Journal of Gerontology: MEDICAL SCIENCES
1998, Vol. 53A, No. 4, M257–M263

Marker Loci Associated With Life Span in Genetically Heterogeneous Mice

Richard A. Miller,[1,4,5] Clarence Chrisp,[3] Anne U. Jackson,[2] and David Burke[2,4]

Departments of [1]Pathology and [2]Human Genetics, [3]Unit for Laboratory Animal Medicine, and [4]Institute of Gerontology, University of Michigan.
[5]Ann Arbor Department of Veterans Affairs Medical Center, Ann Arbor.

Background. Little is known about the number or chromosomal location of genetic loci that might identify individuals destined to have a long life span. Analysis of gene/life span associations in mice, which are short-lived compared to humans, might provide guidance for an analysis of the genetic basis of life span in humans.

Methods. A group of 144 genetically heterogeneous mice, produced by a four-way cross between two F_1 hybrid mouse stocks, was genotyped at 82 loci, and the mice were allowed to live either until their natural deaths or until they became extremely ill. Each mouse was also necropsied to determine the probable cause of death. An analysis of variance was used to seek relationships between life span (dependent variable) and the independent variables sex and allele for each marker locus.

Results. Five markers on different chromosomes were associated with differential longevity in male mice, and two other markers were associated with longevity in female mice. Post hoc probabilities were suggestive but not definitive, reaching $p < .003$ for the four strongest effects. Associations between marker loci and life span were sex-specific in almost all cases, affecting either males or females but not both. The strongest effects led to differences in mean survival of about 20% in the affected sex. The survival curves are consistent with the idea that the markers are linked to loci that influence the mortality risks of the longest-lived animals in the cohort. Associations between markers and life span did not appear to reflect associations of these markers to specific diseases in these mice.

Conclusions. Associations between genetic markers and life span in mice bred by using a four-way cross are strong enough to deserve further analysis and seem to be sex-specific in their effects.

ABOUT 25–35% of the variation in life span is attributable to genetic factors in mice and in humans (1,2), but the number, location, and nature of the responsible loci are all still undetermined. Some studies (1) and some theoretical considerations (3) suggest that there may be a large number of loci that contribute to differences among individuals in longevity; if this is true, then the quantitative effect of any one locus is likely to be small. Other analyses (4,5) are more consistent with the idea that much of the variation in longevity among laboratory mice reflects variation at a fairly small number of loci (e.g., 5–10 loci). Although both heritability of a complex phenotype and the number of effective segregating loci will depend on environmental factors and on the source of genetic variance in the population, a demonstration that individual loci had detectably large effects on life span in a segregating population would have important theoretical and practical implications. For example, such a demonstration would permit the derivation of mouse populations that are closely related and yet exhibit differential survival patterns, thus facilitating the analysis of physiological factors that regulate life span and disease. To date, the analysis of the contributions of defined genetic loci to exceptional longevity in human populations has been limited to the study of alleles known to influence specific late-life illnesses (6). Studies of mouse populations, in which sibships of arbitrarily large size can be produced at reasonable cost, may be helpful in searching for human genes that influence longevity and influence health in old age. Our study was designed to count and map mouse genes that have detectably large effects on life span, and here we report preliminary evidence that such genes may have sex-specific effects.

Materials and Methods

Mice and Husbandry

Mice for this study were the progeny of a cross between (BALB/c × C57BL/6)F_1 females and (C3H × DBA/2)F_1 males; each mouse in the group is genetically distinct but is derived from the same four progenitor genomes, which we abbreviate as BALB, B6, C3H, and DBA. The population is thus the genetic equivalent of a very large sibship. The mice were housed in same-sex groups in one room of a specific pathogen-free colony. Screening tests for pathogens were carried out every 3 months and were repeatedly negative throughout the course of this study.

Exclusion Criteria

The cohort of mice used for this analysis originally contained 170 animals, of which 87 were male and 83 were female. Six cages containing 22 male mice were eliminated from the study at ages less than 12 months because fighting had led to bite wounds. Four other males were also excluded from the study: one because it was sacrificed in error at the age of 18.7 months and three others because they died spontaneously before 8 months of age. Thus the

population of mice included in the study included 61 males and 83 females.

