ALS at the normal time (V-16; Fig. 1). The expression of antioxidant enzymes typically decreases with age (Semsei *et al.*, 1991). Mouse V-9 was 1.3 years old when she was euthanized, whereas V-16 developed ALS at 118 days of age. By Western blot analysis, V-9 expressed less SOD1 protein than V-16 (data not shown). However, as shown in Fig. 3, V-9 and V-16 express similar amounts of mutant SOD1 when compared to the amount of wildtype SOD1. Therefore, it is unlikely that the delayed ALS onset observed in these mice is due to the suppression of the mG86R *SOD1* transgene.

We used 129 autosomal primer pairs that were polymorphic between the FVB/NJ and the C57Bl6/129Sv mice to genotype the mice shown in Fig. 1. Because we observed male to male transmission of the protective factor(s), we excluded the X chromosome from the screening process. Furthermore, because mice with delayed or absent ALS onset occur even after several backcrossings into the FVB/NJ strain, the modifier gene(s) acts in a dominant fashion. At least 3 markers per chromosome, at ~15-cM intervals, were tested. Using these 129 markers, we have excluded ~88% of the C57Bl6/129Sv mouse genome from containing genes that delay ALS onset in the mG86R *SOD1* mice. Lod scores were calculated using the FASTLINK program (Cottingham *et al.*, 1993). Chromosomes 1, 3, 4, 5, 6, 7, 10, 11, 12, 15, 16, 17, 18, and 19 can be entirely excluded by the criteria of excluding linkage when the multipoint lod scores are <-2 across the entire chromosomal region being tested. However, we chose to exclude chromosomal regions for linkage under these

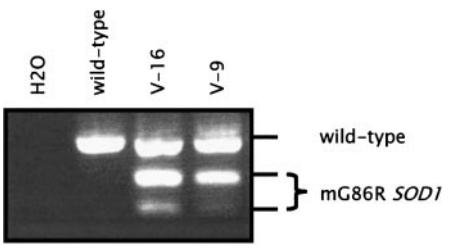

FIG. 3. Expression of mG86R *SOD1.* Spinal cord RNA was RT-PCR amplified and digested with *Fsp*I to differentiate the wildtype and mG86R *SOD1.* No RNA was included in the reaction in the H2O lane. A wildtype mouse was compared to V-16 (normal ALS onset) and V-9 (delayed ALS onset).

