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Coordinated Genetic Control of Neoplastic and Nonneoplastic Diseases in Mice

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Some models of aging imply that late-life diseases, though roughly synchronous, are the result of distinct pathophysiological processes, each in turn influenced by polymorphisms at multiple loci. Other models suggest that the dramatic increase in later life of multiple forms of illness might reflect the outcome of a unitary process, of so-far unknown biochemical nature, that proceeds at a species-specific rate to increase the risk of many forms of disease and disability in parallel. We have previously reported the results of genetic linkage analyses documenting the ability of alleles at *D9Mit110*, *D10Mit15*, and *D12Mit167*, and an allele pair at *D2Mit58* and *D16Mit182* to predict longevity in mice bred as the progeny of (BALB/cJ × C57BL/6J)F1 mothers and (C3H/HeJ × DBA/2J)F1 fathers (the UM-HET3 stock). Here we report the results of post hoc analyses to test the hypothesis that the genes that extend the life span of mice dying of neoplastic diseases also extend the life span of mice that die of nonneoplastic causes. In all four cases we find that the genotype associated with increased survival in mice dying of cancer is also associated for a similar degree of life span extension in mice dying of other causes. For *D9Mit110* and the combination of *D2Mit58* and *D16Mit182*, the difference is statistically significant in both the neoplastic and nonneoplastic mouse groups. The data support the hypothesis that many forms of late-life disease may be influenced by shared pathophysiologic mechanisms that are under coordinated genetic control.

THE effects of aging on most forms of serious disease are similar in kind across mammalian species and also roughly synchronous within each species, and thus they appear to reflect species-specific timing processes of unknown molecular nature. Cataracts, immunodeficiency, osteoarthritic changes, muscle weakness, declines in special senses, changes in connective tissue structure, and diminutions in cardiovascular endurance, as well as a variety of neoplastic and nonneoplastic diseases, are all commonly seen in 80-year-old people, 10-year-old dogs, and 3-year-old mice, but they are rare in young adults of each of these species. The idea that the many signs and symptoms of aging are coordinately regulated is often disputed (1–3) on the grounds that no single process seems likely to regulate synchronous changes in so many different cell types, organs, and extracellular tissues. In contrast, the ability of caloric restriction to retard the great proportion of physiological, biochemical, and pathological features of aging in rodents (4), as well as the observation that longevity of mice can be extended 35–70% by dwarfing mutations at the *Pit-1* and *Prop-1* loci (5,6), seems difficult to reconcile with models in which the manifold features seen in aged mice are timed by essentially independent means.

If it is true that aging is ultimately regulated by a very small number of processes with secondary effects on multiple age-sensitive cells and tissues, then it may be that these processes proceed at different rates among individual members of a given species. In particular, if rates of aging among members of the same species are influenced by alleles at polymorphic genetic loci, then alleles associated with the deceleration of age-related changes in specific cells, tissues, or processes may also be associated with slowed aging in a wide range of age-dependent outcomes.

We have in previous work (7) documented that five polymorphic marker loci, *D2Mit58*, *D9Mit110*, *D10Mit15*, *D12Mit167*, and *D16Mit182*, are predictors of longevity in a genetically heterogeneous four-way cross population (UM-HET3) bred as the progeny of CB6F1 females and C3D2F1 males. Here we report statistical tests of the hypothesis that alleles at these loci that predict relatively long life span are associated with longevity in subpopulations succumbing either to neoplasia or to nonneoplastic disease.

Methods

Mouse Handling

(BALB/cJ × C57BL/6J)F1 females and (C3H/HeJ × DBA/2J)F1 males were purchased from the Jackson Laboratories (Bar Harbor, ME) and mated to produce the study population. Throughout the study, all mice were housed in a single suite of specific-pathogen free (SPF) rooms under identical environmental conditions (12:12 hour light:dark

cycle, 23°C) and given ad libitum access to water and laboratory mouse chow; quarterly tests of sentinel mice showed that the facility remained SPF throughout the period of the study. The animals were maintained for their natural life span or sacrificed when judged by an experienced technician to be moribund. The study population included 278 mice, of which 253 yielded diagnosable cases at necropsy.