In addition, 15 males were found, at necropsy, to be suffering from mouse urinary syndrome (MUS), a syndrome characterized by obstruction of the urethra with expansion and (in extreme cases) rupture of the urinary bladder, which has been observed previously in group-housed males of several inbred strains (7,8) and is thought to represent a response to the social stresses that arise in all-male groups. These males were excluded from most of the analyses described below; their mean age at death was 15.6 ± 4.2 (*SD*) months.

Genotyping Assays

Polymorphic marker loci were selected for polymerase chain reaction (PCR)-based genotyping using data provided by the Mouse Simple Sequence Length Polymorphism (SSLP) Database, Whitehead/MIT Center for Genome Research (Cambridge, MA; http://www.genome.wi.mit.edu/cgi-bin/mouse/) or the Mouse Genome Database 3.1, Mouse Genome Informatics, The Jackson Laboratory (Bar Harbor, ME; http://www.informatics.jax.org/). The 82 SSLP loci represent all chromosomes and provide a marker within 25 cM of approximately 90% of the genome (Jackson, Fornés, Galecki, Miller, & Burke, unpublished data). Sixty-five of the markers are informative for both maternal and paternal alleles; 16, including two X-linked loci, provide information only on maternally inherited alleles, and one provided information only about paternally inherited alleles. Genomic DNA samples were amplified and gel electrophoresed, and SSLP alleles were scored by using conventional methods, as described elsewhere (Jackson, Fornés, Galecki, Miller, & Burke, unpublished data).

Necropsy

Mice were judged to be moribund if, in the judgment of an experienced technician, they were considered unlikely to survive more than another few days. This assessment was based upon several criteria, including continued or severe weight loss, hunched posture, poor grooming, failure to eat or drink, extreme lethargy or partial paralysis, or the presence of a large ulcerated tumor. Mice judged to be moribund were euthanized and subjected to necropsy, as were mice found dead in their cages.

The methods used for pathology have been described in detail earlier (9). In 11 cases the cause of death could not be determined, either because advanced autolysis interfered with histopathological assessment (5 cases) or because it proved to be impossible to assign a probable cause of death from among the multiple lesions found at necropsy (6 cases).

Statistical Analysis

Associations between alleles and life span were evaluated by analysis of variance (ANOVA), using life span as the dependent variable, and taking allele, sex, and the [allele × sex] interaction as independent variables, excluding mice with a diagnosis of MUS. If the ANOVA F test was significant ($p < .05$), post hoc tests were used to assess the significance of the association between allele and longevity in each sex separately. For those 65 loci at which both maternal (BALB vs B6) and paternal (C3H vs DBA) alleles could be distinguished, two separate calculations were performed, one to seek associations between life span and the maternally transmitted allele and one to seek for effects of the alleles transmitted by the father.

Differences between males and females in proportions dying of specific causes were tested by two-tailed Fisher's exact test. Fisher's exact test was also used to assess the probability of associations between inherited alleles and the presence of specific lethal or incidental lesions; maternal and paternal alleles were tested separately, with males and females pooled, and mice with a diagnosis of MUS were excluded. These procedures examine only the effects of alleles that are either fully or partially dominant. Experiment-wise, significance levels were estimated by using a permutation test (10) based upon the backcross model implemented in QTL Cartographer (http://statgen.ncsu.edu) and assessing the maternal and paternal genotypes separately.

RESULTS

Exclusion of Male Mice With Lethal MUS

Early in the course of this study it became clear that a proportion of male mice was dying at early ages with a form of lethal disease rarely seen in most laboratory mouse strains, the mouse urinary syndrome (7,8). This syndrome, which is seen only in males and is virtually never seen in individually housed animals (including those of the genotype used in this study [Miller, Chrisp, and Dysko, unpublished data], is thought to result from the psychological stresses that accompany intermale competition. Figure 1 compares the survival curves for males found at necropsy to have died because of MUS with the survival curves for all other males and for females. To avoid possible confounding

