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Original Article

Genetic Regulation of Female Sexual Maturation and Longevity Through Circulating IGF1

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Abstract

We previously reported that insulin-like growth factor 1 (IGF1) was involved in coregulating female sexual maturation and longevity. To understand the underlying genetic mechanisms, based on the strain survey assays of development and aging traits, we crossed two mouse strains, KK/HlJ and PL/J, and produced 307 female F2 mice. We observed the age of vaginal patency (AVP) and the life span of these females. We also measured circulating IGF1 level at 7, 16, 24, 52, and 76 weeks. IGF1 level at 7 weeks significantly correlated with AVP. IGF1 levels at ages of 52 and 76 weeks negatively correlated with longevity ($p \leq .05$). A gene mapping study found 22, 4 ,and 3 quantitative trait loci for IGF1, AVP, and life span, respectively. Importantly, the colocalization of IGF1, AVP, and life span quantitative trait loci in the distal region of chromosome 2 suggests this locus carries gene(s) that could regulate IGF1, AVP, and life span. In this region, proprotein convertase subtilisin/kexin type 2 has been found to be associated with female sexual maturation in a human genome-wide association study. We verified the roles of proprotein convertase subtilisin/kexin type 2 in regulating IGF1 and AVP by showing that depletion of proprotein convertase subtilisin/kexin type 2 significantly reduced IGF1 and delayed AVP in mice, suggesting that it also might be involved in the regulation of aging.

Key Words: Female sexual maturation—Life span—Genetics—Mouse—*Pcsk2*.

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The evolutionary theory of aging predicts the existence of pleiotropic genes that mediate female sexual maturation (FSM) and life span (1,2). Several lines of evidence support this hypothesis. In the invertebrates, researchers found that allowing only older female Drosophila to reproduce resulted in decreased female fecundity and increased life span in later generations (3,4). In wild mammalian species, ranging from mice to elephants show that the later the initial age of reproduction, the longer the life span (5). The tradeoff holds within species as well. For example, opossums living on a predator-free island in Virginia matured later and aged more slowly than opossums on the "more dangerous" mainland (6). Mice trapped in the wild had delayed FSM and greater female reproduction life span and longevity compared with laboratory mice (7,8). However, the underlying genetic and molecular mechanisms have yet to be clarified.

Our previous studies systematically measured age of FSM and longevity in more than 30 mouse inbred strains (9,10). The age of

FSM and longevity vary dramatically across inbred strains and are negatively correlated. Our studies also verify that IGF1 signaling plays an important role in mediating the tradeoff relationship. Mice with lower circulating IGF1 have delayed FSM and extended longevity compared with mice with higher IGF1 (9). These studies strongly suggest that the genetic regulation of FSM is determined by pleiotropic genes that coregulate life span through effects on basic aging rates. Thus, identifying genes that regulate FSM and IGF1 will help explain the genetic and molecular mechanisms that regulate aging and longevity.

Quantitative trait loci (QTL) analysis is a powerful, hypothesis-free strategy for comprehensively identifying genetic and molecular mechanisms for phenotypes under complex regulation, such as FSM and life span. Sequence analyses have demonstrated that humans and mice share more than 99% of their genes, and that these genes are arranged in a syntenic fashion on chromosomes (11). Thus, it is possible to cross-identify genes that are key regulatory components of biological pathways conserved between rodents and human.

Based on the strain survey studies of development and aging of inbred strains, as well as the knowledge of mouse genome (details are in the Discussion section), we selected two inbred strains, KK/HiJ (KK) and PL/J (PL), and generated 307 F2 females. Using these females, we tested the relationships between FSM, IGF1, and life span, identified QTL associated with these traits that are concordant with human loci, and identified and analyzed a candidate gene, proprotein convertase subtilisin/kexin type 2 (*Pcsk2*), that may regulate these relationships.

Methods

Mice

Strain selection

KK and PL, the parental strains used in the current study, were selected based on the previous strain survey studies (9,10) and the inbred strain genome sequencing data. Our criteria were as follows. First, parental strains must have significantly different IGF1 levels at 6 months. At this time point, strains with lower IGF1 had significantly earlier age of FSM and longer longevity. Given a fixed number of genetic loci determining IGF1 levels, QTL analysis in the intercross between two strains that have a more significant difference in their IGF1 levels will have a greater chance to identify significant QTL than a cross between strains with similar IGF1 levels. Second, the parental strains should not have been used in previous IGF1 QTL studies. A gene mapping study in a cross of new strains will not only increase the chance of identifying new QTL, but also improve our ability to reveal the underlying causal genes by using bioinformatic tools (12), such as haplotype and single nucleotide polymorphism (SNP) analyses. These methods are more powerful when more strains are involved in the identification of a common QTL for a given phenotype. Third, alleles of the parental strains, at locus of *Igf1q8*, should have similar effects on IGF1 level. *Igf1q8* is at 88 Mb on Chr 10. QTL at this locus have the strongest association with IGF1 among previously reported IGF1 QTL (13). Using two strains whose alleles at this locus have similar effects on IGF1 will minimize the variation of IGF1 in the F2 population caused by this locus, thus increasing the opportunity of identifying new QTL that modify circulating IGF1 levels.

KK and PL strains have not been used for gene mapping studies of IGF1, FSM, or life span. KK have significantly higher IGF1 levels at 6 months of age and earlier age of vaginal patency (AVP) than PL mice (404 ± 24 vs 267 ± 13 ng/mL; 30.5 ± 0.5 vs 33.1 ± 0.3 days) (9,10). It is worth noting that, although PL females have delayed AVP and lower IGF1 at 6 months of age, they have significantly shorter longevity than KK females (median life span: 408 vs 593 days) (9). Haplotype analysis shows that alleles of KK and PL at this locus have similar effects on circulating IGF1 (13). Thus, within the F2 population of the KK and PL cross, the *Igf1q8* locus should not be associated with the variation in IGF1 levels.

Mouse colonies

Two females and two males of KK and PL were obtained from the Jackson Laboratory. By crossing KK and PL, we generated reciprocal F1s, KKPL (female KK crossed with male PL), and PLKK (female PL crossed with male KK). To produce F2 mice, we created six breeder pairs of KKPL and six breeder pairs of PLKK. We collected F2 mice from the first seven litters of these breeders. Over 4.5 months, 616 F2 mice at weaning age (21–28 days) were incorporated into the study in staggered cohorts, including 307 females (152 KKPL F2 females and 155 PLKK F2 females) and 309 males (155 KKPL F2 males and 154 PLKK F2 males). Details of breeding information are listed in Supplementary Table 1. Information, including dates of birth, death and vaginal opening, as well as IGF1 levels at each time point of individual F2 mice, has been uploaded into mouse phenome database (www.phenome.jax.org).

B6;129-Pcsk^{2tm1Dfs}/J, carrying a targeted deletion of *Pcsk2*, was obtained from the Jackson laboratory. Heterozygous females and heterozygous males were mated to produce the mice for the current study.