Genotyping

Genotyping was performed by standard polymerase chain reaction (PCR) amplification of genomic DNA from each animal, using marker loci obtained from the Mouse Simple Sequence Length Polymorphism Database, Whitehead/MIT Center for Genome Research (*carbon.wi.mit.edu:8000/ftp/distribution/mouse_sslp_releases/may99*) (8). Polyacrylamide gels were scored by silver staining or by using the ALFExpress automated sequencer as described (9). Analyses made use of 78 marker loci with an average intermarker interval of 23 cM. Of the markers, 69 are fully informative for all four grandparental alleles; the other 9 are informative for only one of the two parents. Genotypes were 95% complete on average for each marker. A full listing of loci as well as the complete genotype and phenotype data sets used in this analysis are available at *sitemaker.med.umich.edu/dtburke/files/253mice_78markers.xls.*

Statistical Analysis for Genome Scan

The genome scan is described in more detail elsewhere (7). In brief, the single-locus scan used an analysis of variance model developed by Xu (10), specifying maternal allele, paternal allele, and maternal allele by paternal allele interaction for fully informative loci, and using only the informative alleles for partially informative loci. To determine whether the genetic effects were sex specific, the calculations were performed on males, on females, and on the pooled-sex data. Statistical significance was based on permutation tested as specified by Churchill and Doerge (11), using 1000 random permutations to estimate experimentwise significance thresholds. Following the single-locus genome scan, a two-locus search was performed by examining all of the possible pairwise allelic interactions in the data. The computational difficulty of the two-locus search necessitated a change in the analytical procedure. Rather than viewing the genome scan data set as 69 four-way informative loci (plus nine biallelic informative loci), the two-locus genome scan defined 147 informative biallelic genotypes per animal (77 maternally informative and 70 paternally informative). Each pairwise combination of biallelic genotypes was examined by using an analysis of variance (ANOVA), using 600 random permutations of the data to estimate experimentwise significance levels. For reasons described in the text, each linkage analysis was conducted twice: once on the entire data set, and once on a data set (EDE, for early deaths excluded) that removed the 20% of the mice dying prior to 657 days of age.

Necropsy Analysis

Mice were inspected at least daily. Mice suspected to be ill (because of weight loss, poor grooming, or visible tumor) were observed twice daily except on weekends. Mice judged by an experienced technician to be so severely ill that survival for more than a few additional days was unlikely were taken to the necropsy suite and humanely euthanized; this group made up 59% of the total. Mice found dead (41%) were also submitted for necropsy. The necropsy protocol has been described in detail elsewhere (12), and it involved both gross inspection and histological examination of sections from 37 organs. Mice dying of lymphoma, fibrosarcoma, hepatocarcinoma, pulmonary adenocarcinoma, mammary adenocarcinoma, pituitary adenoma, histiocytic sarcoma, or endometrial sarcoma, as well as the mice dying of some other, rarer form of malignant neoplasm, were considered to have died from "neoplastic" illnesses. The category "nonneoplastic illnesses" included a miscellany of diagnoses, including congestive heart failure, inanition secondary to tooth degeneration, renal amyloidosis, endometritis, and other, rarer lesions. Mice dying of mouse urinary syndrome were included in neither category.

Post hoc Statistical Testing

The *t* statistic was calculated to compare mean longevity between groups of mice distinguished by the paternal alleles of *D9Mit110* or *D10Mit15*, and the *F* statistic was used to compare among the four genotypic classes distinguished by maternal and paternal alleles of *D12Mit167* or by the combination of paternal alleles at *D2Mit58* and maternal alleles at *D16Mit182.* These post hoc tests were conducted on all mice and then repeated by using subpopulations of mice that had died (a) of a neoplastic illness or (b) of a nonneoplastic illness.