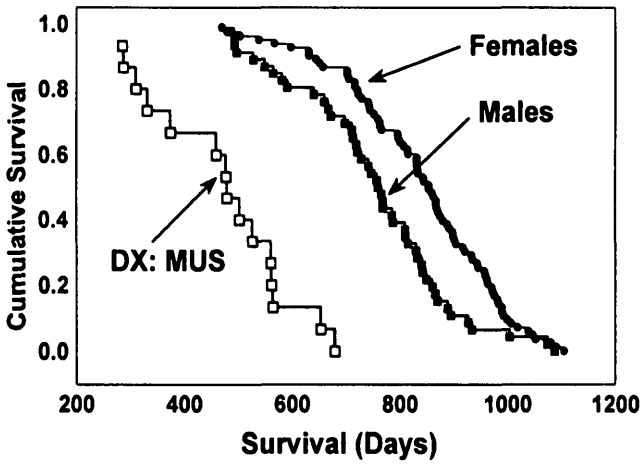

Figure 1. Plot of cumulative survival for three groups of genotyped mice: females, males that died of something other than MUS, and males that died of MUS. Each symbol represents one mouse. Females survive longer than males ($p = .006$ by log-rank test), and males without MUS survive longer than males with MUS ($p < .001$). Median survival of the longest-lived decile of females is 1050 days (range = 1007–1105, $N = 8$). Median survival of the longest-lived decile of males is 1004 days (range = 927–1087, $N = 5$). Median survival of the longest-lived decile of males dying of MUS is 666 days (range = 653–679, $N = 2$).

effects of this unusual syndrome on associations between genes and life span and other diseases, we excluded animals dying of MUS from calculations of genotype/phenotype associations presented throughout this report.

Differences Between Males and Females in Causes of Death

Table 1 shows the main categories of lethal disease observed in the 144 genotyped mice. Mean age at death did not differ among the different diagnostic groups, except for the short life span of mice that died of MUS. However, the proportion of males to females did differ substantially among the different diagnostic categories. Females were significantly more likely than males to die of lymphoma, fibrosarcoma, pituitary adenoma, and mammary carcinoma, and were significantly less likely to die of pulmonary adenocarcinoma or hepatocellular carcinoma.

Associations Between Genetic Markers and Life Span

The initial analysis for life span–associated markers utilized an ANOVA method, in which allele (at the given locus), sex, and the [sex × allele] interactions were treated as the independent variables and life span was treated as the dependent variable, with the MUS mice excluded. A post hoc procedure was then used to estimate the effect of the allele on survival in the sex that was most affected. Seven loci, on six chromosomes, had evidence for sex-specific effects at a criterion of $p < .01$; these are listed in Table 2. Loci with effects on male longevity were found on chromosomes 7, 10, 12, 18, and 19; loci with effects on female longevity were found on chromosomes 16 and 19. In six of these seven cases the interaction parameter was significant at $p < .05$, showing that the effect of allele on longevity differed between males and females. Ten other loci, distributed on chromosomes 1, 2, 4, 10, 11, 15, and 18, were associated with differential longevity with $.01 < p < .05$ (data not shown); in nine of these cases there was a statistically significant ($p < .05$) difference between the effects of the allele in males and females.

Figure 2 illustrates the relationship between genotype and survival for four loci selected from those listed in Table 2. The accompanying survival plots (right-hand column of Figure 2) show that in these instances loci affect not only mean longevity but also the life span of those mice dying at advanced ages. The most potent alleles, for example at D19Mit10, lead to differences in mean survival of about 5 months, or 20% of the mean lifetime of the affected sex.

The data are consistent with the idea that the effects of the strongest alleles may be approximately additive. For the two loci with strongest effects on male offspring, for example, the BALB allele at D7Mit91 confers an additional 117 days of mean longevity, whereas the DBA allele at D19Mit10 confers an additional 139 days; male mice that inherit both of these alleles live, on average, 254 days longer than males that inherit neither allele. (The corresponding effect sizes for females are respectively 31, –10, and –6 days.) For the two loci with strongest effects on female offspring, the BALB allele at D16Mit182 confers an additional 103 days, whereas the BALB allele at D19Mit19 confers an additional 91 days; female mice that inherit both alleles live, on average, 180 days longer than females that inherit neither allele. (The corresponding effect sizes for males are, respectively, –39, –25, and –32 days.)