Mouse husbandry

Mouse husbandry details have been fully described previously (9). Briefly, mice were weaned at approximately 4 weeks of age and housed five/pen in pressurized, individually ventilated polycarbonate cages with high-efficiency particulate filtered air (Thoren Caging Systems Inc., Hazleton, PA) at a temperature of 21–23°C. Cages were located in a barrier facility. Mouse colonies in this facility are monitored four times a year for (and are free of) 15 viruses, 17 bacterial species, two Mycoplasma spp., external and internal parasites, and *Encephalitozoon cuniculi*. The full list of monitored pathogens may be found in our previous publications (9). Bedding was autoclaved white pine shavings (Crobb Box Co., Ellsworth, ME); cages and bedding were changed every 2 weeks. Mice had *ad libitum* access to acidified water (pH 2.8–3.1) and autoclaved pelleted diet with 6% fat (Lab diet 5K52; PMI Nutritional International, Bentwood, MO). To identify mice in the longitudinal group used for aging-related phenotype studies, ear notches were used as individual identification. All experiments were approved by The Jackson Laboratory Animal Care and Use Committee.

Experimental design

We longitudinally measured developmental and aging traits, including body weight and tail length at 1, 2, 3, 4, 8, 12, and 16 weeks; body weight, glucose, and IGF1 levels at 24, 52, and 76 weeks. We also determined the AVP, and measured body composition at 16 weeks and hematology traits at 24 weeks. All measurements are noninvasive. None of the mice died by failed recovery, defined as mice dying within 48 hours after an experiment. The current report focuses on AVP, IGF1, and life span of female F2 mice.

Age of vaginal patency

Beginning at 18 days of age, mice were examined daily until vaginal patency was observed. Ethanol-sterilized forceps were used to allow optimal observation of the genital area (10).

IGF1 ELISA

Mice were fasted for 4 h (8 am to 12 pm) before blood was collected. Approximately 180 μL of whole blood was obtained by submandibular bleeding using heparin-coated microhematocrit tubes. This method does not require anesthesia. Blood was centrifuged at 1,200*g* for 10 minutes at 4°C. Samples were saved at −80°C until analysis. IGF1 levels for the F1 mice were measured by a direct radioimmunoassay (ALPCO Diagnostics, Salem, NH) by the MeCORE Laboratory, Saint Joseph Hospital, Bangor, ME. IGF1 levels of F2 and *Pcsk2* ($^{-/-}$, $^{+/-}$, and $^{+/+}$) mice were measured using ELISA (Cat.#: PMG100; R&D, Minneapolis, MN), following the manufacturer's instructions. IGF1 values that were more than 4 *SD*s from the mean at any time point were considered outliers and excluded from the results. Two out of 1,444 values were discarded.

Life span

Mice were inspected at least once daily. If a mouse was moribund—severely ill and judged likely not to survive another 48 hours—it was euthanized. Criteria for moribundity have been described previously (9). The age at which a moribund mouse was euthanized was used as the best available estimate of its natural life span.

DNA isolation and genotyping

F2 mice were tail tipped (<2 mm) at 16 weeks of age. DNA was extracted using the Gentra kit (Qiagen, Gaithersburg, MD) that utilizes a phenol/chloroform extraction method. DNA was genotyped by the High Throughput Sequenom and Illumina Genotyping facility (http://www.hpcgg.org/) using a 760-SNP array; 221 of these SNPs were polymorphic between KK and PL for an average distance between markers of 6.29 cM, ranging from 0.05 to 33.92 cM. For genotyping that failed in the SNP array analysis, we resequenced 131 SNPs for 385 samples. All genotyping data are available via mouse phenome database . Physical marker positions were determined in NCBI build 37, and genetic positions were estimated from the newly calculated mouse genetic map (14).

Statistical analysis

All statistical analyses were performed using JMP 10.0 (The SAS institute, Cary, NC). Analysis of variance was applied to determine the differences of the phenotypes among groups. To correct for multiple testing, significance was adjusted by the Tukey–Kramer honest significant difference and Bonferroni methods. To determine the associations among the phenotypes, we conducted a Pearson correlation of each of the traits. Survival curves were drawn using the Kaplan–Meier method. Differences were analyzed between curves by the log-rank method.

To determine the effects of nongenetic factors, including dam age, litter size, and mating pair, the phenotypes for the females, life span, IGF1, and sexual maturation, were screened for outliers. Life-span values less than 355 days were removed from the data. Cross-direction was regressed out of all phenotypes and the residuals were retained. A Cox proportional hazards model was fit for life span with dam age, litter size, and mating pair as additive covariates. A linear model was fit separately for age of sexual maturation and circulating IGF1 levels at each time point with dam age, litter size, and mating pair as additive covariates. Analysis of covariance was performed on each model to obtain *p* values for each term, which were then corrected using a Bonferroni correction.

QTL analysis

The genotypes went through a rigorous quality control process in which allele frequencies and recombination rates were inspected. SNPs that did not have a Mendelian 1:2:1 ratio, as determined by a χ^2 test, were removed. Alleles that appeared to have been swapped based on recombination rates were reversed. R/qtl (15) was used to perform QTL mapping. All phenotypes were inverse normally transformed and mapped using the expectation maximization algorithm with sex and cross-direction as additive covariates. Life span was mapped using a Cox proportional hazard model with cross-direction as an additive covariate. Significance thresholds were determined by permuting the phenotype data 1,000 times and recording the maximum logarithm of the odds (LOD) score from each permutation. The genome-wide *p* values were obtained from the 1-α quantiles of the empirical distribution of maximum LOD scores under permutation (16). Support intervals were determined using the Bayesian credible interval of 0.95 (17). The R/qtl data file has been deposited in the QTL Archive under the name Yuan_2013.

Results

FSMand IGF1 Levels of the F1 and F2 Mice

We previously reported a significant difference in plasma IGF1 levels between a pair of reciprocal F1 populations, generated by crossing SM/J (SM) and MRL/MpJ (MRL) mice. This result suggested that the difference of IGF1 level between the reciprocal F1s of SM and MRL might be partially inherited through genes located on the sex chromosomes or mitochondrial DNA, imprinted genes or maternal intrauterine environment (13).

In current study, we found that there was no significant difference in IGF1 levels between the KKPL and PLKK F1s, measured at 4 and 12 weeks (Supplementary Figure 1). AVP were also similar between the reciprocal F1s (Supplementary Table 2, Supplementary Figure 2). Similar to the F1s, between the two reciprocal F2 populations, there was no significant difference in AVP and IGF1 levels at any time point (Supplementary Figures 2 and 3, and Supplementary Tables 2 and 3). Therefore, in the following analysis, AVP and IGF1 data at the same time point for KKPL and PLKK F2 mice were combined. We also conducted an *F* test to determine if nongenetic factors—including parents, age of dam, and size of litter—affect life span, AVP, and circulating IGF1 levels. Dams and Sires that produced the F2 mice in this study ranged in age from 55 to 226 and from 205 to 335 days, respectively (Supplementary Table 1). No significant association was found between these nongenetic factors and AVP or IGF1 levels. These results suggest that, between KK and PL strains, the genetic differences located on autosomal chromosomes may be the dominant reasons for the differences in IGF1 levels and AVP.