Results

Early Mortality in Male Mice as a Result of Urinary Syndrome

The goal of our study was to map the genes that influenced longevity in UM-HET3 mice and to see whether the loci that regulated life span did so through effects on specific classes of late-life disease. Early in the study we noted an unexpectedly high rate of mortality in the first 18 months of life, limited to male mice. Most of the males dying at these ages were found, at necropsy, to exhibit the signs of the mouse urinary syndrome (MUS) previously noted to occur in group-housed males of certain inbred stocks (13,14), which is characterized by bite wounds on the genitals, the formation of a coagulum in the urethra, and bladder rupture. This syndrome is thought to represent the outcome of adjustments in dominance hierarchy in all-male groups. Survival curves (Figure 1) illustrated that mice dying with MUS had a median survival of less than 500 days, whereas female mice and males dying of other causes had median survival of approximately 800 days. Indeed, this urinary syndrome was responsible for 58% of the male deaths prior to 657 days of age (the age at which 20% of all mice had died), but only 5% of the male deaths after this age. Because our principal interests concerned the genetics of aging and late-life illness, we conducted our genome scans twice: once on the entire data set, and once on a truncated data set (EDE) limited to those mice that survived more than 657 days.

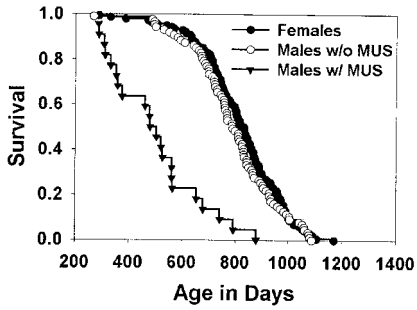

Figure 1. Mortality curves for female mice, male mice dying of mouse urinary syndrome (MUS), and all other male mice. Each symbol represents an individual mouse dying at the age indicated.

Summary of Genome Scan Results

Table 1 summarizes the key points of our quantitative trait locus (QTL) analysis, which has been reported in more detail in a separate manuscript. Three loci—*D9Mit110*, *D10Mit15*, and *D12Mit167*—were found to have a significant ability to predict longevity by using a permutation method (11) to control experimentwise Type I error rates at $p < .05$. *D9Mit110* (C3 allele > D2 allele at $p < .01$) and *D10Mit15* (D2 allele > C3 allele at $p < .05$) were found to be associated with differential longevity only in males, and only when animals dying earlier than 657 days were excluded (EDE data set). *D12Mit167* was found to be associated with differential longevity in both male and female mice, attaining statistical significance ($p < .01$) only in the truncated EDE population. For *D12Mit167*, longevity was influenced by an interaction between maternally and paternally inherited alleles, with the combination B6/C3 associated with longest life span and the C/C3 combination associated with shortest life span. In addition, a genomewide search for pairs of loci that influenced life span together showed that mice with both C3 allele at *D2Mit58* and the C allele at *D16Mit182* lived substantially longer than mice with other allele combinations at these two loci; by a permutation test, the difference approached the conventional criterion at $p = .06$. These four genotypes—three single loci and a locus-pair combination—were then studied further by post hoc testing to see whether the genotypes differed in their effects on different classes of lethal illnesses.

Summary of Necropsy Results

Of the 278 cases that came to necropsy, the pathologist was able to infer a likely cause of death in 253. In some cases, diagnosis was impossible because of postmortem autolysis, and in others, two or more diseases contributed to the death or terminal illness. Table 2 summarizes the results of the necropsy analysis. There is a wide range of lethal illnesses, consistent with the genetically heterogeneous nature of the population. Neoplasia of various sorts was responsible for the deaths of 88% of the females. Among males, mouse urinary syndrome was responsible for 27% of the deaths and other nonneoplastic illnesses for 18%. Females were particularly likely to die of lymphoma and fibrosarcoma, and males were likely to die of hepatocarcinoma and pulmonary adenocarcinoma.

