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Genetic analysis of lifespan in hybrid progeny derived from the SAMP1 mouse strain with accelerated senescence

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Abstract

The SAMP1 mouse, a senescence-accelerated mouse prone (SAMP) strain, shows accelerated senescence coupled with a short lifespan as a genetic trait, and has been used in gerontological research. The accelerated senescence and short lifespan of SAMP strains is considered to be under the control of multiple genes. To identify the chromosomal regions encompassing the genes for the accelerated senescence and short lifespan, we performed whole genome scanning with polymorphic marker loci in a progeny from a cross between the SAMP1 strain and normal B10.BR strain. A genetically recessive effect of the amyloidogenic *Apoa2*^{c} allele from SAMP1 on chromosome 1 to shorten the lifespan was demonstrated in the progeny, consistent with the previous report. The recessive effect was observed also at *D1Mit67*, *D5Mit267*, *D6Mit384* and *D19Mit33*, suggesting the presence of genes for accelerated senescence in the SAMP strains around these loci. Other markers on chromosomes 8, 14, 16, and 17, however, exhibited a dominant or additive effect to shorten or prolong the

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lifespan, demonstrating a complex genetic control of the trait. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Senescence-accelerated mouse prone (SAMP) strains show accelerated senescence and various age-associated disorders as genetic traits, and have been used in gerontological research (reviewed by Takeda et al., 1997). The SAM mice, consisting of nine accelerated senescence-prone SAMP strains, and four senescence-accelerated mouse resistant (SAMR) strains with a normal senescence profile, have been established from a progeny of inadvertent crossing between AKR/J and unspecified strain(s). Coupled with the accelerated senescence, SAMP strains show markedly shorter average lifespan (9.7 months), compared with SAMR strains (13.3 months). The accelerated senescence and short lifespan of SAMP strains is considered to be under the control of multiple genes, fixed in the nine SAMP strains during the inbreeding process. These genes are supposed to be recessive, because F_1 hybrids between SAMP strains and normal strains had a normal phenotype. The studies to elucidate the mechanism of accelerated senescence in these mice have revealed distinctive features in SAM, such as the changes in chromosomes and nucleic acids with age (Yamagishi et al., 1985; Nisitani et al., 1990; He and Yasumoto, 1994; Tobita et al., 1994; Odagiri et al., 1998), peroxidation of lipids and proteins, and antioxidant enzymes (Edamatsu et al., 1995; Park et al., 1996; Choi et al., 1999), age-associated mitochondrial dysfunction (Fujibayashi et al., 1998; Nakahara et al., 1998; Nishikawa et al., 1998), and abnormality in cultured fibroblasts of SAMP mice (Hosokawa et al., 1994; Lecka-Czernick et al., 1997; Fujisawa et al., 1998). However, it is not clear whether or not these phenotypes are associated directly with the accelerated senescence and short lifespan of SAMP mice. One exception is the senile AApoAII amyloidosis caused by the *Apoa2*^{c} allele of apolipoprotein AII (*Apoa2*) gene. Two previous observations suggested genetic association of the *Apoa2*^{c} of SAMP1 mice with the senescence process. (i) The *Apoa2*^{c} allele was associated genetically with a high degree of senescence of mice in (SAMP1 × SAMR1)F_2 (Naiki et al., 1993). (ii) Senile amyloidosis shortened the lifespan by about 24% in R1.P1–*Apoa2*^{c} congenic mice (Higuchi et al., 1996).

Genetics may offer a powerful approach to the elucidation of mechanisms underlying the accelerated senescence of SAMP mice. Recently, genetic profiles of nine SAMP and four SAMR strains were revealed with microsatellite marker loci, providing a basis for genetic analysis of accelerated senescence and lifespan (Xia et al., 1999). They also revealed strain distribution patterns (SDPs) at these loci. The SDPs at *D14Mit92*, *D16Mit30*, and *D17Mit176* exhibited perfect agreement with the SDP of accelerated senescence-'prone' and '-resistant' phenotypes. Thus the chromosomal regions around these four loci might be associated with the accelerated senescence of SAMP strains. In this study, we performed whole genome

scanning with polymorphic marker loci in hybrid progeny derived from SAMP1 to identify the chromosomal regions associated with the lifespan.