The significance levels shown in Table 2 are nominal and do not take into account adjustments needed for simultaneous assessment of multiple linked and unlinked loci. A permutation test (10) was used to assess the experiment-wise significance of these associations. None of the associations shown in Table 2 meet the experiment-wise significance threshold at $p < .05$, although the effect of D16Mit182 on female longevity was marginally significant at $p < 0.1$. Thus the relationships shown in Table 2 and Figure 2 need to be considered tentative rather than definitive at this stage of the analysis.

Associations Between Genetic Markers and Pathological Findings

It was important to determine whether the relationships

Table 1: Mean Life Spans and Sex Ratios for Various Lethal Illnesses Among Genotyped Mice

Diagnosis	Total	Males	Females	% Male	Fisher Exact *p* Value*	Survival (mean ± *SD*)
Lymphoma	35	4	31	11	<.0001	839 ± 164
Other neoplasms	18	7	11	39	.80	786 ± 125
Fibrosarcoma	18	3	15	17	.02	859 ± 115
Pulmonary CA†	11	10	1	91	.0008	812 ± 154
Nonneoplastic	12	7	5	58	.36	744 ± 166
Mammary CA	11	1	10	9	.025	842 ± 110
Hepatocellular CA	7	7	0	100	.002	817 ± 73
Pituitary adenoma	6	0	6	0	.038	811 ± 130
Undetermined	11	7	4	64	.20	688 ± 197
Total without MUS	129	46	83	36		808 ± 150
[Males w/o MUS]	46					753 ± 147
[Females]	83					839 ± 83
MUS	15	15	0	100	<.0001	470 ± 128
Total	144	61	83	42		773 ± 180

*Significance test for the hypothesis that the distribution of males and females with the indicated diagnosis is the same as the distribution for all other mice (excluding MUS).

†CA = carcinoma.

Table 2. Associations Between Genetic Markers and Life Span in Four-Way Cross Mice

Marker	Long-Lived Allele*	Affected Sex**	Allele Effect† (*p* value)	Sex Difference‡ (*p* value)	*N* (mice)
D7Mit91	BALB	M	.003	.113	44
D10Mit40	DBA	M	.008	.043	46
D12Mit38	BALB	M	.002	.046	45
D16Mit182	BALB	F	.002	.010	74
D18Mit55	DBA	M	.004	.049	44
D19Mit19	BALB	F	.005	.034	78
D19Mit10	DBA	M	.001	.012	34

*Long-lived allele: the grandparental strain contributing the allele associated with increased longevity.

**Affected sex: the sex in which the locus has the stronger effect on life span.

†Post hoc probability that the difference in life span would be obtained by chance; tested by Student-Newman-Keuls test for males and by least-significant-difference test in females (which were more numerous); not corrected for effects of comparisons at many loci.

‡Interaction *p*, derived from ANOVA, which tests the likelihood that the association between the marker and life span differs between males and females.

seen in Table 2 and Figure 2 could be explained by a genetic predisposition to one or more specific forms of common late-life illness. If, for example, a specific marker locus was linked to genes that tended to promote a form of early-life lymphoma, this effect by itself might lead to an association between the marker locus and life expectancy. To assess this possibility, associations between genotype and terminal pathology were sought both for lethal and for incidental findings at necropsy. Figure 3 displays those associations in which the Fisher exact test statistic met the arbitrary criterion $p \leq .01$. At this significance level three loci are associated with an increased risk of lethal lymphoma (chromosomes 1, 10, and 19). Two loci are associated with either lethal (D8Mit42) or incidental (D7Mit76) fibrosarcoma. Associations are also seen between genetic markers and diagnoses of pituitary adenoma, mammary (breast) adenocarcinoma, pulmonary (lung) carcinoma, and hepatocellular carcinoma (liver). It is noteworthy that the B6 allele at D10Mit40 is associated with lymphoma, whereas the BALB allele at the same locus is associated with pulmonary carcinoma. At D8Mit42, the allele from the BALB strain is associated with risk of lethal pituitary adenoma, whereas the C3H allele is associated with lethal fibrosarcoma.

A similar analysis revealed an association between marker locus D6Mit268 and MUS. These data are also included in Figure 3. Of the 14 mice with MUS for which a paternal allele at D6Mit268 could be determined, 13 had received the C3H allele and only one the DBA allele ($p < .002$).