Analysis of Mean Life Span in Genotype-Stratified Subgroups Dying of Neoplastic and Nonneoplastic Causes

We used an ANOVA to determine if genotype predicted mean life span for specific diagnosis groups in each of the four genetic systems that were previously shown (see Table 1) to be predictors of all-cause longevity. For *D9Mit110* and *D10Mit15*, we used a Student's *t* statistic to compare the two relevant genotypic classes. For *D12Mit167* (at which maternal and paternal alleles interact) and for the combination of *D2Mit58* plus *D16Mit182*, the *F* statistic was used to compare among the four distinct genotypes. Table 3 presents the outcome of these calculations. In each of the four cases there was a significant association between genotype and mean longevity when all causes of death were considered together, which is consistent with the more stringent experimentwise inferences summarized in Table 1. The effect size, calculated as the difference in mean longevity between the longest-lived genotype and the genotype associated with the shortest life span, ranged from 80 days for *D10Mit15* (among EDE males) to 173 days for the combination of *D2Mit58* plus *D16Mit182* (among females). When only those mice dying of neoplasia were considered, there was again a significant effect of genotype in all four cases, reflecting the high frequency of neoplastic illnesses among lethal diagnoses. Of greater interest, these genotypes were also associated with increased life span among mice dying of nonneoplastic illnesses. The effect sizes were similar among these nonneoplastic cases to effects calculated for mice dying of cancer, and they ranged from 76 days (*D10Mit15*) to 165 days (for *D2Mit58* plus *D16Mit182*). Despite the small number of cases, the genotypic effects were statistically significant ($p < .05$) for *D9Mit110* and for the combination of *D2Mit58* plus *D16Mit182*. The effects for the other two situations, though similar in magnitude to those seen among mice dying of neoplasia, are not statistically significant and should be considered tentative until they have been examined further in separate experiments.

Table 1. Summary of Genome Scan Results for All Causes of Death

Locus or Locus Pair	Population Tested	Longest Lived	Shortest Lived	Experimentwise *p*
D9Mit110	EDE males	C3	D2	< .01
D10Mit15	EDE males	D2	C3	< .05
D12Mit167	EDE, both sexes	B6C3	CC3	< .01
D2Mit58 + *D16Mit182*	Females	C3 at *D2Mit58* + C at *D16Mit182*	D2 at *D2Mit58* + B6 at *D16Mit182*	< .06

Note: EDE = early deaths excluded, i.e., excluding all mice dying prior to 657 days, the age past which 80% of the mice survived.

Table 2. Synopsis of Necropsy Outcomes

Cause of Death	% of Diagnosable Cases	
	Virgin Females	Virgin Males
Congestive heart failure	2	6
Endometrial sarcoma	3	0
Fibrosarcoma	18	5
Hepatocarcinoma	1	12
Histiocytic sarcoma	3	3
Lymphoma	34	8
Mammary adenocarcinoma	7	1
Mouse urinary syndrome	0	32
Pituitary adenoma	6	0
Pulmonary adenocarcinoma	2	14
Miscellaneous, nonneoplastic	15	15
Miscellaneous, neoplastic	9	5
Diagnosable cases (no.)	136	117
Total cases (no.)	147	131

Note: The table shows necropsy-based diagnoses (as percentage of the diagnosable cases) from the 253 mice for which the pathologist was able to infer a likely cause of death.

Figure 2 shows survival curves for mice stratified by the two genotypic criteria that were found to be statistically significant in Table 3. The top panels show survival curves for EDE males differing at *D9Mit110* (left to right: all mice, neoplastic deaths, and nonneoplastic deaths), and the bottom panels show analogous results among females for the longest-lived and shortest-lived genotypes at *D2Mit58* plus *D16Mit182*. These survival plots are consistent with the summary statistics shown in Table 3, showing extended longevity for mice dying of either neoplastic or nonneoplastic causes in each case. We also calculated effect sizes for specific forms of neoplasia and noted a few points of interest, although we recognize that the small number of cases for any one form of tumor provides little statistical power for hypothesis testing. For *D9Mit110*, for example, we noted equivalent effect sizes for mice dying of lymphoma (136 days), pulmonary adenocarcinoma (209 days, $p = .001$), and hepatocellular carcinoma (65 days), but a lack of effect for mice dying of fibrosarcoma (−76 days, $n = 6$ mice). For the *D2Mit58* plus *D16Mit182* combination, effect sizes were similar for each common form of tumor: lymphoma (186 days), fibrosarcoma (277 days), and mammary adenocarcinoma (200 days, $p = .02$).

