Distinct physical condition and social behavior phenotypes of CD157 and CD38 knockout mice during aging

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Abstract

The ability of CD38 and CD157 to consume nicotinamide adenine dinucleotide (NAD) has received much attention because aging-induced elevation of CD38 expression plays a role in the senescence-related decline in NAD levels. Therefore, it is of interest to examine and compare the effects of age-associated changes on the general health and brain function impairment of Cd157 and Cd38 knockout (CD157 KO and CD38 KO) mice. Body weight and behaviors were measured in 8-week-old (young adult) or 12-month-old (middle-aged) male mice of both KO strains. The locomotor activity, anxiety-like behavior, and social behavior of mice were measured in open field, and three-chamber tests. Middle-aged CD157 KO male mice gained more body weight than young adult mice, while little or no body weight gain was observed in middle-aged CD38 KO mice. Middle-aged CD157 KO mice displayed increased anxiety-like behavior and decreased sociability and interaction compared with young adult KO mice. Middle-aged CD38 KO mice showed less anxiety and hyperactivity than CD157 KO mice, similar to young adult CD38 KO mice. The results reveal marked age-dependent changes in male CD157 KO mice but not in male CD38 KO mice. We discuss the distinct differences in aging effects from the perspective of inhibition of NAD metabolism in CD157 and CD38 KO mice, which may contribute to differential behavioral changes during aging.

1. Introduction

Immune-related CD38 and CD157 are similar molecules that share enzymatic functions [1–4]. Both molecules possess cyclase activity and can metabolize nicotinamide adenine dinucleotide (NAD) into ADP-ribose and cyclic ADP-ribose (cADPR), which are important second messengers that can potentially activate TRPM2 channels and trigger Ca^{2+} mobilization in ryanodine receptor-sensitive Ca^{2+} pools [1–3,5]. This increase in the intracellular free Ca^{2+} concentration has been shown to facilitate oxytocin release into the brain and the systemic circulation at the posterior pituitary gland [6,7]. When cADPR-sensitive CD38-dependent oxytocin release is disrupted, abnormal social behavior can be induced in mice with a null mutation in the Cd38 gene (CD38 KO mice). CD38 KO pups exhibited a higher level of locomotor activity and a lower number of ultrasonic vocalizations than wild type (WT) pups [1,8,9]. Young adult (approximately 8–12 weeks old) CD38 KO mice showed increased locomotory activity, deficits in social memory (amnesia), reductions in anxiety-like behavior, and impairment in parental behavior [6,10]. However, relatively little is known about behavioral changes in aged CD38 KO mice.

In contrast, Cd157 knockout (CD157 KO mice), which comprises a model of pre-motor symptoms of Parkinson’s disease, can be characterized by increased anxiety-related and depression-like behavior or social avoidance in young adulthood [11–14]. A decreased number of calls and a poor vocal repertoire were also detected in CD157 KO pups [15]. Additionally, little or no data exist regarding behavioral changes in aged CD157 KO mice.

Aging is associated with impairments in a wide range of physical and brain functions in humans and animals [16–22]. Furthermore, 10–15 month-old mice can be considered as middle-aged, during which time senescence processes and reproductive decline begin to occur [23,24]. With aging, gains in body
mass [25–28] and decreases in locomotor activity are frequently observed [25,27–31] but see [32]. However, some studies have reported increases in anxiety-related [27–29,31] and depression-like behavior [31] and decreases in social interaction [16,27,31]. During middle age, mice may begin to display a decline in cognitive function and memory loss [28,30,31,33–35]. Although this does not always occur, especially in early middle-aged mice [27,28], it is worth examining age-related changes in middle-aged mice instead of examining older mice (24 months).

Recently, the ability of CD38 to metabolize NAD has received much attention because elevated CD38 expression plays a role in the senescence-related decline in NAD levels [36–38]. Interestingly, aged CD38 KO mice have been reported to exhibit better general health than WT mice of the same age [36]. Moreover, inhibition of the catalytic activity of CD38 by small molecule inhibitors or monoclonal antibodies can also promote a beneficial effect by sustaining higher NAD levels in aged mice [38].