2. Materials and methods

2.1. Breeding and rearing condition of mice

SAMP1 mice were from a colony at the Field of Regeneration Control, Institute for Frontier Medical Science, Kyoto University. B10.BR–$H2^k$/SgSnSlc (B10.BR) congenic strain was used as a mating partner, as the C57BL/10, a recipient strain of the B10.BR, has a long mean lifespan (826 days in males and 693 days in females; http://www.informatics.jax.org/external/festing/mouse/docs/C57BL.shtml). Another merit of the strain is that it has the same $H2^k$ haplotype on the *H2* locus as the SAMP1, and hence could exclude the possible influence of the *H2* locus on the lifespan (Salazar et al., 1995). B10.BR mice were purchased from JAPAN SLC, Inc. (Hamamatsu, Japan). The (B10.BR × SAMP1) hybrid mouse population, consisting of 14 (B10.BR × SAMP1)F_1 × SAMP1 backcross (BC) mice, 27 (BC × BC) intercross and 30 (BC × BC) × BC mice was produced. All these mice were raised under conventional conditions at the animal facility of the Field of Regeneration Control, Institute for Frontier Medical Science, Kyoto University, with a 12-h light/dark cycle and room temperature of 24 ± 2°C Mice had free access to the commercial diet (CE-2, Nihon CLEA) and tap water. Mice were inspected daily. Tissues were taken from carcasses and fixed in 10% buffered formalin. The severity of AApoAII deposition in the major organs (liver, heart, kidneys, spleen, and stomach) was evaluated by determining the amyloid index.

2.2. Genotyping for microsatellite marker loci and Apoa2

Oligonucleotide primers for the mouse microsatellite markers were purchased from Research Genetics (Huntsville, AL, USA). Map positions of the markers on the chromosomes, and total length of each chromosome were based on the Chromosome Committee Report (http://www.informatics.jax.org/ccr). Genomic DNA was extracted from the liver. The PCR amplification was carried out in a 25 μl reaction containing 200 μM each dNTP, 1X buffer containing 1.5 mM-$MgCl_2$, 0.1 μM each primer, 0.625 unit of *Taq* polymerase (Promega), and 125 ng genomic DNA. The cycling parameters for PCR were, initial denaturation for 1 min at 94°C; followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C; final elongation for 1 min at 72°C. PCR products were resolved by 4% (1% agarose and 3% NuSieve GTG) agarose gel electrophoresis and examined for polymorphism between B10.BR and SAMP1 strains. Eighty of the polymorphic microsatellite markers distributed over autosomes and chromosome X (Table 1) were used for the genetic association test. The average interval between loci, or between locus and centromere or telomere on each chromosome was 15.5 cM.

SAMP1 and B10.BR mice have *Apoa2*^{c} and *Apoa2*^{b} alleles at the *Apoa2* locus, respectively (Higuchi et al., 1991). Mice were genotyped for the *Apoa2* locus by utilizing the *Cfr*13I-site polymorphism between the *Apoa2*^{c} and *Apoa2*^{b} alleles (Naiki et al., 1993).

2.3. Statistical analysis

Difference in mean lifespan was evaluated by Student's t-test. Survival curves were drawn by the Kaplan and Meier method, and compared by the Logrank test. These tests were performed by using a StatView J-4.5 software.

3. Results

3.1. Profile of hybrid mouse cohort for the lifespan

We obtained a hybrid mouse cohort consisting of 34 males and 37 females from a cross between B10.BR and SAMP1 mouse strains. No gross abnormalities were observed in the carcasses, except for the AApoAII amyloidosis. The lifespan of the mice ranged from 230 to 833 days with a mean value $\pm$ standard deviation (S.D.) of 490.3 ± 151.9 days. The mean lifespan of males and females were 463.8 ± 125.2, and 514.8 ± 160.9 days, respectively. There was no significant sexual difference in the mean lifespan ($P = 0.14$).