Table 3. Summary of Post hoc Tests for Survival After Stratification for Cause of Death

Cause of Death	Effect Size (d)	*p*(*t*)	*N*
Locus *D9Mit110*			
All	88	0.001	77
Neoplastic	90	0.004	60
Nonneoplastic	119	0.03	15
Locus *D10Mit15*			
All	80	0.003	78
Neoplastic	91	0.003	50
Nonneoplastic	76	0.25	17
Locus *D12Mit167*			
All	93	0.002	166
Neoplastic	85	0.04	109
Nonneoplastic	126	0.19	38
Locus pair: *D2Mit58* + *D16Mit182*			
All	173	0.0001	132
Neoplastic	180	0.001	97
Nonneoplastic	165	0.04	22

Notes: Each post hoc analysis was conducted only for the population of mice in which the strongest genetic effect was noted (see Table 1). Effect size is calculated as the mean longevity of mice with the longest-lived genotype minus the mean longevity of mice in the shortest-lived genotype; *p* values represent two-sided *t* tests for biallelic contrasts (*D9Mit110* and *D10Mit15*), and *F* tests for *D12Mit167* and the *D16Mit182* × *D2Mit58* locus pair, because in each of these systems there are four distinguishable genotype classes. Here *N* gives the number of mice evaluated for the entire population, for the neoplastic deaths, or for the nonneoplastic deaths.

Discussion

Our results show that genetic loci identified by genome scanning as associated with differences in overall life span in the UM-HET3 four-way cross population lead to similar degrees of life-span difference in nonoverlapping groups of mice: (a) those that die of some form of cancer; and (b) those that die of a nonneoplastic illness. In all four situations (Table 3), the degree of life-span difference, calculated as the mean life span in the longest-lived genotype minus the mean longevity in the shortest-lived genotype, was similar in the neoplastic and the nonneoplastic groups, and in two cases, the genetic effect was statistically significant by *t* test or ANOVA *F* test in both groups of mice.

The analysis presented in this paper focuses on a narrow question: What is the genetic influence on age at death in groups of mice that differ in cause of death? In particular, the results do not bear on the question of whether these loci, or others, affect the likelihood that an individual mouse will die of neoplasia, or of a specific variety of neoplastic or nonneoplastic disease. It is possible, for example, that one or more loci segregating in our population could influence the risk or the rate of progression of lymphoma, or of breast or lung cancer, or of fatal congestive heart failure. Detection of such loci will require deployment of specific statistical tools for computation of experimentwise confidence levels for qualitative trait loci and adjustment for competing risks. Such an evaluation is now underway, but it is beyond the scope of the current report.

We interpret our results as support for two hypotheses: first, that some unknown physiological or biochemical pathway (the "aging process") influences the progression of multiple forms of late-life illnesses; second, that this process is itself under the influence of detectable polymorphic genetic loci. According to these hypotheses those mice that are, for whatever reasons, going to die of neoplastic disease will do so earlier or later depending on whether they inherit the alleles that speed or slow the aging process. Furthermore, those mice that are destined to die of a nonneoplastic illness will also do so earlier or later depending on inheritance of the same set of polymorphic, age-regulating alleles.