General health outcomes in aging CD157 KO mice have not been previously reported. Therefore, we first examined middle-aged CD157 KO mice and then determined similarities and differences in aging-induced changes between CD38 KO and CD157 KO mice, especially with respect to body weight and social behavior. CD38 KO and CD157 KO mice were studied simultaneously because their genotypes differ (Cd38−/−/Cd157+/+ and Cd38+/+/Cd157−/−, respectively), and we expected distinct outcomes because of the differential deletion of these NAD-related genes. The effects of these molecules on aging can be determined by directly comparing CD157 and CD38 KO mice without using aged WT mice as controls.

2. Materials And Methods

2.1. Animals

Cd157/Bst1−/− (CD157 KO on a C57BL/6 background) mice were described previously [10,39]. CD157 KO mice were maintained by crossbreeding homozygous mutant mice. Cd38−/− (CD38 KO) mice with an ICR genetic background were described previously [6,40]. Most experiments were performed using a congenic method on selected adult males of the homozygous KO groups. All CD157 KO and CD38 KO mice were born in our laboratory colony. Pups were weaned at 21–28 days of age and housed in same-sex groups of five animals/cage in the animal center of our university under standard conditions (24°C; 12/12-h light/dark cycle, with lights on at 8:45 a.m.) with ad libitum access to food and water. The mice were fed with Charles River formula chow with a standard caloric content (Oriental Yeast Co. Ltd, Cat # CRF-1, Tokyo, Japan). Behavioral testing was performed on mice aged 8 or 12 weeks (designated as young adult) or 12 months (designated as middle-aged). All animal experiments were carried out in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan and were approved by the Kanazawa University Committee on Animal Experimentation.
2.2. Open field test

An Open field test was performed as described previously [14,41]. Briefly, the open field chamber consisted of a square wooden box (550 × 600 × 400 mm), and the inner surfaces were covered with polypropylene sheets. The open field was divided into an inner zone (300 × 300 mm) and the periphery. First, a mouse was placed in the arena for 10 min (session 1, as a habituation period) and then returned to its home cage.

In session 2 (with a non-social object), an empty wire cage was placed in the center of the arena. The mouse was placed in the open field for 10 min, during which time stress was induced by the non-social target. Then, the mouse was returned to its home cage.

In the session 3, a naïve WT male mouse (a BL6 mouse was used with CD157 KO mice, and an ICR mouse was used with CD38 KO mice) was placed in a wire cage at the center. The time spent in the inner and outer zones, total distance travelled, and immobility time were analyzed using a digital video system and ANY-maze video tracking software (Stoelting Co., Wood Dale, IL, USA). After each session, the test chambers were sprayed with 1% sodium hypochlorite and 70% ethanol and cleaned with paper towels [14]. The time interval between sessions was 2–3 min.

2.3. Three-chamber test

A preference test for social targets of mice was performed using a three-chamber box [14]. The apparatus consisted of a rectangular, three-chambered box covered with clear polycarbonate. Dividing walls had doorways allowing access into each chamber. At the end of each test, the apparatus was sprayed with 1% sodium hypochlorite and 70% ethanol and wiped clean with paper towels. The following procedure was used for the social behavior test:

(1) Habituation. The day before the test, mice were habituated in an empty apparatus for 20 min. Target mice were also habituated for 20 min in small cages. On the day of the experiment, the test mouse was first placed in the middle chamber and allowed to explore for 5 min with free access to all parts of the arena. Each of the two sides contained an empty wire cage (70 mm × 90 mm × 70 mm with bars spaced 5 mm apart). Zones located at 2.5 cm intervals around the wire cages were considered zones of direct interaction (cage zone)