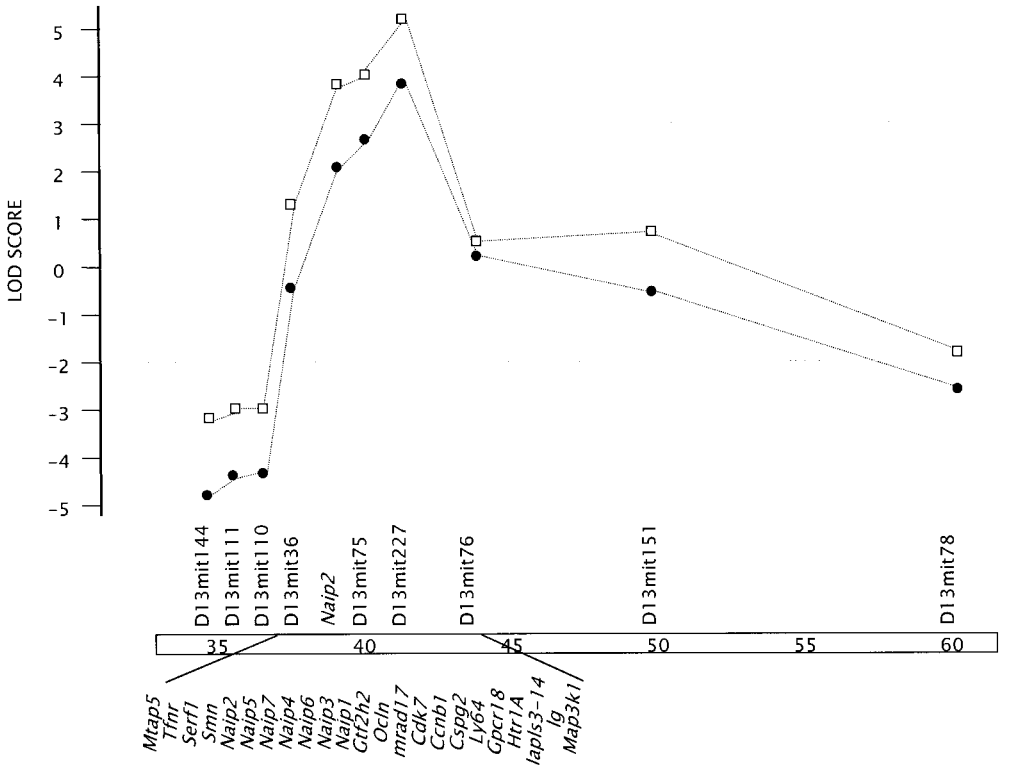

FIG. 4. Chromosome 13 linkage. Squares show male-specific lod scores. Circles show combined male and female lod scores. The highest lod scores were obtained with *Naip2*, D13mit75, and D13mit75. In male mice, there are recombinants with D13mit36 and D13mit76 (Fig. 5). The genes that map between these loci are shown under the cartoon of chromosome 13.

criteria only when the nonparametric lod (NPL) scores were also negative. The NPL score is a measure of whether affected individuals within a pedigree inherit identical alleles more often than expected by chance (Kruglyak *et al.*, 1996). NPL scores were calculated using the GENEHUNTER program (Kruglyak *et al.*, 1996). Using these more conservative criteria, only chromosomes 4, 5, 7, 11, 12, 17, and 19 were completely excluded.

Due to the way that the breeding was conducted, mice IV-2, IV-7, and IV-13 must be either homozygous for FVB/NJ alleles or heterozygous for a C57Bl6 or 129Sv allele at every marker. Because 13 of the 16 offspring of these mice demonstrated delayed ALS onset (Fig. 1), it is very unlikely ($P < 0.0085$) that there is only a single dominant modifier locus. It is more likely that two or more genetic loci prevented ALS in these offspring. However, we observed strong evidence for linkage at only a single chromosomal locus.

The best evidence for linkage was observed on mouse chromosome 13 between D13mit36 and D13mit76 (Figs. 3 and 4). A lod score of 5.07 was observed at D13mit227 when the male offspring were analyzed independently. This lod score was reduced to 3.82 when female offspring were included in the analysis (Fig. 4). A polymorphic primer set within the nearby *Naip2* gene resulted in lod scores of 3.87 and of 2.10 for males and for males plus females, respectively. Recombinants were observed at D13mit36 and D13mit76 (VI-1; Fig. 5) flanking this interval. Interestingly, both the C57Bl6 and the 129Sv chromosome 13 alleles were associated with delayed ALS onset (Fig. 5). The genotyping results for VI-1 at the *Naip2* locus and V-20 at D13mit227 were inconclusive. In each case, the FVB/NJ allele and an allele not present in either parent were observed. For this reason, this allele is designated with a question mark. At each of the other loci tested, Mendelian inheritance was observed not only in VI-1 and V-20 but also in all of the other mice genotyped. This finding suggests that the sizes of the dinucleotide repeats amplified with these primer sets altered during meiosis.

With the exception of V-20, all of the male mice with delayed ALS onset inherited C57Bl6- or 129Sv-derived chromosome 13 alleles (Fig. 5). Four female mice with delayed ALS onset did not inherit chromosome 13 alleles (V-2, V-14, VI-2, and VI-10; Fig. 1) from either the C57Bl6 or the 129Sv mice between D13mit36 and D13mit76. Therefore, as predicted, additional loci that modify the penetrance of mG86R *SOD1*-mediated ALS remain to be identified. Furthermore, the protective effect of the chromosome 13 locus appears to have reduced penetrance in females (Fig. 4) as four female mice who inherited these alleles developed ALS at the normal time (III-6, IV-10, VI-4, and VI-11; Fig. 1). It is also not clear whether the chromosome 13 protective alleles act as true dominants. Only III-6 (Fig. 1) was homozygous for the C57Bl6 chromosome 13 alleles but she developed ALS at the normal time. Females may have reduced penetrance with this modifier locus or