The model makes a number of other testable predictions. For example, it predicts that mice inheriting the allele combinations associated with greatest longevity ought to show relatively decelerated rates of change in multiple physiological systems, that is, slower immune senescence, slower cataract development, slower rates of change in patterns of liver gene expression, and so on. Tests of these ideas will

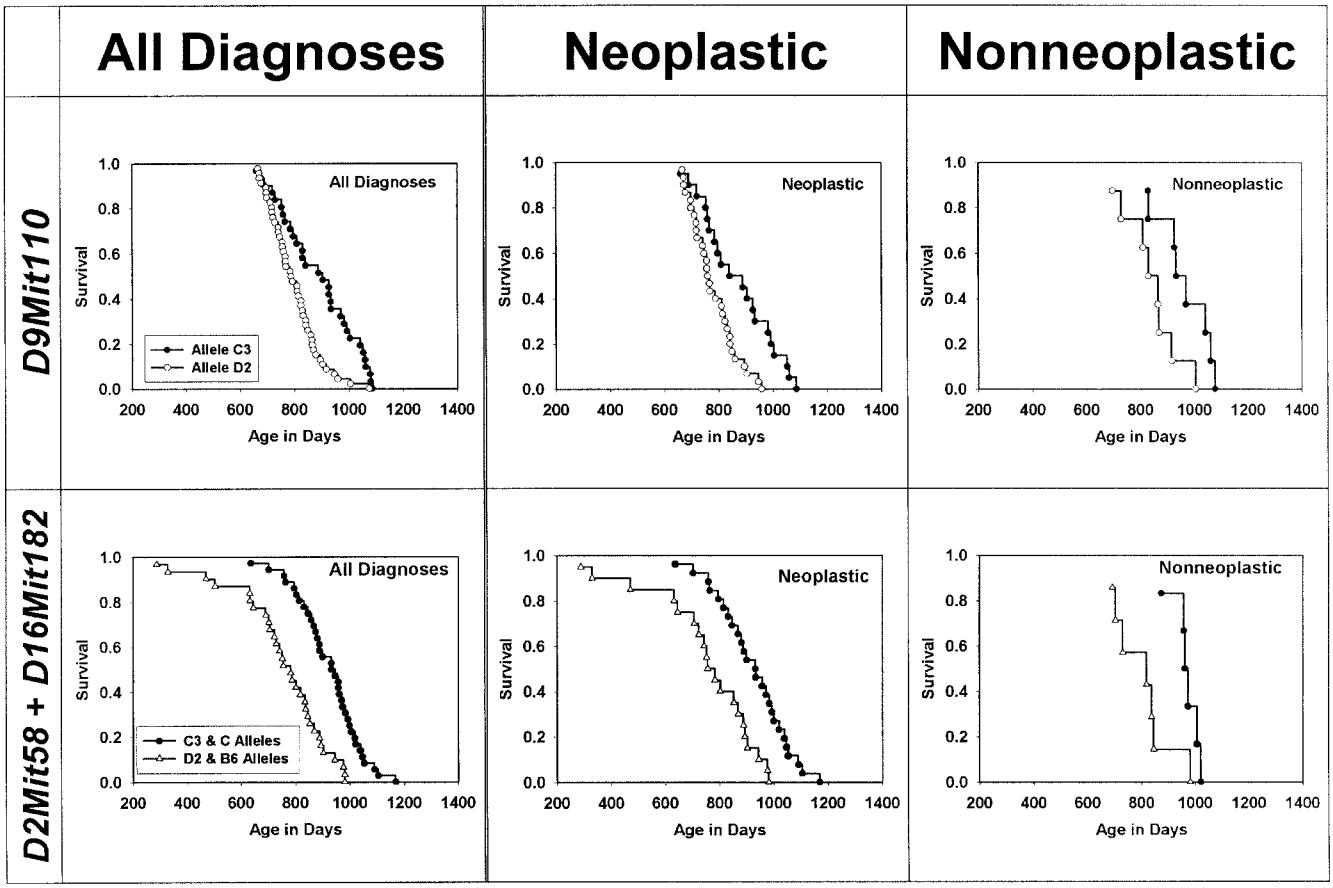

Figure 2. Mortality curves for mice stratified by allelic differences at the locus (or locus pair) indicated in the left-hand margin. The curves for *D9Mit110* show male mice in the early deaths excluded group, stratified by paternal alleles (C3 = C3H/HeJ; D2 = DBA/2J). The curves for the combination of *D2Mit58* + *D16Mit182* show female mice; here the genotypes represent first the paternal allele of *D2Mit58* (C3 or D2), followed by the maternal allele at *D16Mit182* (C or B6). The center column shows the survival curves for the subsets of these mice dying of neoplastic disease; the right column shows those dying of nonneoplastic causes.

require larger numbers of mice, because in each case it seems plausible that the end points—immune function, lens opacity, and the like—may be influenced by a wide range of genetic and environmental factors that make it more difficult to detect the hypothesized effects of aging and the polymorphisms that influence its rate.

Conclusions

By showing that genetic polymorphisms can influence the timing of at least two broad classes of late-life illnesses, our findings provide support for the idea that the synchronous development of multiple diseases late in the life span, including cancers, reflects shared pathophysiological processes that can be accelerated or decelerated by alleles at a modest number of polymorphic loci. It will be of interest in future work to determine if mice selected, at birth, for combinations of alleles that bestow reduced late-life mortality risk also exhibit retarded rates of change in immune function, muscle function, gene expression, hormonal balance, and other features of aging. If these genetic associations are replicated in other stocks of heterogeneous mice, including populations derived from progenitors not previously selected by laboratory domestication (15), the data may help to guide searches for human aging loci with pleiotropic effects on multiple forms of cancer and on nonneoplastic hazards of aging.