(2) Sociability. After habituation, an unfamiliar mouse (Stranger 1 (Str1); a naïve C57BL/6 male) was placed in the wire cage (in the left chamber); the other wire cage (in the right chamber) was left empty, and the test mouse was placed in the center compartment of the social test box and allowed to explore for a 5-min session, with free access to the two side chambers. The amount of time spent in the cage zone in each chamber was measured using a digital video system and ANY-maze software.
(3) At the end of the 5-min sociability test, each mouse was further tested in the third 5-min session to quantitate preference for spending time with a new stranger. The new unfamiliar mouse (Str2; an experiment-naïve C57BL/6 male mouse) was placed in the wire cage (in the right chamber) that was empty during the previous 5-min session. The test mouse had a choice between the first, already-investigated, now-familiar mouse (Str1) and the novel unfamiliar mouse (Str2).

As described above, the amount of time spent in each chamber and in the direct interaction zones was measured using a digital video system and ANY-maze software. At the end of each test, the three-chamber box was cleaned as described above. The mean time interval between sessions was 2–3 min.

2.4. Statistical analysis

GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Data are expressed as the mean ± S.E.M. Comparisons were performed between young adult and middle-aged mice. The statistical analysis was performed by multiple Student’s *t*-tests or two-way analysis of variance (ANOVA) followed by post hoc Bonferroni tests. In all analyses, *p* < 0.05 indicated statistical significance.

3. Results

We examined age-dependent body weight gain in male CD157 and CD38 KO mice (Fig. 1). Twelve-month-old (designated as middle-aged) CD157 KO mice had a significantly higher body weight (Fig. 1A) and body mass index (BMI; Fig. 1B) than 8-week-old mice (young adult): *t*(12) = 10.37, *p* < 0.0001 for body weight; *t*(12) = 9.369, *p* < 0.0001 for BMI.

In sharp contrast, middle-aged (12 month-old) CD38 KO male mice had a similar range of body weight to that of young adult mice (8 weeks old) (Fig. 1C; *t* = 0.4878, *p* = 0.6406). The BMI of middle-aged CD38 KO mice was slightly but significantly lower than that of young adult mice (Fig. 1D; *t*(7) = 2.417, *p* = 0.0463). Therefore, middle-aged CD38 KO mice did not gain body mass but rather exhibited a decrease in body mass, while middle-aged CD157 KO mice became obese.

3.1. Locomotion and anxiety-related behavior in a new environment in the open field test

Next, we measured locomotor activity and social behavior in an open field test (Supplementary Figure 1). Anxiety-related behavior was examined in the habituation stage of the open field test, in which mice were exposed to a new environment. The distance traveled (Fig. 2A) and average speed (Fig. 2B) were significantly lower in middle-aged male CD157 KO mice than in young adult male CD157 KO mice (*t*(14) = 3.637, *p* = 0.0027 for distance; *t*(14) = 3.683, *p* = 0.0025 for average speed). The immobilization time was also longer in middle-aged mice (Fig. 2C; *t*(14) = 5.006, *p* = 0.0002). Middle-aged CD157 KO mice remained near the wall of the open field and did not cross the central area. Thus, the average time in the inner zone was significantly lower in middle-aged mice (Fig. 2D; *t*(14) = 2.8370, *p* = 0.0132).
CD38 KO male mice displayed a similar pattern of locomotion with aging to CD157 KO mice, including less distance traveled in the arena (Fig. 2E, $t_{(12)} = 2.300, p = 0.0402$), a lower average speed (Fig. 2F, $t_{(12)} = 2.260, p = 0.0432$), and a longer immobility time (Fig. 3G, $t_{(12)} = 2.979, p = 0.0115$). Middle-aged and young adult CD38 KO mice remained in the center area for a similar amount of time, as evidenced by the lack of a significant difference between the two groups (Fig. 2H, $t_{(12)} = 0.7930, p = 0.4432$). The results showed that middle-aged CD157 KO mice displayed decreased locomotor activity and severe anxiety-related behavior, while middle-aged CD38 KO mice displayed reduced locomotion with less anxiety-related behavior.