3.2. Evaluation of the effect of the *Apoa2*^{c} allele to shorten the lifespan

We demonstrated previously that senile amyloidosis shortened the lifespan by about 24% in R1.P1–*Apoa2*^{c} congenic mice (Higuchi et al. 1996). We first investigated if the effect of the *Apoa2*^{c} allele from SAMP1 on chromosome 1 to shorten the lifespan was reproduced in the mouse cohort used in this study. The mice homozygous for the SAMP1 allele ($n = 26$), heterozygotes ($n = 37$), and homozygotes for the B10.BR allele (B/B; $n = 8$) at the *Apoa2* locus had a mean lifespan of 429, 508, and 607 days, respectively (Table 1). Student's t-test revealed that the mean lifespan of mice with P/P genotype was shorter than that of mice with the other two genotypes ($P = 0.02$ and 0.0001 for P/P vs. P/B, and P/P vs. B/B, respectively). The mean lifespan of mice with P/B and B/B genotypes was not different ($P = 0.12$). Comparison of survival curves (Fig. 1) also revealed a significant difference between mice with the P/P genotype and P/B or B/B genotype ($P = 0.005$ and 0.002 for P/P vs. P/B, and P/P vs. B/B, respectively). No difference was observed between mice with P/B and B/B genotypes ($P = 0.242$). Mice with P/P, P/B, and B/B genotypes at the *Apoa2* locus had a mean amyloid index of 2.20, 0.64, and 0.98, respectively. The Mann–Whitney U-test revealed that the mean amyloid index of mice with the P/P genotype was higher than that of mice with the other two genotypes ($P = 0.002$ and 0.0123 for P/P vs. P/B, and P/P vs. B/B, respectively). The mean amyloid index of mice with the P/B and B/B genotypes was

Table 1
List of marker loci associated with lifespan

Marker locus	Position	Effect[a]	Mean lifespan in days (number of mice)			Logrank test of survival curves
			P/P[b]	P/B[b]	B/B[b]	
D1Mit67	9.0 cM	(↓) recessive	459 (52)	< 552 (19)	(0)	Not different
D1Mit265	74.3 cM	(↓) recessive	458 (44)	< 527 (24)	= 494 (3)	Not different
D1Mit110	87.9 cM	(↓) recessive	429 (26)	< 508 (37)	= 547 (8)	Different between P/P and P/B
Apoa2	92.6 cM	(↓)recessive	429 (26)	< 508 (37)	= 607 (8)	Different between P/P and P/B
D1Mit150	100 cM	(↓) recessive	429 (26)	< 508 (37)	= 607 (8)	Different between P/P and P/B
D5Mit349	8.0 cM	(↓) recessive	447 (39)	< 553 (26)	= 504 (6)	Not different
D5Mit267	24.0 cM	(↓) recessive	433 (37)	< 562 (29)	= 500 (5)	Different between P/P and P/B
D5Mit197	36.0 cM	(↓) recessive	453 (39)	< 538 (27)	= 528 (5)	Not different
D6Mit384	28.0 cM	(↓) recessive	461 (42)	< 537 (27)	= 466 (2)	Not different
D8Mit190	21.0 cM	(↑) additive	533 (37)	> 448 (32)	= 371 (2)	Not different
D14Mit129	14.5 cM	(↓) dominant	491 (39)	= 466 (28)	< 653 (4)	Not different
D16Mit28	13.2 cM	(↓) dominant	472 (39)	= 495 (30)	< 767 (2)	Not different
D17Mit176	22.5 cM	(↑) additive	566 (23)	> 469 (38)	= 399 (10)	Different among three genotypes
D17Mit117	29.4 cM	(↑) additive	526 (31)	= 480 (31)	> 381 (9)	Different between P/B and B/B
D19Mit36	52.0 cM	(↑) dominant	449 (14)	= 523 (45)	> 414 (12)	Different between P/B and B/B

[a] Effect of the SAMP1 allele with lifespan, either to shorten (↓), or prolong (↑), and either in additive, dominant, or recessive manner.