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References

1. Masoro EJ. Aging: current concepts. In: Masoro EJ, ed. *Handbook of Physiology. Section 11. Aging.* New York: Oxford University Press; 1995:3–21.
2. Holliday R. *Understanding Ageing*. Cambridge, UK: Cambridge University Press; 1999.
3. Smith JM. The causes of ageing. *Proc R Soc London Ser B.* 1962;157: 115–127.

4. Weindruch R, Walford RL. *The Retardation of Aging and Disease by Dietary Restriction.* Springfield, IL: Charles C Thomas; 1988.
5. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature.* 1996;384:33.
6. Miller RA. Kleemeier Award Lecture: are there genes for aging? *J Gerontol Biol Sci.* 1999;54A:B297–B307.
7. Jackson AU, Galecki AT, Burke DT, Miller RA. Mouse loci associated with life span exhibit sex-specific and epistatic effects. *J Gerontol Biol Sci*. 2002;57A:B9–B15.
8. Dietrich WF, Miller J, Steen R, et al. A comprehensive genetic map of the mouse genome [published erratum appears in *Nature*. May 9, 1996;381(6578):172]. *Nature.* 1996;380:149–152.
9. Jackson AU, Fornes A, Galecki A, Miller RA, Burke DT. Longitudinal QTL analysis of T cell phenotypes in a population of four-way cross mice. *Genetics.* 1999;151:785–795.
10. Xu S. Mapping quantitative trait loci using four-way crosses. *Genet Res Camb.* 1996;68:175–181.
11. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics.* 1994;138:963–971.
12. Chrisp CE, Turke P, Luciano A, Swalwell S, Peterson J, Miller RA. Lifespan and pathology in genetically heterogeneous (four-way cross) mice: a new model for aging research. *Vet Pathol.* 1996;33:735–743.
13. Tuffery AA. Urogenital lesions in laboratory mice. *J Pathol Bacteriol.* 1966;91:301–309.
14. Everitt JI, Ross PW, Davis TW. Urologic syndrome associated with wire caging in AKR mice. *Lab Anim Sci.* 1988;38:609–611.
15. Miller RA, Dysko R, Chrisp C, et al. Mouse (Mus musculus) stocks derived from tropical islands: new models for genetic analysis of life history traits. *J Zool.* 2000;250:95–104.

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