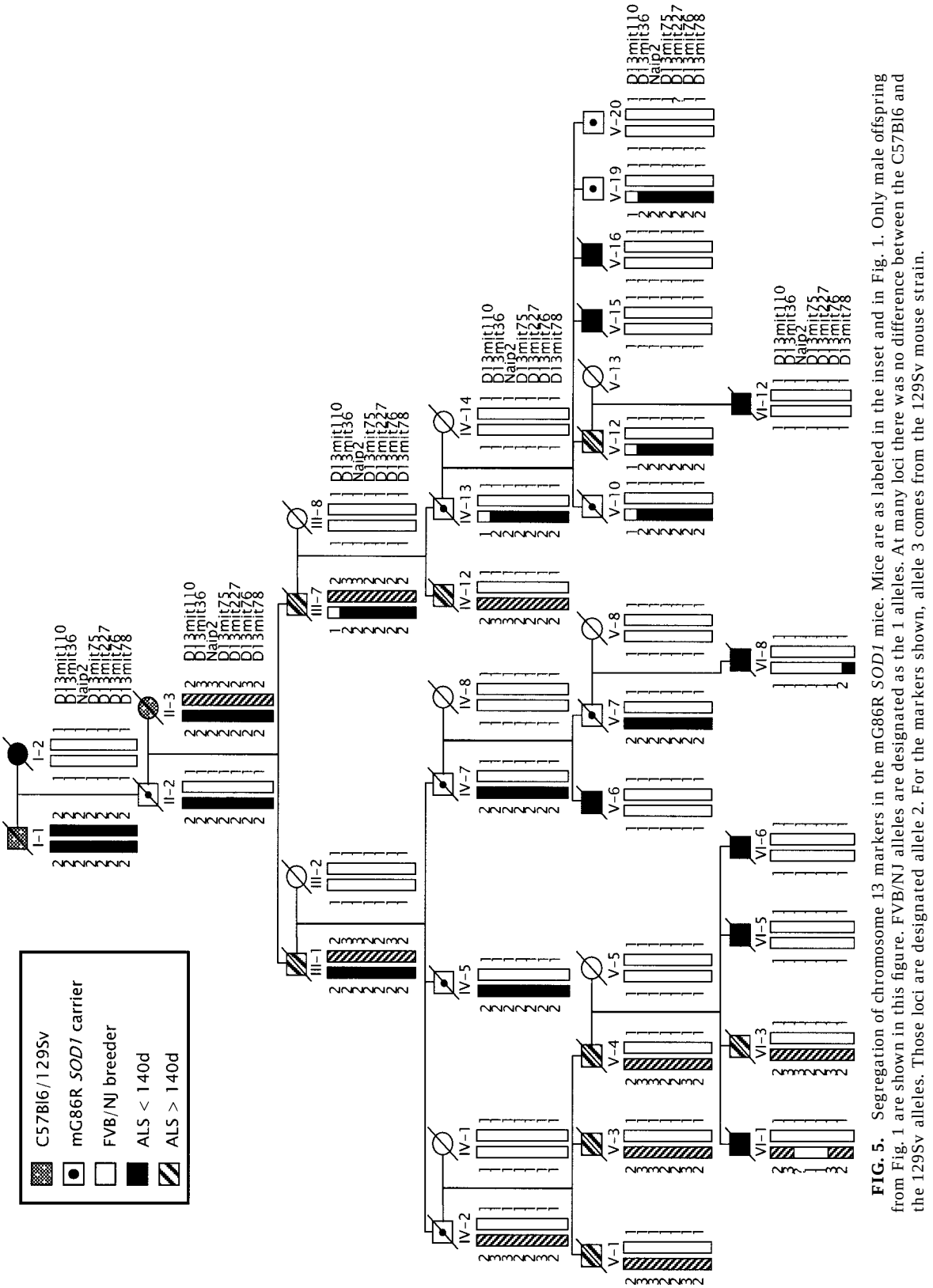

FIG. 5. Segregation of chromosome 13 markers in the mG86R *SOD1* mice. Mice are as labeled in the inset and in Fig. 1. Only male offspring from Fig. 1 are shown in this figure. FVB/NJ alleles are designated as the 1 alleles. At many loci there was no difference between the C57Bl6 and the 129Sv alleles. Those loci are designated allele 2. For the markers shown, allele 3 comes from the 129Sv mouse strain.

