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# Differences in saccharin preference and genetic alterations of the *Tas1r3* gene among senescence-accelerated mouse strains and their parental AKR/J strain

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## HIGHLIGHTS

- Differences in saccharin preference were found among SAM strains and AKR/J.
- Several polymorphisms in chromosome 4 were observed among SAM strains and AKR/J.
- SAM strains have similar polymorphic patterns in *Tas1r3* to those of C57BL/J.

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## ABSTRACT

The senescence-accelerated mouse (SAM) is used as an animal model of senescence acceleration and age-associated disorders. SAM is derived from unexpected crosses between the AKR/J and unknown mouse strains. There are nine senescence-prone (SAMP) strains and three senescence-resistant (SAMR) strains. Although SAMP strains exhibit strain-specific and age-related pathological changes, the genes responsible for the pathologic changes in SAMP strains have not been comprehensively identified. In the present study, we evaluated sweet taste perception using the two-bottle test. We compared genotypes of the taste related gene, *Tas1r3*, using SAM strains and the parental AKR/J strain. The two-bottle test revealed that SAMR1 (R1), SAMP6 (P6), SAMP8 (P8), and SAMP10 (P10) mice were saccharin-preferring strains, whereas AKR/J did not prefer saccharin. All genotypes of the R1, P6, P8, and P10 strains at the polymorphic sites in *Tas1r3*, which is known to influence saccharin preference, were identical to those of C57BL6/J, a well-known saccharin-preferring strain, and were completely different from those of the parental AKR/J strain. These genetic alterations in SAM strains appear to arise from an unknown strain that is thought to have been crossed with AKR/J initially.



## 1. Introduction

The senescence-accelerated mouse (SAM) is a group of inbred mouse strains used as animal models for senescence acceleration and age-associated disorders. SAM was derived from unexpected crosses between the AKR/J strain and unknown mouse strain(s) [1], from which nine phenotypically distinct senescence-prone (SAMP) and three senescence-resistant (SAMR) strains were established through selective breeding based on graded scores for senescence, life span, and pathological phenotypes [2–4]. SAMP mice exhibit a short life span, early senescence onset, and strain-specific age-related pathological changes. In contrast, SAMR mice show normal aging and are used as controls. Among these strains, SAMP6 (P6) is predominantly used as a model of senile osteoporosis because these mice exhibit low bone mass and slow bone loss [5]. Recently, we have also reported some behavioral alterations in P6 mice, including increased motor activity [6–8], motor coordination deficits [9], low anxiety [6], and enhanced memory [7,10]. SAMP8 (P8) and SAMP10 (P10) have age-related deficits in learning and memory [11–14], emotional disorders [15,16], and altered circadian rhythms [16]. Because these phenotypes accelerate with aging, P8 and P10 are frequently used as models to study age-related changes in higher brain function.

Past genetic analyses have revealed several common genetic mutations, as well as distinct mutations, among SAMP strains [17–20]. Although various genetic mutations in SAMP strains are known, the full suite of genes responsible for the accelerated senescence and pathological changes have not been identified. Naiki et al. reported that combinations of multiple gene mutations were responsible for the SAMP1 phenotypes [21], implying that combinations of multiple gene mutations must be examined.

Because sweet taste perceptions in SAM strains have not been studied, we performed the two-bottle test to identify new behavioral differences within SAM strains. We compared the genotypes of a taste related

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gene using SAM strains and the parental AKR/J strain in order to identify new genetic mutations and new polymorphic loci within SAM strains. These data may help determine the unknown strain thought to have been crossed with AKR/J initially.

## 2. Materials and methods

### 2.1. Mice

AKR/J mice were obtained from CLEA Japan Inc. (Tokyo, Japan). R1, P6, P8, and P10 mice were purchased from Japan SLC (Shizuoka, Japan). All mice were housed individually in microisolation cages (19.6 × 30.6 × 16.6 cm) under a 12:12-hour light:dark cycle (dark period, 20:00 to 08:00) until used. Food and water were available ad libitum. The RIKEN Institutional Animal Care and Use Committee approved all experimental procedures.

### 2.2. Saccharin preference tests

Animals were given the choice between water and saccharin solutions (0.32, 1.6, and 8.0 mM) after an initial habituation to two bottles of water for 3 days in the home cage. Nozzles with spill-prevention balls were attached to the bottles. Mice and bottles (containing saccharin solution or water) were weighed at the beginning of the experiment. During the 3-day consecutive experiments, bottles were switched from one side of the cage to the other side to reduce the effects of side preference. After completion of the experiment, the volume of fluid consumed was recorded. During the experiments, evaporation/spillage was estimated from two bottles placed in an empty cage, one containing water and the other containing the appropriate saccharin solution. These control bottles were also switched from one side of the cage to the other side daily. The intake volume of each fluid was calculated by subtracting the evaporation/spillage volume from the total reduced volume.

### 2.3. Marker analyses

Extraction of genomic DNA from tail clips was performed using the MELT™ Total Nucleic Acid Isolation System (Ambion Inc., Austin, TX, USA) according to the manufacturer's instructions. Custom-made PCR primers for seven microsatellite markers (Fig. 1) spanning chromosome 4 were purchased from Invitrogen (Carlsbad, CA, USA). Genetic map positions (cM) from the centromere of the markers were obtained from the mouse genome database (http://www.informatics.jax.org). Using the thermal cycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), PCR amplifications were conducted in 20 μl reaction volumes containing approximately 100 ng DNA, 1× PCR buffer, 0.05 mM dNTPs, 0.2 mM of each primer, and 0.4 U DNA polymerase (KOD FX, TOYOBO Co., Ltd., Osaka, Japan). PCR cycling parameters were as follows: one cycle of 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 45 s, followed by 72 °C for 10 min. PCR products were electrophoresed on 4% agarose gels in 1× TBE buffer, stained with ethidium bromide, and photographed using UV light. Genotypes that were doubtful or unclear were reamplified.