Life Span of the F2 Mice

KKPL and PLKK F2 females have significantly longer life spans than parental inbred strains (Table 1, Figure 1; log-rank test: $p < .01$). The longevities in the F2 mice might be extended due to the diminished number of homozygosities with deleterious effects. Although the mean life span of KKPL F2 females is significantly longer than PLKK F2 females (703 ± 13.1 vs 665 ± 16.9 days, $p = .04$), their median life spans are similar (711 vs 691 days). A log-rank test shows that their survival curves are not significantly different ($p = .78$).

We calculated the mortality rates for the KK, PL, KKPL F2, and PLKK F2 females at the ages of 400, 600, 800, 900, and 1,000 days. As shown in Table 1, at all five time points, the mortality rates of KKPL and PLKK F2 females are similar but significantly lower than the mortality rates of the KK and PL females.

Table 1. Age-Specific Mortality Rates (%)

Mice	Number	Life Span (d)		Mortality Rate at Different Ages*				
		Mean (STE)	Median (95% CI†)	400 d	600 d	800 d	900 d	1,000 d
KK‡	32	575 (24.3)	593 (551–614)	16 (11–22)	60 (48–73)	95 (88–99)	99 (97–100)	100 (99–100)
PL‡	32	462 (23.9)	408 (386–512)	29 (21–39)	83 (72–92)	100 (98–100)	100 (100–100)	100 (100–100)
KKPL F2	152	703 (13.1)	711 (684–724)	7 (5–9)	33 (28–38)	73 (67–78)	88 (83–92)	96 (94–98)
PLKK F2	155	665 (16.9)	691 (654–721)	7 (6–10)	34 (29–39)	74 (69–79)	89 (85–92)	97 (94–98)

Notes: *Mortality rate is calculated by using the function of fit parametric survival in JMP. Data are shown as mortality rate (%) and 95% confidence interval of the mortality rate in parentheses. The mortality rates are significantly different if their confidence intervals are not overlapped ($p < .05$).

†Confidence interval.

‡Data has been reported previously (9).

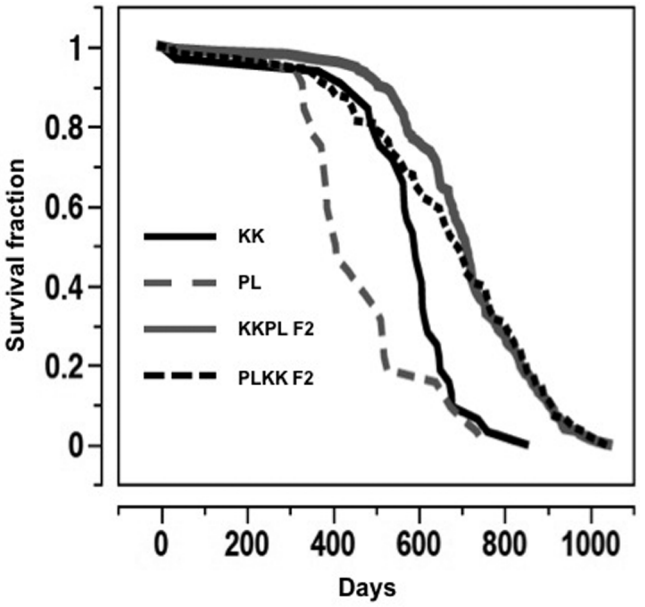


Figure 1. Survival curves of KK, PL, as well as KKPL and PLKK F2 females. Log-rank test for all four survival curves shows that there is significant difference ($p < .01$). Log-rank test for the survival curves of KKPL and PLKK F2 shows there is no significant difference ($p = .78$).

Correlations Among AVP, IGF1, and Life Span

In both F2 populations, IGF1 levels were highest at 7 weeks and lowest at 24 weeks, followed by increasing levels at 52 and 76 weeks (Supplementary Figure 3, Supplementary Table 3). Among different time points, IGF1 levels are significantly correlated ($p < .05$, Supplementary Table 4), suggesting that genetic factors may regulate IGF1 levels continuously through development and the aging process. All p values are adjusted by Bonferroni correction.

Among the five time points, IGF1 levels negatively correlated with life span (Figure 2A–E); at the age of 52 weeks, the correlation is significant ($R^2 = .08$, $p < .01$). The correlation of life span with IGF1 at 76 weeks is suggestive ($R^2 = .03$, $p = .05$). The correlation of life span with IGF1 at 24 weeks, which was reported to be associated with life span across inbred strains (9), is insignificant in the current study ($p = .51$).

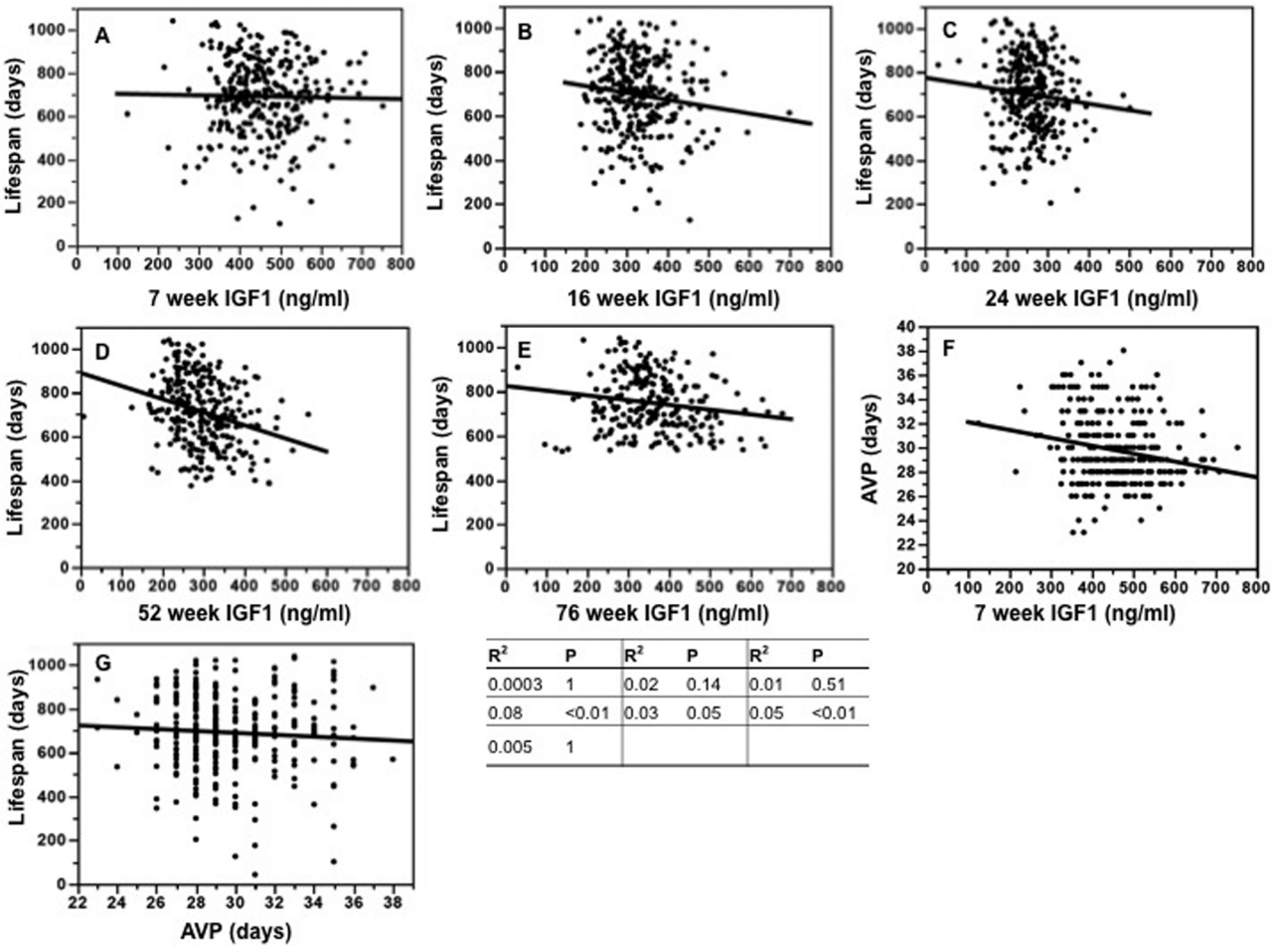
Consistent with our previous report that manipulating IGF1 level by genetic methods could alter the age of FSM (10), we found in our current study that females with a higher IGF1 level at 7 weeks of age have significantly earlier AVP ($R^2 = .05$, $p < .01$, Figure 2F), suggesting that IGF1 plays an important role in the regulation of AVP in the F2 mice. However, in these F2 mice, we did not find significant correlation between AVP and longevity (Figure 2G), which has been found across mammalian species (3) and across inbred strains of *Mus musculus* (18). Interestingly, in a post hoc analysis, a positive correlation between AVP and life span ($p = .02$, $R^2 = .14$) was found in long-lived mice (36 of 307), whose life spans are longer than 900 days. This result suggests that AVP might correlate with the maximum life span in a given population. This hypothesis needs to be further verified.