### 3.2. Anxiety-related behavior in the open field test with a non-social object

When a non-social object (empty wire cage) was placed in the center area of the open field, the locomotor activity of middle-aged CD157 KO mice was similar to that observed in the habituation stage: distance traveled (Fig. 3A, $t_{(14)} = 2.611, p = 0.0205$), average speed (Fig. 3B, $t_{(14)} = 2.652, p = 0.0189$), and time spent immobile (Fig. 3C, $t_{(14)} = 3.027, p = 0.0091$). Additionally, middle-aged CD157 KO mice tended to spend less time in the center zone of the arena (Fig. 3D, $t_{(14)} = 1.609, p = 0.1299$).

In contrast, when a non-social object was placed in the center of the open field arena, middle-aged CD38 KO mice exhibited a locomotor activity level similar to that of young adult KO mice: distance traveled (Fig. 3E, $t_{(12)} = 0.6049, p = 0.5565$), average speed (Fig. 3F, $t_{(12)} = 0.5904, p = 0.5659$), time spent immobile (Fig. 4G, $t_{(12)} = 0.3589, p = 0.7259$), and time spent in the inner zone (Fig. 3H, $t_{(12)} = 0.4551, p = 0.6571$). Additionally, in the presence of the non-social object, age-dependent changes were observed in middle-aged CD157 KO mice, but such changes were not present in middle-aged CD38 KO mice.

### 3.3. Anxiety-related behavior in the open field test with a social object

Social behavior was assayed in the third stage of the open field test. The social object (an unfamiliar WT mouse of the same sex) was placed in a wire cage at the center of the arena (Fig. 4). Middle-aged CD157 KO mice displayed a significantly decreased distance traveled (Fig. 4A; $t_{(14)} = 2.827, p = 0.0135$), lower average speed (Fig. 4B; $t_{(14)} = 2.793, p = 0.0144$), and increased time spent immobile (Fig. 4C; $t_{(14)} = 3.088, p = 0.0080$). Moreover, middle-aged CD157 KO mice remained for a markedly shorter time in the inner zone of the arena but without significance (Fig. 4D; $t_{(14)} = 1.973, p = 0.0686$).

Surprisingly, middle-aged male CD38 KO mice displayed nearly the same level of activity as young adult KO mice in the social stage of the open field test. The parameters in Fig. 4E–H were indistinguishable between young and middle-aged mice: distance traveled (Fig. 4E; $t_{(12)} = 0.4042, p = 0.6931$), average speed (Fig. 4F; $t_{(12)} = 0.3755, p = 0.7138$), time immobile (Fig. 4: $t_{(12)} = 0.7427, p = 0.4720$), and time spent in the inner zone (Fig. 4H, $t_{(12)} = 0.3348, p = 0.7436$).
It was clear that in all stages in the open field test, anxiety-related behavior was more severe in middle-aged CD157 KO male mice than in young adult CD157 KO mice. However, although middle-aged male CD38 KO mice displayed anxiety-related behavior during the habituation stage, no age-associated changes were observed in middle-aged CD38 KO mice in the open field test with the non-social and social targets.

3.4. Sociability test in a three-chamber apparatus

A three-chamber test was performed to evaluate social behavior (Fig. 5). In the sociability stage, mice usually choose to stay with a social target rather than a non-social target [43]. In CD157 KO mice (Fig. 5A), two-way ANOVA showed a significant effect of age ($F_{1,22} = 10.19, p = 0.0042$), effect of object ($F_{1,22} = 10.38, p = 0.0039$) and age × object interaction ($F_{1,22} = 9.097, p = 0.0064$). Young adult male CD157 KO mice spent significantly more time with the mouse (Str1) than with the object (Bonferroni’s post hoc comparison $p < 0.001$). Middle-aged CD157 KO mice spent equal time with the social and non-social objects. Moreover, the time spent with Str1 by middle-aged CD157 KO mice was significantly shorter than that for young adult mice (Bonferroni’s post hoc test $p < 0.001$).