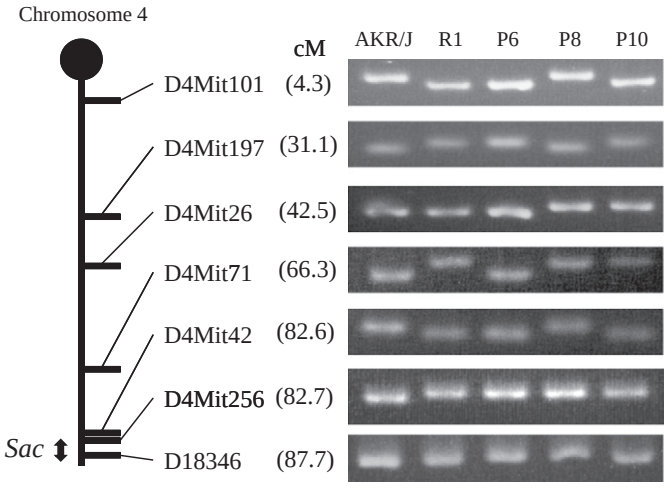


**Fig. 1.** The polymorphism patterns of AKR/J, R1, P6, P8, and P10 with seven microsatellite markers on chromosome 4. The *Sac* locus is between D4Mit256 and D18346. Different band sizes between saccharin-preferring strains (R1, P6, P8, and P10) and non-saccharin-preferring strain (AKR/J) were observed for D4Mit256, while there were three types of band sizes (AKR/J type, R1 and P10 type, and P6 and P8 type) for D18346.

### 2.4. DNA sequencing

Six primer pairs were designed to amplify fragments that included eight *Tas1r3* coding region sites that were previously reported to be regions associated with saccharin preference [24]. To design the primer pairs, mouse genomic DNA sequences were purchased from Ensembl Genome Browser (http://asia.ensembl.org/Mus_musculus/Info/Index). The DNA sequence analysis software GENETYX-SV/RC version 16.0 (Genetyx Co., Ltd., Shibuya, Tokyo, Japan) was used to align and compare sequences. Genomic DNA from each strain was extracted as described above and used as a template to generate PCR products used for sequencing reactions. The primer sequences used for sequencing are available upon request. Cycle sequencing using BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA) was followed by analysis using the 3730 × l DNA Analyzer (Applied Biosystems).

### 2.5. Statistical analyses

Two-way analysis of variance (ANOVA) with Bonferroni post hoc tests was used for the analyses of saccharin preference using Prism 4 (GraphPad software, La Jolla, CA, USA). All data are presented as the mean ± SEM. The alpha level was 0.05.

## 3. Results

### 3.1. Saccharin preference

The saccharin preference ratios for the five mouse strains are presented in Fig. 2a. The preference ratios of AKR/J ($n = 13$) for the 0.32, 1.6, and 8.0 mM saccharin were 0.466 ± 0.036, 0.470 ± 0.020, and 0.141 ± 0.006, respectively. The preference ratios of R1 ($n = 13$) for the 0.32, 1.6, and 8.0 mM saccharin were 0.890 ± 0.012, 0.972 ± 0.003, and 0.877 ± 0.033, respectively. The preference ratios of P6 ($n = 8$) for the 0.32, 1.6, and 8.0 mM saccharin were 0.581 ± 0.012, 0.769 ± 0.012, and 0.564 ± 0.032, respectively. The preference ratios of P8 ($n = 8$) for the 0.32, 1.6, and 8.0 mM saccharin were 0.541 ± 0.005, 0.820 ± 0.005, and 0.578 ± 0.008, respectively. The preference ratios of P10 ($n = 12$) for the 0.32, 1.6, and 8.0 mM saccharin were 0.789 ± 0.021, 0.925 ± 0.007, and 0.640 ± 0.014, respectively. The dose × strain interactions [$F(8, 98) = 20.12$, $P < 0.0001$], dose effects [$F(2, 98) = 182.10$, $P < 0.0001$], and strain effects [$F(4, 98) = 288.02$, $P < 0.0001$] are shown. For every dose, the saccharin preference of the AKR/J strain was significantly lower compared with that of the other four strains (0.32 mM, AKR/J vs. R1, $P < 0.001$, AKR/J vs. P6, $P < 0.001$, AKR/J vs. P8, $P < 0.05$, AKR/J vs. P10, $P < 0.001$; 1.6 mM, AKR/J vs. R1, $P < 0.001$, AKR/J vs. P6, $P < 0.001$, AKR/J vs. P8, $P < 0.001$, AKR/J vs. P10, $P < 0.001$; 8.0 mM, AKR/J vs. R1, $P < 0.001$, AKR/J vs. P6, $P < 0.001$, AKR/J vs. P8, $P < 0.001$, AKR/J vs. P10, $P < 0.001$, determined by Bonferroni post hoc tests). Among the four SAM strains, there were differences in the preference ratios. At the 0.32 and 1.6 mM saccharin doses, R1 and P10 preferred saccharin compared with P6 and P8 (0.32 mM, R1 vs. P6, $P < 0.001$, R1 vs. P8, $P < 0.001$, P10 vs. P6, $P < 0.001$, P10 vs. P8, $P < 0.001$; 1.6 mM, R1 vs. P6, $P < 0.001$, R1 vs. P8,