QTL for IGF1, AVP, and Life Span

We applied a whole-genome scan for IGF1 levels, AVP, and life span (Supplementary Figure 4). Table 2 shows significant ($p < .05$), suggestive ($.05 < p < .1$), and marginal ($.1 < p < .63$) QTL. All QTL were named according to the guidelines of QTL nomenclature (19).

IGF1 QTL

As shown in Table 2, Supplementary Figures 4 and 5, we identified nine significant, three suggestive, and 10 marginal IGF1 QTL in this study. Five QTL—*Igf1q17*, *23*, *24*, *29*, and *35*, on Chr 2, 14, 16, and X—have not been previously reported. IGF1 QTL were identified at the distal region of Chr 8 and proximal region of Chr 11 at all five time points. The most significant IGF1 QTL identified in this study is *Igf1q16* on proximal Chr 11 (LOD = 8.29, $p < .001$), which accounts



R^2	P	R^2	P	R^2	P
0.0003	1	0.02	0.14	0.01	0.51
0.08	<0.01	0.03	0.05	0.05	<0.01
0.005	1				

Figure 2. Correlations among IGF1, AVP, and life span. Correlations between life span and IGF1 at 7, 16, 24, 52, and 72 weeks (A-E); correlation between AVP and IGF1 at 7 weeks (F); correlation between life span and AVP (G). All the *p* values are adjusted by Bonferroni correction. AVP = age of vaginal patency; IGF = insulin-like growth factor.

for 6.98% of the variation in IGF1 levels at age 7 weeks. The effect of this QTL declines with aging. In the same region, 2.74% of the variation in IGF1 at age 76 weeks is due to *Igf1q33*. Interestingly, the association between IGF1 and the distal region of Chr 8 increases with age. At 7 weeks, *Igf1q15* was marginally associated with IGF1 (LOD = 2.61, $p = .35$). At 52 weeks, *Igf127* explains a 3.84% variation of IGF1, which is the second-most significant IGF1 QTL identified in this study (LOD = 7.92, $p < .001$). These results suggest that these regions carry genes that may regulate IGF1 through the development and aging processes, but with different efficiencies at different ages.

AVP QTL

We identified one significant QTL for AVP on the distal region of Chr 2 (*Vpq4*) and three marginal QTL on Chr 6, 15, and 18 (*Vpq5*, 6, 7). A previous study reported an AVP QTL on Chr 6 (20). The other three QTL have not been reported. At *Vpq4* and 6, PL alleles associate with delayed AVP; at *Vpq5* and 7, the PL allele associate with earlier AVP.

Life-span QTL

We identified one marginal life-span QTL, *Ls5*, on Chr 12, that has not been previously reported. At this locus, the KK allele associates with increased longevity. We also detected one significant and one marginal life-span QTL, *Ls3*, and *Ls4*, on Chr 2 and Chr 7, respectively, which have been reported in other mouse crosses (21–23). At both loci, the PL allele associates with longer longevity than the KK allele.

We previously reported that KK females had significantly higher IGF1 and earlier AVP than PL females (9,10). Thus, it was not surprising that we found that KK alleles of most of IGF1 QTL associate with higher IGF1; and at two of four AVP QTL, KK alleles associate with earlier AVP. However, we also found that at one IGF1 QTL, *Igf1q18*, the PL allele is associated with a higher IGF1 level; and at the other two AVP QTL, KK alleles associated with delayed AVP. These results suggest that the circulating IGF1 level and AVP are complex traits that are the outcomes of the co-ordination of multiple loci.

Interestingly, across inbred strains, the strains with higher IGF1 had shorter longevity; however, KK mice, despite their higher IGF1, lived significantly longer than PL mice (Table 1, Figure 1) (9). This suggests that there are IGF1-independent mechanisms involved in the determination of the differences in longevity between KK and PL mice. The current study focuses on investigating the genetic coregulation of FSMand longevity through IGF1; thus, the promising candidate genes and loci should associate with reduced IGF1, delayed AVP, and extended longevity.