Discussion

This article presents the first results from our ongoing study searching for relationships between mouse genetic markers and life span and includes data on the genetic associations of common late-life diseases in our four-way cross population. Although the sample size (46 males and 83 females) for this initial cohort is too small to generate unambiguous evidence for loci that regulate mouse life span, the strongest associations seen are sufficiently impressive (seven loci with $p \leq .01$ in Table 2) to provide a rationale for further study using more closely spaced markers and larger numbers of animals.

Tests for associations between a single trait (life span) and a large number of marker loci are likely to produce type I errors, i.e., an impression of significant effect despite the lack of a true association between genotype and trait; confident assessment of these apparent linkages must await the outcome of a replicate study now in progress. Approximately 300 tests were carried out to seek relationships between markers and life span: 162 (81 loci tested in males and 81 tested in females) for markers inherited from the mother and another 132 (66 loci × 2 sexes) for paternally inherited markers. Chance alone is expected to produce one such association that exceeds a criterion of $p = .0033$ in a group of 300 such tests (.0033 = 1/300). Four of the associations listed in Table 2 met or exceeded this criterion. Thus our data are consistent with the idea that the mouse genome may contain a fairly small number of independently segregating loci with detectable effects on longevity and suggest that further analysis of marker loci on chromosomes 7, 10, 12, 16, 18, and 19 (from Table 2) may be rewarding.

The finding that the genetic effects on life span were in nearly all instances specific either for males or for females was striking. Theoretical speculation about the causes of aging often focuses on mechanisms, such as free radical production, glycation-based damage to macromolecules, somatic mutations, and accumulation of senescent cells, that seem likely to be equally hazardous in both males and females. For example, if a particular marker were linked to a locus that retarded aging and lengthened life span via improved protection against free radical mediated damage, one would expect it to be associated with increased longevity in both sexes. Our data suggest that the loci that influence life span and age-associated illness in males may be different from those that affect females. In this context it is noteworthy that quantitative trait locus (QTL) mapping of *Drosophila* genes has also identified loci with strong influences on male longevity but no effects on the life span of female flies (11) as well as other loci with effects in female flies only.

Our results can be usefully compared to those of two other groups that have also attempted to find relationships between genetic variation in laboratory mice and life span. Covelli and his colleagues (5) have investigated F_1, F_2, and backcross populations generated from two heterogeneous stocks that had been selected for differences in early life antibody responses to sheep erythrocytes but were later found also to differ in mean and maximal longevity. Their data indicated that a fairly small number of independently segregating loci—between 3 and 10, depending on assumptions of the analysis—seemed to account for the differences between the two parental stocks in humoral immunity and in longevity. Puel et al. (4) then carried out a QTL study of F_2 mice generated from the same parental stocks, seeking associations between marker loci and antibody responses. They reported evidence for strong effects of five loci, and