[b] Genotype of mice, either homozygous for *SAM*P1 allele (P/P), heterzoygous (P/B) or homozygous for B10.BR allele (B/B). Other marker loci examined were, *D1Mit123* (21.0 cM), *D1Mit332* (43.1 cM), *D1Mit26* (62.1 cM), *D2Mit32* (13.0 cM), *D2Mit56* (38.0 cM), *D2Mit525* (61.2 cM), *D2Mit213* (105 cM), *D3Mit203* (11.2 cM), *D3Mit51* (35.2 cM), *D3Mit82* (64.1 cM), *D3Mit19* (87.6 cM), *D4Mit236* (12.1 cM) *D4Mit27* (42.5 cM), *D4Mit308* (57.4 cM), *D4Mit42* (81.0 cM), *D5Mit239* (58.0 cM), *D5Mit139* (69.0 cM), *D5Mit224* (87.0 cM), *D6Mit138* (0.68 cM), *D6Mit29* (36.5 cM), *D6Mit201* (74.0 cM), *D7Mit57* (4.0 cM), *D7Mit85* (26.5 cM), *D7Mit62* (44.0 cM), *D7Mit206* (60.0 cM), *D8Mit69* (31.0 cM), *D8Mit200* (58.0 cM), *D9Mit43* (4.0 cM), *D9Mit154* (21.9 cM), *D9Mit166* (41.0 cM), *D9Mit35* (52.0 cM), *D9Mit120* (69.0 cM), *D10Mit247* (7.0 cM), *D10Mit44* (27.0 cM), *D10Mit42* (44.0 cM), *D10Mit180* (64.0 cM), *D11Mit74* (0.0 cM), *D11Mitt174* (20.0 cM), *D11Mit112* (32.0 cM), *D11Mit288* (55.0 cM), *D11Mit168* (71.0 cM), *D12Mit83* (6.0 cM), *D12Mit34* (29.0 cM), *D12Mit8* (58.0 cM), *D13Mit3* (10.0 cM), *D13Mit167* (36.0 cM), *D13Mit76* (61.0 cM), *D13Mit204* (72.0 cM), *D14Mit111* (7.5 cM), *D14Mit193* (40.0 cM), *D15Mit87* (19.2 cM), *D15Mit71* (46.7 cM), *D16Mit38* (28.5 cM), *D16Mit50* (53.5 cM), *D16Mit71* (70.7 cM), *D17Mit19* (3.0 cM), *D17Mit123* (56.7 cM), *D18Mit123* (20.8 cM), *D18Mit185* (43.0 cM), *D19Mit69* (6.0 cM), *D19Mit39* (24.0 cM), *D19Mit66* (41.0 cM), *DXMit166* (15.1 cM), *DXMit117* (50.8 cM), *DXMit20* (70.0 cM).

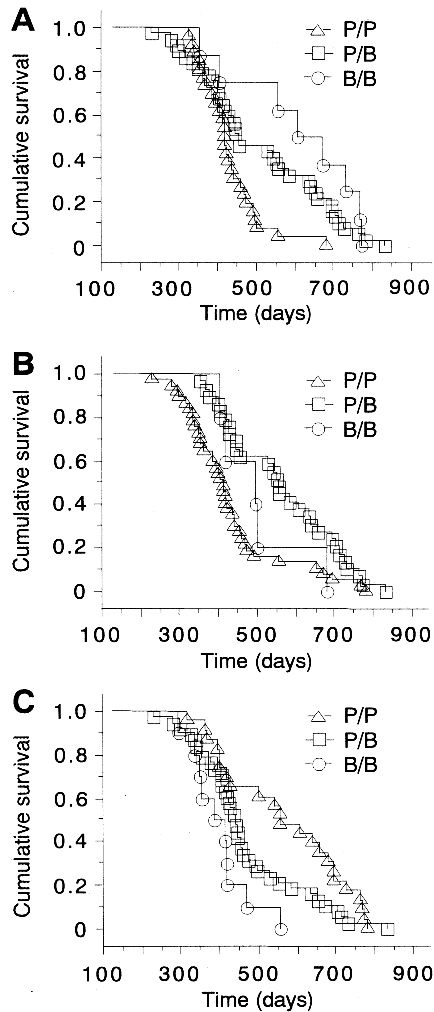

Fig. 1. Survival curves of mice with three genotypes at *Apoa2(A)*, *D5Mit267(B)*, and *D17Mit176(C)*. P/P, P/B, and B/B stand for mice homozygous for the SAMP1 allele, heterozygotes, and mice homozygous for the B10.BR allele, respectively.