Table 2. AVP, IGF1, and Life-Span QTL Identified in F2 Females

Phenotype		QTL	Peak		LOD	Sig.*	Perc.var† (%)	Candidate Region (Mb)		High Allele
			Chr	Mb				Proximal	Distal	
IGIF1	7 wk	*Igf1q14*	2	137.1	3.36	0.091	1.21	69.3	176.1	KK
	7 wk	*Igf1q15*	8	105.1	2.61	0.349	1.07	3.1	131.5	KK
	7 wk	*Igf1q16*	11	29.2	8.29	0	6.98	3.5	37.5	KK
	16 wk	*Igf1q17*	2	76.9	5.33	0.004	4.01	59.3	137.1	KK
	16 wk	*Igf1q18*	6	85.4	2.9	0.222	0.32	62.5	103.8	PL
	16 wk	*Igf1q19*	8	90.5	4.34	0.018	2.48	67.8	113.4	KK
	16 wk	*Igf1q20*	11	3.5	3	0.184	3.75	3.5	36.6	KK
	24 wk	*Igf1q21*	8	100.3	4.56	0.01	1.32	78.9	115.5	KK
	24 wk	*Igf1q22*	11	11	4.06	0.03	4.69	5.2	20.7	KK
	24 wk	*Igf1q23*	14	49.9	2.67	0.328	1.98	19.8	94.4	KK
	24 wk	*Igf1q24*	16	11.5	2.42	0.472	1.66	3.3	96.1	KK/PL
	24 wk	*Igf1q25*	17	34.5	3.52	0.067	1.13	9.3	41.1	KK
	52 wk	*Igf1q26*	2	114.5	2.96	0.194	1.45	57.8	142.6	KK
	52 wk	*Igf1q27*	8	94.1	7.92	0	3.84	88.6	111.1	KK
	52 wk	*Igf1q28*	11	5.7	3.16	0.138	4	3.5	19.2	KK
	52 wk	*Igf1q29*	16	11.5	2.95	0.198	2.98	3.3	23.9	KK/PL
	76 wk	*Igf1q30*	7	53.9	2.45	0.445	2.94	25.5	135.9	KK
	76 wk	*Igf1q31*	8	100.3	4.31	0.019	1.61	82.2	119.7	KK
	76 wk	*Igf1q32*	9	48.1	3.28	0.113	1.42	31.8	63.8	KK
	76 wk	*Igf1q33*	11	14.5	3.47	0.074	2.74	3.5	19.2	KK
	76 wk	*Igf1q34*	17	31.7	4.34	0.018	2.34	18	45.1	KK
	76 wk	*Igf1q35*	X	138.8	3.71	0.046	3.76	106.4	138.8	KK
AVP		*Vpq4*	2	75.9	5.26	0.004	4.02	62	146.3	PL
		Vpq5	6	69.2	3.27	0.113	0.21	50.9	92.7	KK
		Vpq6	15	49.2	3.09	0.156	1.09	7.7	63.6	PL
		Vpq7	18	37.5	2.42	0.469	1.58	11	70.7	KK
Life span		*Ls3*	2	124.6	4.28	0.019	NA	106.6	140.9	PL
		Ls4	7	72.1	2.56	0.383	NA	48.2	146.9	PL
		Ls5	12	45.2	2.71	0.314	NA	17.3	65.8	KK

Notes: AVP = age of vaginal patency; QTL = quantitative trait loci.
*Significance of the QTL determined by permutation test (n = 1,000).
†Percentage of variation in the traits that the QTL could explain.

Colocalizations of AVP, IGF1, and longevity QTL—As shown in Figure 3, *Ls 3*, *Igf1q14*, and *Vpq4* are colocalized in the distal region of Chr 2. *Igf1q14* and *Vpq4* on Chr 2 may have two peaks; one is at 75 Mb, while the other is at 130 Mb. The distal peak colocalizes with *Ls3*. Importantly, the PL allele at this locus associates with lower IGF1, delayed AVP, and extended longevity (p < .05; Figure 3D–F). Females with homozygous PL alleles at this locus have 6.8% reduced IGF1, 3.8% delayed AVP, and a 9.9% increase in median lifespan compared with mice homozygous for the KK allele (IGF1: 395.4 vs 368.6 ng/mL, AVP: 29.96 vs 31.09 days, median life span: 656 vs 721 days).

As shown in Supplementary Figure 6, previous analysis of a four-way cross, (BALB/cByJ x B6) × (C3H/HeJ x DBA/2J), also detected life span and IGF1 QTL colocalizing to the distal region of Chr 2 (24). An IGF1 QTL at this locus was also identified in Du6i × DBA/2J F2 mice (25). Interestingly, a body weight QTL was also identified in this region in 129P3/J (129) × C57BL/6ByJ (B6) F2 mice (26). These colocalizations strongly suggest this locus is involved in regulating IGF1, development and aging; and, the PL allele at this locus associates with lower IGF1, delayed AVP, and increased life span.

Identifying *Pcsk2* as a Candidate That Regulates FSM Through the Regulation of IGF1

Human genome-wide association study (GWAS) identified 35 genes associated with age at menarche (27). Notably, the homologous region of one GWAS peak (rs852069), which is 84 kb from *PCSK2*, is at 143 Mb of Chr 2 in the mouse genome, corresponding to the peaks for *Vpq4*, *Igf1q14*, *and Ls3* (Supplementary Figure 6). To test if *Pcsk2* is involved in the regulation of AVP and IGF1, we measured IGF1 and AVP in the *Pcsk2* knockout and control females.

We found that IGF1 is significantly lower in homozygous *Pcsk2* knockout female mice ($^{-/-}$) compared with heterozygous knockout ($^{-/+}$) and wild-type ($^{+/+}$) females at 8 weeks of age (231.4 ± 15.5 vs 353.2 ± 10.7, 309.2 ± 17.9 ng/mL, p < .01; Figure 4A). Along with the reduced IGF1, *Pcsk2*$^{-/-}$ females have significantly delayed AVP compared with *Pcsk2*$^{-/+}$ and *Pcsk2*$^{+/+}$ females (36.2 ± 0.8 vs 31.3 ± 0.5 and 31.8 ± 0.8 days; log-rank test p < .01; Figure 4B).

Discussion

Optimized Genetic Study

The genetic study of the current report was designed based on previous strain survey studies and the inbred strain genome sequencing data as described in the Methods section. This design has improved the power to identify novel QTL because the parental strains, KK and PL, had significant differences in their IGF1 level and AVP and had not been used for QTL study. Importantly, the similarity of the effects of the KK and PL alleles of *Igf1q8* (88 Mb on Chr 10) on IGF1 level eliminates the association between this locus with the variation of IGF1 in the F2 mice. As we predicted, in the KKPL and