TABLE 1

Markers with Positive NPL Scores

Locus	Chr	cM	NPL (male)	NPL (male + female)
D1mit178	1	35	**2.09 (*P* = 0.036)**	**0.98 (*P* = 0.036)**
D1mit332	1	44	**2.32 (*P* = 0.033)**	**1.54 (*P* = 0.026)**
D1mit216	1	49	**2.32 (*P* = 0.033)**	**1.54 (*P* = 0.026)**
D1mit136	1	60	1.46 (*P* = 0.080)	**1.54 (*P* = 0.026)**
D1mit137	1	66	1.46 (*P* = 0.080)	**1.12 (*P* = 0.032)**
D1mit425	1	82	1.30 (*P* = 0.099)	**0.88 (*P* = 0.041)**
D1mit111	1	92	1.30 (*P* = 0.099)	**0.88 (*P* = 0.041)**
D3mit55	3	11	−0.15 (*P* = 0.0437)	**1.00 (*P* = 0.035)**
D3mit295	3	15	−0.11 (*P* = 0.415)	**1.20 (*P* = 0.030)**
D8mit216	8	61	1.71 (*P* = 0.071)	−0.05 (*P* = 0.163)
D8mit13	8	69	1.19 (*P* = 0.115)	−0.29 (*P* = 0.244)
D8mit42	8	73	1.19 (*P* = 0.115)	−0.29 (*P* = 0.244)
D13mit110	13	37	**2.89 (*P* = 0.025)**	−0.12 (*P* = 0.237)
D13mit36	13	37	**3.12 (*P* = 0.023)**	**0.95 (*P* = 0.050)**
Naip2	13	38	**3.12 (*P* = 0.023)**	**0.95 (*P* = 0.050)**
D13mit75	13	40	**3.46 (*P* = 0.018)**	0.46 (*P* = 0.100)
D13mit227	13	41	**4.33 (*P* = 0.010)**	**1.48 (*P* = 0.041)**
D13mit76	13	43	**3.28 (*P* = 0.020)**	**3.51 (*P* = 0.004)**
D13mit151	13	50	**3.61 (*P* = 0.016)**	**1.50 (*P* = 0.040)**
D13mit78	13	59	**3.46 (*P* = 0.017)**	**1.51 (*P* = 0.049)**
D18mit200	18	10	**3.13 (*P* = 0.018)**	0.51 (*P* = 0.075)
D18mit202	18	14	**2.79 (*P* = 0.025)**	**1.39 (*P* = 0.029)**
D18mit91	18	20	**2.79 (*P* = 0.025)**	**1.43 (*P* = 0.028)**
D18mit81	18	26	**2.79 (*P* = 0.025)**	**1.43 (*P* = 0.028)**

the C57Bl6 allele may be more protective in the heterozygous state, synergistically interacting with the 129Sv or FVB/NJ alleles. To test directly the penetrance of the protective effect and effect of the homozygous or heterozygous state we will need to breed the C57Bl6 or 129Sv chromosome 13 alleles into FVB/NJ mG86R *SOD1* mice in the absence of other C57Bl6 or 129Sv genetic material.

There are several chromosomal regions that could not be excluded from containing genes that modify the ALS phenotype because part or all of the pedigree resulted in positive NPL scores. Cumulative NPL scores for either the male offspring alone or the male plus female offspring are shown in Table 1. Only loci that generated NPL scores of at least 1 are shown. NPL scores with *P* values less than 0.05 are highlighted in boldface type. Almost all of the chromosome 13 markers used gave significant NPL scores when male mice were analyzed independently. This result is in concordance with the high parametric lod scores obtained with these markers when only male mice were analyzed using the FASTLINK program (Fig. 4). After chromosome 13, chromosomes 1 and 18 are the most likely chromosomes to contain modifier loci that can prevent ALS onset. Only the 129Sv-derived chromosome 1 alleles are associated with positive NPL scores. The chromosome 18 alleles from the uricase background were not transmitted by III-7 to IV-13 (Fig. 1). When this branch of the pedigree is omitted from the GENEHUNTER analysis, the NPL scores obtained for the chromosome 18 markers are >3.83 ($P = 0.016$). With the exception of the chromosome 13-linked loci and marker D8mit215, mice with delayed ALS onset did not inherit alleles from the uricase mice more often than mice with normal ALS onset. Therefore, if some of these potential modifier loci delay ALS onset in the mG86R *SOD1* mice, the penetrance of these loci may be as low as 50%.