not different ($P = 0.31$). These results indicated that the genetically recessive effect of the *Apoa2*^{c} allele was successfully reproduced in the mouse cohort, ascertaining the validity of the mouse cohort for a genetic study of the lifespan. However, it was not clear whether or not the amyloidosis was the cause of the mouse's death. The marker loci linked to the *Apoa2* gene also showed the recessive effect to reduce the lifespan, presumably owing to the influence of the *Apoa2*. The influence of *Apoa2* was detected at *D1Mit110* and *D1Mit265*, but not at *D1Mit26*, which are 4.7, 18.3, and 30.5 cM apart from *Apoa2*, respectively. We could not examine the influence of *Apoa2* in the region distal to the gene, because none of the markers examined was

polymorphic between B10.BR and SAMP1, except for *D1Mit150*. All mice had the same genotype at *D1Mit150*, and hence the same correlation as the *Apoa2* locus.

3.3. Identification of chromosomal regions associated with lifespan

Correlation of each 80 microsatellite marker locus distributed throughout the genome, except for chromosome Y, with the lifespan was then evaluated. Student's t-test revealed the correlation of the following ten marker loci on eight chromosomes, besides *Apoa2* and linked marker loci with lifespan (Table 1); *D1Mit67*, *D5Mit349*, *D5Mit267*, *D6Mit384*, *D8Mit190*, *D14Mit129*, *D16Mit28*, *D17Mit176*, *D17Mit117* and *D19Mit36*. The results of the Logrank comparison of survival curves were consistent with the results by Student's t-test for *D5Mit267*, *D17Mit176*, *D17Mit117* and *D19Mit36*, but not for the rest (Fig. 1).

4. Discussion

There have been few systematic studies on the genetic factors involved in the senescence process and lifespan in mice, partly due to the complexity of the phenomenon (Yunis et al., 1984; Gelman et al., 1988; de Haan et al., 1998). The SAMP1 mouse strain used in this study, has a short lifespan. However, no pathological abnormality common to SAMP mice has been found. The cause of death of SAMP mice has not yet been specified as well. Therefore, we simply measured lifespan as a phenotype to be analyzed in this study. To facilitate the detection of the chromosomal regions associated with the lifespan, we used the B10.BR strain, which was supposed to have a very long lifespan contrary to the SAMP1, as a mating partner. We have to admit that the transmission of alleles was biased so that there were more SAMP1 alleles and fewer B10.BR alleles in the mouse cohort, because it was not an F_2 set, but a mixture of progeny of backcross and intercross of backcross. The genes responsible for the accelerated senescence are supposedly recessive genetically. If it were the case, the recessive effects would be reflected in the difference between the mouse cohort with only P/P and P/B genotypes. In fact, the genetically recessive effect of the *Apoa2*^{c} allele from SAMP1 was detected successfully in the cohort. Another problem inherent to the genetic analysis in the mouse cohort was the possibility to fail to detect the locus correlated with the phenotypes. In general, a QTL with 5 or 10% explained variance can be mapped on an interval of 40 or 20 cM, respectively, with a minimum population of 200 backcross or F_2 mice (van Ooijen, 1992). The fact that the influence of the *Apoa2* gene was detected at the locus, which was 18.3 cM apart, suggested that at least one marker in every 36 cM would be required to detect QTL with as strong influence as the *Apoa2*. A large interval of 36 cM between *D2Mit525* (61.2 cM) and *D2Mit213* (105 cM) was left without marker loci. We examined eight microsatellite markers between the loci, but they were not polymorphic between B10.BR and SAMP1.