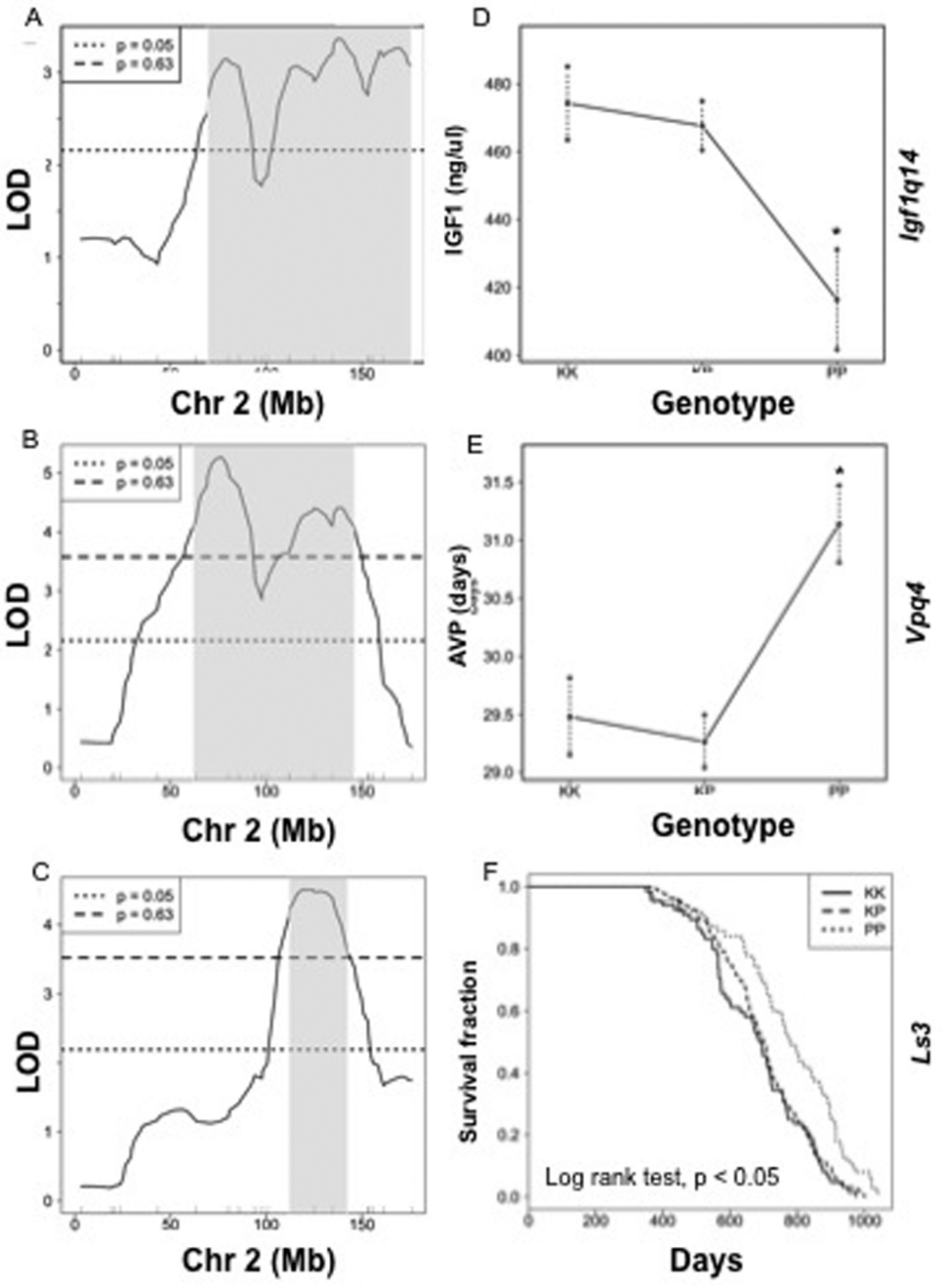


Figure 3. IGF1, AVP, and life-span QTL on Chr 2. (A–C) Show the colocalization of IGF1, AVP, and life-span QTL in the distal region of Chr 2. (D–F) Show that at this locus, the mice that carry PL homozygous allele have lower IGF1, delayed sexual maturation, and longer longevity than mice with heterozygous alleles (PL/KK) or KK homozygous allele ($p < .05$). AVP = age of vaginal patency; QTL = quantitative trait loci.

PLKK F2 females, no IGF1 QTL was found at this locus through the development and aging processes; while we identified five IGF1 QTL (*Igf1q17*, *23*, *24*, *29*, and *35*), three AVP QTL (*Vpq4*, *6*, and *7*), and one life-span QTL (*Ls5*) have not been previously reported.

KK and PL have not been used previously for QTL analysis. It has been demonstrated that bioinformatics tools are more effective for identifying a causal gene when more strains are involved in the identification of a common QTL for a given phenotype (12). In the current study, we found that IGF1 QTL on Chr 2, 6, 7, 8, 11, and 17; AVP QTL on Chr 6; and life-span QTL on Chr 2 and 7 colocalize with previously reported QTL (13,20–25,28,29). Thus, our study may facilitate future investigations to identify the underlying causal genes of these QTL. Taken together, our study demonstrates an optimized gene mapping study design using strain survey and bioinformatics resources.

Continuous and Time-Specific Genetic Regulation of IGF1 in Development and Aging Processes

Positive and negative effects of IGF1 on aging have been demonstrated. IGF1 protects cells from apoptosis, and therefore could

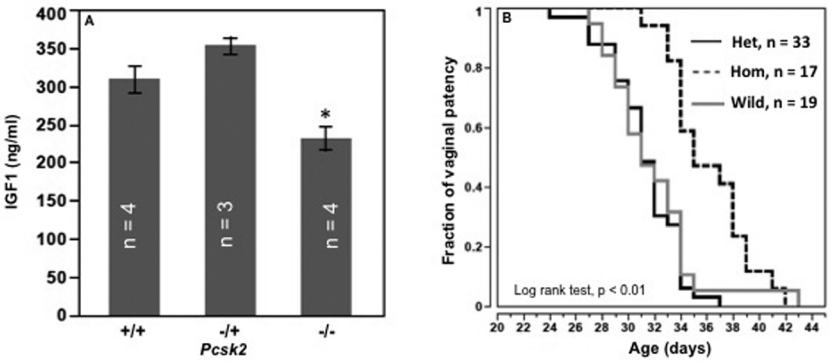


Figure 4. *Pcsk2* knockout females have reduced IGF1 and delayed AVP. Circulating IGF1 is significantly lower in *Pcsk2*$^{-/-}$ than *Pcsk2*$^{+/-}$ and wild-type controls (A; *$p < .01$). Accompanied with lower IGF1, *Pcsk2*$^{-/-}$ females have significantly delayed AVP (B; log-rank test, $p < .01$).

delay neuron degeneration diseases and improve heart function (30,31). In rodents and humans, insulin sensitivity decreases with aging (32,33). IGF1 is an insulin-sensitizing hormone and plays an important role in reducing risks of metabolic disorders in aging (34). Increased IGF1 is also positively associated with bone density and muscle strength (35). However, increased IGF1 levels are associated with higher risk of malignancy (reviewed in Samani and colleagues (36)) and firmly established in mouse models (37–40). Thus, understanding the genetic regulation of IGF1 at different ages is critical to fully grasping the relationship of IGF1 with aging and to identify genetic approaches to delay aging and extend longevity.

In our current study, we found that across all five time points examined, IGF1 levels in individual mice are significantly correlated with each other, suggesting that there are common mechanisms regulating IGF1 through the development and the aging process. The QTL analysis identified that the distal region of Chr 8 (*Igf1q15*, *19*, *21*, *27*, *and 31*) and the proximal region of Chr 11 (*Igf1q16*, *20*, *22*, *28*, *and 33*) are associated with IGF1 levels throughout the development and aging processes, suggesting these regions carry genes that could regulate IGF1 continuously. We have also identified age-specific IGF1 QTL. For example, the loci of 85.4 Mb on Chr 6 (*Igf1q18*), 49.9 Mb on Chr 14 (*Igf1q23*), and 138 Mb on Chr X (*Igf1q35*) were identified as QTL for IGF1 at ages 16, 24, and 76 weeks, respectively.

Among all IGF1 QTL identified in this study, only at *Igf1q18*, the PL allele associates with higher IGF1 than KK allele. It is interesting that this locus has also been associated with IGF1 levels at 16 weeks of age in the C3H X B6 F2 mice; and B6 allele associated with higher IGF1 than C3H allele (29), although C3H inbred mice have significantly higher IGF1 than B6. These results suggest that the concentration of IGF1 is determined by many loci and strains with lower IGF1 may carry alleles that increase IGF1. At the locus of *Igf1q18*, PL and B6 strains may carry the same IGF1-reducing allele.