For CD38 KO mice (Fig. 5D), two-way ANOVA revealed a significant effect of age ($F_{1,14} = 13.65, p = 0.0024$), effect of object ($F_{1,14} = 42.77, p < 0.0001$), and age × object interaction ($F_{1,14} = 5.721, p = 0.0314$). CD38 KO mice of both ages spent more time with the social object than with the non-social object (Bonferroni’s post hoc $p < 0.0001$ for young adult mice, $p < 0.05$ for middle-aged mice). The interaction time with Str1 by middle-aged CD38 KO mice was shorter than that for young adult KO mice (Bonferroni’s post hoc $p < 0.01$). The results showed that in male CD38 KO mice, interest in both social and non-social targets seems to decrease in middle age, but sociability was not lost, even at middle age.

3.5. Social novelty preference in the three-chamber test

Mice usually show interest in new social targets, which is termed a social novelty preference. Both young adult and middle-aged CD157 KO mice spent nearly equal time with Str1 and Str2, although the time spent in the cage with a new target mouse (Str2) was slightly longer than that spent with the familiar mouse (Str1), without significance (Fig. 5B; two-way ANOVA revealed a significant effect of age ($F_{1,22} = 9.647, p = 0.0052$) but not an effect of object ($F_{1,22} = 0.5934, p = 0.4493$), or an object × age interaction ($F_{1,22} = 0.0007, p = 0.9793$). Regarding the total time spent in the social target zone, the time spent by middle-aged CD157 KO mice was significantly shorter than that of young adult mice (Fig. 5C; $t_{(11)} = 2.601, p = 0.0246$), indicating that social interaction significantly decreased with aging.

In CD38 KO mice, two-way ANOVA showed a significant effect of age ($F_{1,14} = 9.244, p = 0.0088$) and object ($F_{1,14} = 10.29, p = 0.0063$) but not an age × object interaction ($F_{1,14} = 0.1537 p = 0.7009$). Both age groups of CD38 KO mice spent the same time with Str1 and Str2. Nevertheless, less total time was spent in the social contact zone by middle-aged mice (Fig. 5F; $t_{(7)} = 3.182, p = 0.0154$).
The results of the three-chamber test indicate that middle-aged CD157 KO mice did not display sociability and social novelty preference; however, middle-aged CD38 KO mice remained sociable and showed a tendency of social novelty preference, similar to WT mice.

4. Discussion

In the present study, we demonstrated the effect of aging on mice lacking CD157 or CD38 using a social behavioral test battery, as summarized in Fig. 6. Because of differences in the genetic background (CD157 KO mice had a C57BL6 background and CD38 KO mice had an ICR background), it is difficult to perform a simple comparison between these two KO models, but changes induced by aging within the same strain can be observed.

Because no reports of middle-aged CD157 KO mice exist to our knowledge, it is not possible to compare our current results with other reports. However, aging effects in CD38 KO mice have been well studied [36,44], but these mice were produced on an ICR genetic background. Few reports are available regarding aging in the ICR strain [1,6,40]. In this respect, the current report is unique.

For animals, including laboratory mice, during healthy aging, an increase in body weight associated with increasing fat mass is relatively a natural phenomenon [45,46]. In our study, male middle-aged CD157 KO mice had higher body weight and BMI values than young adult mice. The weights of middle-aged (12 months old) C57BL6 male mice have previously been reported to be 34.4 ± 4.6 g [47] or 39.6 ± 4.7 g (The Jackson Laboratory https://www.jax.org/jax-mice-and-services/strain-data-sheet-pages/body-weight-chart-aged-C57BL6). Male middle-aged (1 year old) CD157 KO mice (on the C57BL6 genetic background) had a high body weight (43.6 ± 4.2 g). However, this value cannot be simply compared with reported weights of middle-aged WT C57BL6 mice because the environment and chow composition are not same. It is necessary to examine middle-aged CD157 KO mice and C57BL6 mice under the same conditions to investigate obesity in the future.