Multiple marker loci exhibited association with lifespan. The mode of effect on the lifespan varied depending on the locus, suggesting the complex genetic control of the trait. Among them, *D1Mit67*, *D5Mit267*, *D6Mit384* and *D19Mit33* deserve attention, as the effects of the SAMP1 allele on these loci were suggested to be recessive. Especially the recessive effect was ascertained both by Student's *t*-test and the Logrank test of the survival curves for *D5Mit267*. It is, however, premature to conclude that the regions around these loci truly contained causative genes for the accelerated senescence and consequently shortened the lifespan. For example, although *Apoa2* acted to shorten the lifespan, we know that it is not a major gene for the accelerated senescence and short lifespan of *SAMP* strains, because only four SAMP strains had the $Apoa2^{c}$ allele.

Xia and colleagues deduced that the chromosomal regions around *D14Mit92* (45.0 cM), *D16Mit30* (36.5 cM) and *D17Mit176* (22.5 cM), were associated with the accelerated senescence and short lifespan of SAMP strains, based on the observation that all SAMP strains share the same allele at these loci (Xia et al., 1999). It was worthy of note that the association of the *D17Mit176* was again observed in this study. We could not examine *D16Mit30* and other neighboring loci, because they were not polymorphic between B10.BR and SAMP1. The position of *D16Mit28* (13.2 cM), which exhibited association in this study was somewhat apart from *D16Mit30*. No association was observed at *D16Mit38* (28.5 cM). Thus, the effect of the gene(s) around *D16Mit28* would be irrelevant to that around *D16Mit30*.

On chromosome 14, a dominant effect of allele from SAMP1 to reduce the lifespan was observed at *D14Mit129* (14.5 cM), but no association was observed at *D14Mit193* (40.0 cM), which is located closer to *D14Mit92* (45.0 cM).

The allele from SAMP1 at *D17Mit176* exhibited an effect to increase rather than reduce the lifespan. It may not be strange though, because we do not have to assume that all genes from SAMP1 work to reduce the lifespan. Actually the SDP at *D17Mit176* are reversed to that of *D14Mit92*. All nine SAMP strains had the allele from AKR/J at *D17Mit176*, while all four SAMR strains had the allele from the unspecified (B) strain. On the contrary, all nine SAMP strains had the allele from the unspecified strain at *D14Mit92*, while all four SAMR strains had the allele from AKR/J. The results could be explained if we assume that the allele from B or B10.BR strains work to shorten the lifespan, while the allele from AKR/J counteract it.

The information obtained in this study would serve as a basis for identification of the genes for accelerated senescence and short lifespan in SAMP mice. First of all, we will have to confirm whether or not the chromosomal regions identified in this study truly contain the genes, responsible for the accelerated senescence. It would also be intriguing to see the combined effects of these loci, because the accelerated senescence of the SAMP strains is considered to be under the control of multiple genes. We are now making congenic strains for each chromosomal region for this purpose. New mouse strains, which would be more useful for gerontological research may also be generated, by changing the combination of the regions. Meanwhile, we are examining positional candidate genes, looking for the specific

change in SAMP strains. We have examined five genes, superoxide dismutase 3 (*Sod3*) on chromosome 5, glutathione reductase 1 (*Gr1*) on chromosome 8, superoxide dismutase 1 (*Sod1*) on chromosome 16, superoxide dismutase 2 (*Sod2*) on chromosome 17, and anti-oxidant protein 1 (*Aop1*) on chromosome 19. We focused on antioxidant enzymes, because some of them have been reported to influence longevity in other species (Orr and Sohal, 1994; Parkes et al., 1998), and SAMP strains were actually under an increased oxidative stress (Park et al., 1996; Butterfield et al., 1997). No nucleotide differences, however, were observed in the coding regions of the genes in the SAMP1 strain. Further examination of the positional candidates should help us to identify the genes, which might help to elucidate the mechanism of the normal senescence process as well.

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