Pcsk2, a Candidate Gene, Coregulates FSM and Longevity Through the Regulation of IGF1

Lin28b was identified as a regulator of FSM through combined human and mouse gene mapping studies (41). Using the same strategy, we identified another potential regulator, *Pcsk2*. Genes of the *Pcsk* family are essential for processing prohormones to produce mature hormones. *In vitro* studies suggested that *Pcsk1* and *Pcsk3* were involved in processing the progrowth hormone-releasing hormone polypeptide (42). Therefore, they may regulate growth hormone and downstream hormone production, including IGF1 and possibly also insulin. *Pcsk2* was reported to be involved in processing insulin, glucagon, somatostatin, and amylin (43–45). Here, we show for the first time that IGF1 is significantly reduced when *Pcsk2* is depleted. However, whether the suppression of IGF1 by the depletion of *Pcsk2* is due to the interruption of IGF1 processing or interruption of upstream proteins, such as growth hormone and growth hormone-releasing hormone, requires further investigation.

The correlations between IGF1 and AVP with longevity, along with the colocalizations of IGF1, AVP, and life-span QTL in the distal region of Chr 2, as well as the evidence that *Pcsk2*$^{-/-}$ females have significantly reduced IGF1 and delayed AVP suggests that *Pcsk2* is involved in the regulation of aging via IGF1 signaling. Previous studies in nematodes support this hypothesis. Mutations in egg-laying defective 3 (*egl-3*), orthologous to *Pcsk2* in *Caenorhabditis elegans*, induce egg-laying defects, as evidenced by the increased number of eggs retained *in utero* (46,47). Importantly, suppressing *egl-3* could significantly extend longevity in worms in a *daf-16*-dependent manner (48,49). These results, along with the fact that *PCSK2* associates with age at menarche in women, make this gene a promising candidate for mediating the evolutionarily conserved tradeoff relationship between reproduction and longevity.

Concordance of Human and Mouse Loci

The identifications of *Lin28b* in the previous report (41) and *Pcsk2* in the current study as regulators of FSM demonstrate that combining the human GWAS and mouse QTL study could accelerate the identification of causal genes for QTL. In the current study, we also found concordance of longevity and IGF1 QTL between human and mouse, suggesting that human and mouse share some common mechanisms of regulating these complicated traits.

Human GWAS found 24 suggestive loci associated with longevity (50). In a previous report, using Liftover (http://genome.ucsc.edu/), we identified their homologous regions in the mouse genome. The human GWAS longevity peaks colocalized with mouse longevity QTL. Fisher's exact test shows that the probability of the colocalization is not by chance (18). Among the three life-span QTL in the current report, *Ls4* not only colocalizes with mouse longevity QTL previously mapped in the four-way cross, (BALB × B6) × (C3H × D2) and among B6 × D2 recombinant inbred strains (51,52), but also colocalizes with a GWAS peak for human longevity (rs8029244) (50). This is 1.5 Mb from a well-known longevity gene, insulin-like growth factor 1 receptor.

For IGF1 level, the human GWAS in middle-aged and older men and women found SNPs in *IGFBP3*, *IGFALS*, *SORCS2*, and

FOXO3 associated with IGF1 levels (53). *Igfbp3* locates in the proximal region of Chr 11, colocalizing with *Igf1q16*, *20*, *22*, *28*, and *33*. *Igfals* locates at 24.88 Mb of Chr 17, colocalizing with *Igf1q25* and *33*. Although IGF1 QTL localizing near *Sorcs2* and *Foxo3* was not seen in this study, these loci associate with IGF1 QTL in other mouse crosses (24,25,28).

Summary

The current study, designed based on mouse strain survey studies, was optimized to identify novel QTL. Our results support the hypothesis that FSM and longevity might be coregulated through IGF1 signaling. Using QTL method and combining human GWAS result, we identified that *Pcsk2* is a regulator of FSM and IGF1. Thus, it may also play a role in regulating aging and longevity. The concordance of IGF1, AVP, and life-span QTL between human and mouse suggests that mouse and human share common genetic mechanisms that regulate development and aging. Therefore, combining human and mouse genetic studies could accelerate the identification of the causal genes.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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References

1. Charlesworth B. Evolutionary mechanisms of senescence. *Genetica*. 1993;91:11–19.
2. Williams GC. Pleiotropy, natural selection, and the evolutional of senescence. *Evolution*. 1957;11(4):4.
3. Harvey PH, Zammuto RM. Patterns of mortality and age at first reproduction in natural populations of mammals. *Nature*. 1985;315:319–320.
4. Austad S. Retarded senescence in an insular population of Virginia opossums (Didelphis virginiana). *J Zool*. 1992;229(4):14.
5. Miller RA, Harper JM, Dysko RC, Durkee SJ, Austad SN. Longer life spans and delayed maturation in wild-derived mice. *Exp Biol Med (Maywood)*. 2002;227:500–508.
6. Flurkey K, Brandvain Y, Klebanov S, et al. PohnB6F1: a cross of wild and domestic mice that is a new model of extended female reproductive life span. *J Gerontol A Biol Sci Med Sci*. 2007;62:1187–1198.
7. Luckinbill LS. Selection for delayed senescence in Drosophila melanogaster. *Evolution*. 1984;38:8.
8. Rose MR. Laboratory evolution of postponed senescence in Drosophila melanogaster. *Evolution*. 1984;38:7.
9. Yuan R, Tsaih SW, Petkova SB, et al. Aging in inbred strains of mice: study design and interim report on median lifespans and circulating IGF1 levels. *Aging Cell*. 2009;8:277–287. doi:10.1111/j.1474-9726.2009.00478.x
10. Yuan R, Meng Q, Nautiyal J, et al. Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *Proc Natl Acad Sci USA*. 2012;109:8224–8229. doi:10.1073/pnas.1121113109
11. Mouse Genome Sequencing C, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature*. 2002;420(6915):520–562.
12. Burgess-Herbert SL, Cox A, Tsaih SW, Paigen B. Practical applications of the bioinformatics toolbox for narrowing quantitative trait loci. *Genetics*. 2008;180:2227–2235. doi:10.1534/genetics.108.090175
13. Leduc MS, Hageman RS, Meng Q, et al. Identification of genetic determinants of IGF-1 levels and longevity among mouse inbred strains. *Aging Cell*. 2010;9:823–836. doi:10.1111/j.1474-9726.2010.00612.x
14. Cox A, Ackert-Bicknell CL, Dumont BL, et al. A new standard genetic map for the laboratory mouse. *Genetics*. 2009;182:1335–1344. doi:10.1534/genetics.109.105486
15. Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics*. 2003;19:889–890.
16. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics*. 1994;138:963–971.
17. Sen S, Churchill GA. A statistical framework for quantitative trait mapping. *Genetics*. 2001;159:371–387.
18. Yuan R, Peters LL, Paigen B. Mice as a mammalian model for research on the genetics of aging. *ILAR J*. 2011;52:4–15.
19. Maltais LJ, Blake JA, Chu T, Lutz CM, Eppig JT, Jackson I. Rules and guidelines for mouse gene, allele, and mutation nomenclature: a condensed version. *Genomics*. 2002;79:471–474. doi:10.1006/geno.2002.6747
20. Krewson TD, Supelak PJ, Hill AE, et al. Chromosomes 6 and 13 harbor genes that regulate pubertal timing in mouse chromosome substitution strains. *Endocrinology*. 2004;145:4447–4451. doi:10.1210/en.2004-0543
21. de Haan G, Gelman R, Watson A, Yunis E, Van Zant G. A putative gene causes variability in lifespan among genotypically identical mice. *Nat Genet*. 1998;19:114–116. doi:10.1038/465
22. Jackson AU, Galecki AT, Burke DT, Miller RA. Mouse loci associated with life span exhibit sex-specific and epistatic effects. *J Gerontol A Biol Sci Med Sci*. 2002;57:B9–B15.
23. Miller RA, Chrisp C, Jackson AU, Galecki AT, Burke DT. Coordinated genetic control of neoplastic and nonneoplastic diseases in mice. *J Gerontol A Biol Sci Med Sci*. 2002;57:B3–B8.
24. Hanlon P, Lorenz WA, Shao Z, et al. Three-locus and four-locus QTL interactions influence mouse insulin-like growth factor-I. *Physiol Genomics*. 2006;26:46–54. doi:10.1152/physiolgenomics.00247.2005
25. Brockmann GA, Kratzsch J, Haley CS, Renne U, Schwerin M, Karle S. Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F(2) variance of growth and obesity in DU6i x DBA/2 mice. *Genome Res*. 2000;10:1941–1957.
26. Reed DR, Li X, McDaniel AH, et al. Loci on chromosomes 2, 4, 9, and 16 for body weight, body length, and adiposity identified in a genome scan of an F2 intercross between the 129P3/J and C57BL/6ByJ mouse strains. *Mamm Genome*. 2003;14:302–313.
27. Elks CE, Perry JR, Sulem P, et al.; GIANT Consortium. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet*. 2010;42:1077–1085. doi:10.1038/ng.714
28. Harper JM, Galecki AT, Burke DT, Pinkosky SL, Miller RA. Quantitative trait loci for insulin-like growth factor I, leptin, thyroxine, and corticosterone in genetically heterogeneous mice. *Physiol Genomics*. 2003;15:44–51. doi:10.1152/physiolgenomics.00063.2003
29. Rosen CJ, Churchill GA, Donahue LR, et al. Mapping quantitative trait loci for serum insulin-like growth factor-1 levels in mice. *Bone*. 2000;27:521–528.
30. Li Q, Li B, Wang X, et al. Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. *J Clin Invest*. 1997;100:1991–1999. doi:10.1172/JCI119730
31. Janssen JA, Stolk RP, Pols HA, Grobbee DE, Lamberts SW. Serum total IGF-I, free IGF-I, and IGFB-1 levels in an elderly population: relation to cardiovascular risk factors and disease. *Arterioscler Thromb Vasc Biol*. 1998;18:277–282.
32. DeFronzo RA. Glucose intolerance and aging. *Diabetes Care*. 1981;4:493–501.
33. Escriva F, Agote M, Rubio E, et al. In vivo insulin-dependent glucose uptake of specific tissues is decreased during aging of mature Wistar rats. *Endocrinology*. 1997;138:49–54.

34. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet*. 2002;359:1740–1745. doi:10.1016/S0140-6736(02)08655-5
35. Juul A, Bang P, Hertel NT, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab*. 1994;78:744–752.
36. Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocr Rev*. 2007;28:20–47. doi:10.1210/er.2006-0001
37. Yang XF, Beamer WG, Huynh H, Pollak M. Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. *Cancer Res*. 1996;56:1509–1511.
38. Pollak M, Blouin MJ, Zhang JC, Kopchick JJ. Reduced mammary gland carcinogenesis in transgenic mice expressing a growth hormone antagonist. *Br J Cancer*. 2001;85:428–430. doi:10.1054/bjoc.2001.1895
39. Wu Y, Cui K, Miyoshi K, et al. Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors. *Cancer Res*. 2003;63:4384–4388.
40. Bol DK, Kiguchi K, Gimenez-Conti I, Rupp T, DiGiovanni J. Overexpression of insulin-like growth factor-1 induces hyperplasia, dermal abnormalities, and spontaneous tumor formation in transgenic mice. *Oncogene*. 1997;14:1725–1734. doi:10.1038/sj.onc.1201011
41. Zhu H, Shah S, Shyh-Chang N, et al. Lin28a transgenic mice manifest size and puberty phenotypes identified in human genetic association studies. *Nat Genet*. 2010;42:626–630. doi:10.1038/ng.593
42. Posner SF, Vaslet CA, Jurofcik M, Lee A, Seidah NG, Nillni EA. Stepwise posttranslational processing of progrowth hormone-releasing hormone (proGHRH) polypeptide by furin and PC1. *Endocrine*. 2004;23:199–213. doi:10.1385/ENDO:23:2-3:199
43. Jonsson A, Isomaa B, Tuomi T, Eriksson JG, Groop L, Lyssenko V. Effect of a common variant of the PCSK2 gene on reduced insulin secretion. *Diabetologia*. 2012;55:3245–3251. doi:10.1007/s00125-012-2728-5
44. Wang J, Xu J, Finnerty J, Furuta M, Steiner DF, Verchere CB. The prohormone convertase enzyme 2 (PC2) is essential for processing pro-islet amyloid polypeptide at the NH2-terminal cleavage site. *Diabetes*. 2001;50:534–539.
45. Winsky-Sommerer R, Grouselle D, Rougeot C, et al. The proprotein convertase PC2 is involved in the maturation of prosomatostatin to somatostatin-14 but not in the somatostatin deficit in Alzheimer's disease. *Neuroscience*. 2003;122:437–447.
46. Trent C, Tsuing N, Horvitz HR. Egg-laying defective mutants of the nematode Caenorhabditis elegans. *Genetics*. 1983;104:619–647.
47. Kass J, Jacob TC, Kim P, Kaplan JM. The EGL-3 proprotein convertase regulates mechanosensory responses of Caenorhabditis elegans. *J Neurosci*. 2001;21:9265–9272.
48. Hamilton B, Dong Y, Shindo M, et al. A systematic RNAi screen for longevity genes in C. elegans. *Genes Dev*. 2005;19:1544–1555. doi:10.1101/gad.1308205
49. Ch'ng Q, Sieburth D, Kaplan JM. Profiling synaptic proteins identifies regulators of insulin secretion and lifespan. *PLoS Genet*. 2008;4:e1000283. doi:10.1371/journal.pgen.1000283
50. Newman AB, Walter S, Lunetta KL, et al. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. *J Gerontol A Biol Sci Med Sci*. 2010;65:478–487. doi:10.1093/gerona/glq028
51. Miller RA, Chrisp C, Jackson AU, Burke D. Marker loci associated with life span in genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci*. 1998;53:M257–M263.
52. Lang DH, Gerhard GS, Griffith JW, et al. Quantitative trait loci (QTL) analysis of longevity in C57BL/6J by DBA/2J (BXD) recombinant inbred mice. *Aging Clin Exp Res*. 2010;22:8–19.
53. Kaplan RC, Petersen AK, Chen MH, et al. A genome-wide association study identifies novel loci associated with circulating IGF-I and IGFBP-3. *Hum Mol Genet*. 2011;20:1241–1251. doi:10.1093/hmg/ddq560