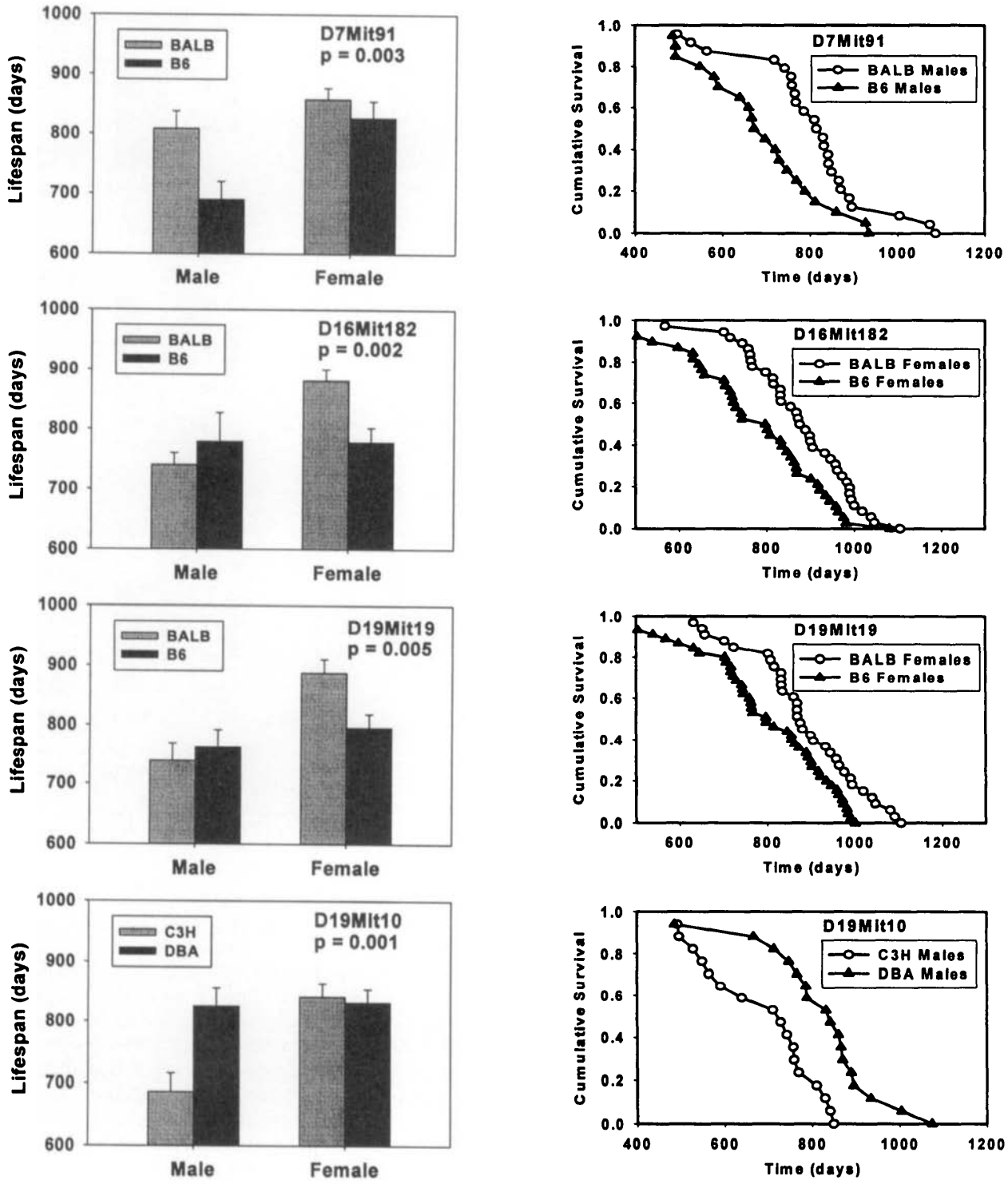

Figure 2. Associations between marker loci and longevity. The panels on the left show mean life span (± *SE*) for male and female mice (MUS excluded) in groups distinguished by the allele at the locus indicated. The *p* values represent the significance of the genetic effect in the affected sex and were calculated as described for Table 2. The panels on the right show cumulative survival curves for the affected sex. Table 2 contains a more complete list of such associations.

weaker effects of three loci, distributed throughout the genome. Gelman and her colleagues (1) have reported life span analysis of a set of recombinant inbred mouse lines derived from a cross between B6 and DBA inbred parents. Two statistical approaches, one based on marginal effects of discriminatory loci considered individually and the other using a proportional hazards model to construct sets of independently predictive loci, were used to seek assignments for chromosomal regions with effects on the shape of the survival curve or on mean survival. Taken together, these approaches produced evidence for effects of genes on eight different autosomes; studies of other mouse strains