DISCUSSION

In people, ALS disease severity is clearly modified by other genetic and environmental factors because clinical variation occurs among patients with the same *SOD1* genotype and who are members of the same family (Andersen *et al.,* 1997). While the A4V *SOD1* mutation generally results in a very rapid progression of the ALS phenotype, only 91% of the individuals who inherit this mutation develop symptoms of ALS (Cudkowicz *et al.,* 1997). Furthermore, the D90A *SOD1* mutation is recessive in some genetic backgrounds, but it is dominant in others. The recessive D90A chromosomes share a common founder haplotype, suggesting that there is a linked human chromosome 21 protective factor that makes this mutation recessive in this specific genetic background (Al-Chalabi *et al.,* 1998). We observed no evidence for a modifier locus on mouse chromosome 16 from the C57Bl6/129Sv mice in the region syntenic to human chromosome 21 near *SOD1*. This probably excludes the mouse homologue of the human gene that protects people who carry the recessive D90A *SOD1* mutation, assuming that this human chromosome 21 gene is contained within the homologous region of mouse chromosome 16.

Similar to these examples of phenotypic variation in people, the phenotypes observed in transgenic mice are highly dependent upon the mouse strain. Like our results with the mG86R *SOD1* ALS mice, the expression of the hG37R *SOD1*-mediated ALS phenotype is dependent upon the mouse genetic background. Delayed and variable onset occurs when the mice are in a pure C57BL/6J background. To control the variability in phenotype expression, The Jackson Laboratory (http://jaxmice.jax.org) maintains these mice by breeding hemizygous carriers to C57BL/6J × C3H/HeJ F1 mice.

Previous experiments using other ALS mouse models demonstrated that alterations in the expression levels of *Bcl-2, NF-L,* and *NF-H* delayed ALS onset. Surprisingly, most of these genes were excluded as candidate genes that delay ALS onset in the mG86R *SOD1* mice. The mouse neurofilament heavy (*NF-H*) and light (*NF-L*) genes on chromosomes 11 and 14 were excluded. The only previously tested modifier gene that was not excluded is *Bcl-2*, which maps to mouse chromosome 1 near D1mit136. We obtained an NPL score of 1.54 ($P = 0.026$) at D1mit136, suggesting that polymorphisms in the endogenous mouse *Bcl-2* gene could

also be involved in the delayed ALS onset seen in the mG86R *SOD1* mice.

As shown in Fig. 4, the region of chromosome 13 linked to delayed ALS onset contains a number of promising candidate genes including *Smn*, multiple copies of *Naip*, and *Serf1* (a putative modifier of human spinal muscular atrophy; Scharf *et al.*, 1998). Deletions of *SMN* and *NAIP* are associated with human spinal muscular atrophy, a disease that is characterized by the loss of lower motor neurons (Scharf *et al.*, 1998; Endrizzi *et al.*, 1999). While mutations in *SMN* and/or *NAIP* have not been shown to cause ALS (Parboosingh *et al.*, 1999), both diseases are characterized by the loss of motor neurons. Polymorphisms in these genes or their altered expression might help protect mice from the effects of mutant SOD1. In the 129Sv mouse strain, there are seven *Naip* genes in this region of mouse chromosome 13 (Growney *et al.*, 2000). At least three of these are predicted to be expressed in the central nervous system (Endrizzi *et al.*, 1999; Huang *et al.*, 1999). Also in this chromosomal region are transcription factors (*Tfnr* and *Gtf2h2*), cell cycle control proteins (*Ccnb1, Cdk7, Mrad17*), a serotonin receptor (*Htr1A*), and a brain expressed microtubule-associated protein (*Mtap5*).

The identification of the gene(s) that suppresses the development of ALS will have important consequences. The genetic differences between the mouse strains effect a "genetic cure" for ALS in the mG86R *SOD1* mice. If we can identify the gene(s) that reduces the penetrance of this mutation, we may be able to elucidate the mechanisms by which mutations in *SOD1* lead to ALS. If the functional changes induced by these genetic differences can be mimicked by pharmaceutical therapeutics, a treatment of ALS and perhaps other neurodegenerative disorders in people may be possible.

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