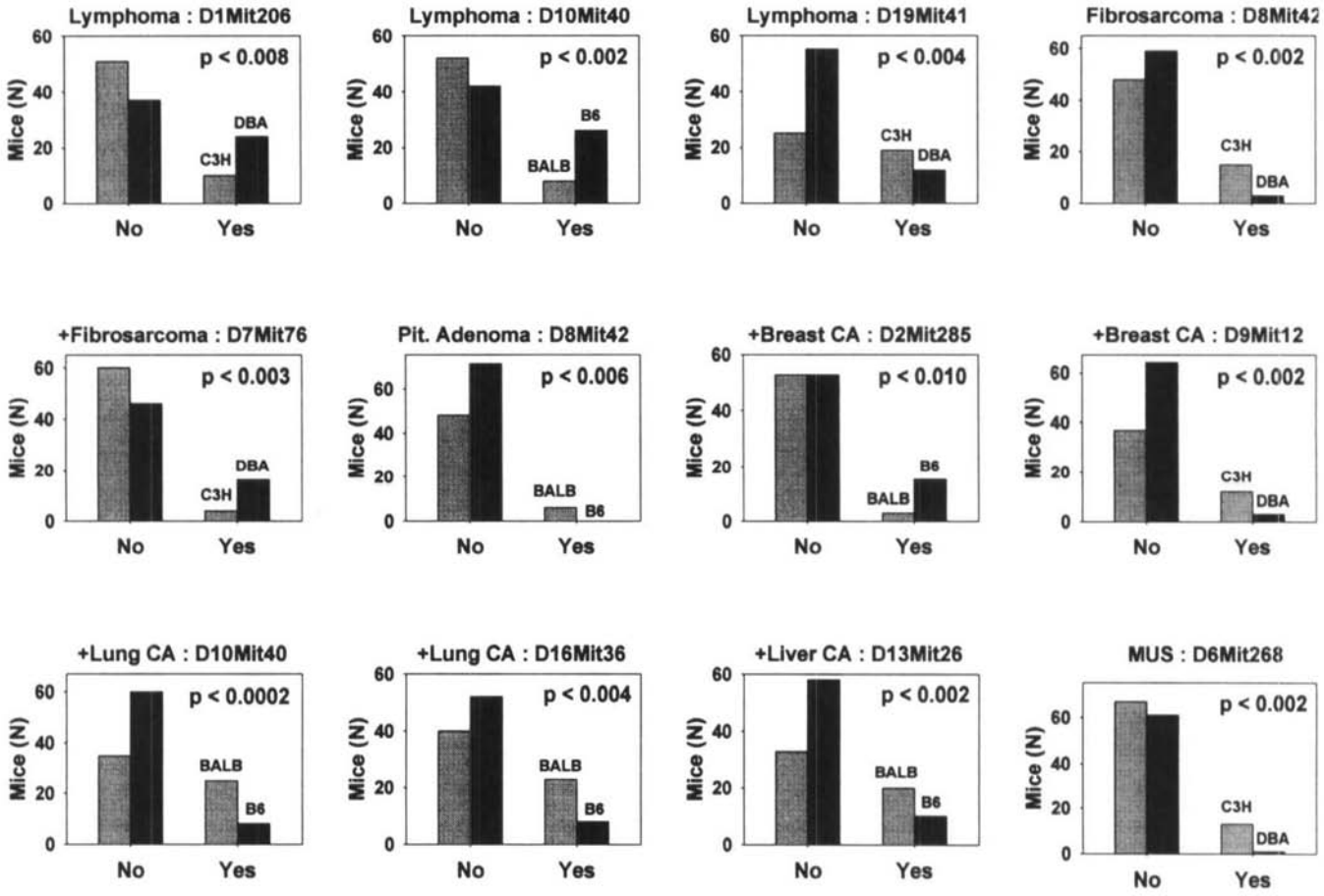

Figure 3. Associations between markers and diagnoses. Each panel shows the number of mice with ("Yes") and without ("No") the indicated diagnosis for each of the two alleles indicated in the panel. Pit = Pituitary. Only alleles from the parent whose alleles were associated with the diagnosis are shown. The *p* value reflects the result of an analysis, by Fisher exact test, that the association between allele and diagnosis would be obtained by chance, without correction for multiple comparisons; only associations where $p \leq .01$ are illustrated. A "+" sign before the diagnosis indicates that the calculation includes both lethal and nonlethal diagnoses; the other panels include lethal diagnoses only. Mice with MUS were excluded from the calculations except for the

may well provide evidence for alleles with similar effects on other chromosomes. Attempts to seek correspondences between our results and those of the Puel and Gelman analyses are not likely to be very helpful at this early stage, in part because of differences in the sources of genetic variation among the mouse populations studied, but we note that our marker D18Mit55 in Table 2 is 10 cM away from a marker implicated in the Puel study and that D7Mit91 and D12Mit38 are, respectively, 7 cM and 22 cM away from loci implicated in the Gelman analyses.

The hunt for human loci that contribute to late-life illnesses has already achieved some successes, including discoveries of genes that influence risks of Alzheimer's disease, adult onset diabetes, a variety of forms of neoplasia, and other prominent disease syndromes. The associations between disease and marker loci shown in Figure 3 do not meet accepted standards for statistical confidence after adjustment for multiple comparisons. Evaluation of possible gene/disease associations at high statistical power will require analysis of additional numbers of mice; this work is now in progress. Nonetheless, the available data tend not to support the idea that the associations between a genetic marker and life span are attributable to a corresponding association between the marker and a specific form of late-life illness. In most cases the life span–associated markers listed in Table 2 are not associated strongly with any one form of illness (Figure 3). D10Mit40 is an exception, appearing in both Table 2 and Figure 3, but the life span result represents an association between the paternally inherited DBA allele and survival in males, whereas the disease association concerns the maternally inherited BALB allele and lymphoma, a disease rarely seen in our male mice.

Analysis of larger sample sizes will be needed to show whether the associations between markers and life span are equally strong after stratification for cause of death. It will be of interest to discriminate among loci that alter the timing of all disease processes—if any such loci exist—from those that affect only the timing of neoplasia or other specific forms of late-life disease. It will also be of great interest to see if loci that predict longevity also are associated with individual differences in the rate of change of age-sensitive physiological traits, such as loss of protective immunity, decline in muscle strength, and accumulation of biochemical lesions including oxidation damage to proteins and nucleic acids. Linkage evidence from these murine studies may also prove useful in guiding a search for human

genes, in homologous chromosomal intervals, that influence the likelihood of disease-free survival into old age.

ACKNOWLEDGMENTS

This work was supported by National Institute on Aging Grants AG11687 and AG08808.

We thank Erin Belloli, Alison Fornés, Luann Linsalata, Lisa Mullins, and Joyce Peterson for technical assistance; Andrzej Galecki for statistical advice; Robert Dysko for veterinary consultation; and James Neel for comments on a draft of the manuscript.

Address correspondence to Dr. Richard A. Miller, Room 5316 CCGCB, Box 0940, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0940. E-mail: millerr@umich.edu

REFERENCES

1. Gelman R, Watson A, Bronson R, Yunis E. Murine chromosomal regions correlated with longevity. *Genetics*. 1988;118:693–704.
2. McGue M, Vaupel JW, Holm N, Harvald B. Longevity is moderately heritable in a sample of Danish twins born 1870–1880. *J Gerontol Biol Sci*. 1993;48:B237–B244.
3. Rose MR. *Evolutionary Biology of Aging*. New York: Oxford University Press; 1991.
4. Puel A, Groot PC, Lathrop MG, Demant P, Mouton D. Mapping of genes controlling quantitative antibody production in Biozzi mice. *J Immunol*. 1995;154:5799–5805.
5. Covelli V, Mouton D, Di Majo V, Bouthillier Y, Bangrazi C, Mevel JC, Rebessi S, Doria G, Biozzi G. Inheritance of immune responsiveness, life span, and disease incidence in interline crosses of mice selected for high or low multispecific antibody production. *J Immunol*. 1989;142:1224–1234.
6. Schachter F, Faure-Delanef L, Guenot F, Rouger H, Froguel P, Lesueur-Ginot L, Cohen D. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet*. 1994;6:29–32.
7. Everitt JI, Ross PW, Davis TW. Urologic syndrome associated with wire caging in AKR mice. *Lab Anim Sci*. 1988;38:609–611.
8. Tuffery AA. Urogenital lesions in laboratory mice. *J Pathol Bacteriol*. 1966;91:301–309.
9. Chrisp CE, Turke P, Luciano A, Swalwell S, Peterson J, Miller RA. Life span and pathology in genetically heterogeneous (four-way cross) mice: a new model for aging research. *Vet Pathol*. 1996;33:735–743.
10. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics*. 1994;138:963–971.
11. Nuzhdin SV, Pasyukova EG, Dilda CL, Zeng ZB, Mackay TF. Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proc Nat Acad Sci USA*. 1994;94:9734–9739.

Received July 21, 1997
Accepted January 19, 1998