Body weight gain is an important issue. We performed a preliminary experiment to examine this issue by obtaining CD38 KO mice on a C57BL6 background from the Jackson Laboratory, designated as CD38BL6 KO, for two reasons. First, use of CD38BL6 KO mice on a C57BL6 background allows comparisons with CD157 KO mice. Additionally, in an independent experiment, mice were fed in a different location with similar normal chow. The mean body weights of 9-month-old mice were 34.6 ± 2.2 g in the C57BL6 group (n = 6), 38.0 ± 2.9 g in the CD157 KO group (n = 6), and 33.7 g in the CD38BL6 KO group (n = 6). C57BL6 and CD157 KO mice had similar body weights at the age of 8 weeks, but at the age of 9 months, the KO mice exhibited a greater body weight (Supplementary Figure 2; two-way ANOVA revealed a significant effect of age $F_{1,16} = 100.5$, $p < 0.0001$ but a marginal effect of genotype, $F_{1,16} = 4.043$, $p = 0.0615$ and genotype × age interaction, $F_{1,16} = 2.571$, $p = 0.1284$). CD157 KO mice tended to gain more body weight than initially expected.
Although male middle-aged CD38 KO mice had the same body weight as young adult KO mice, their BMI was significantly lower. According to recent studies, CD38 KO mice are resistant to a high-fat diet (Wang et al., 2018) or a high-fat, high-sucrose diet [48], both of which usually induce obesity in WT mice. It can be assumed that CD38 deficiency leads to increased NAD levels, which results in activation of NAD-dependent pathways that can suppress adipocyte differentiation and lipogenesis in adipose tissue [49].

The most important information obtained from the BMI is related to mouse adiposity, but the results are not always consistent. For example, in one study, the BMI was highly correlated with the amount of dissected adipose tissue [50], but in another study, such a correlation was not observed [51]. A lower BMI in middle-aged CD38 KO mice indicates lower adiposity; however, further examination of lipids in CD38 KO mouse tissues is required.

In ongoing experiments, CD157 KO mice are being fed with a high-fat, high-calorie diet rather than normal chow to determine whether CD157 KO mice show characteristics of obesity because such a diet can be used to examine obesity.

Middle-aged mice usually display less locomotor activity in tests than young mice [25,27–31]. However, middle-aged mice have been shown to have similar muscular strength to young mice [26,27,52]. Therefore, the decreased locomotor activity in KO mice (Supplementary Figure 1) may reflect an emotional abnormality rather than a physical weakness. This may be applicable to middle-aged CD157 KO mice, which showed decreased locomotor activity and increased time spent immobile, compared with young adult KO animals, in all stages of the open field test (Fig. 6).

Recent studies have shown that young CD157 KO mice display deficits in social behavior [11,14] (Fig. 6). In the three-chamber test, young adult CD157 KO mice displayed typical rodent behavior in the sociability stage (choice between social and nonsocial behavior), but at one year of age, CD157 KO mice spent equal time with the social and nonsocial objects. In the social preference stage, young CD157 KO mice could not discriminate between a familiar and a novel social object, as previously reported [11]. The same pattern was observed for middle-aged CD157 KO mice, but this age group spent markedly less time near the social object. These results indicate social deficits or avoidance behaviors in CD157 KO mice.

**Conclusion**

In the current study, we revealed dramatic changes in social behavior in middle-aged CD157 KO mice and only a slight change in CD38 KO mice. In this case, the obvious marked decline in social behavior in middle-aged CD157 KO mice may indicate that an initial social behavior abnormality progresses during maturation/or the aging process. This process seems to be accelerated by increased CD38 expression, as observed in other cells [53]. This concept is supported because little change with aging was observed in middle-aged CD38 KO mice, likely because of a lack of CD38.

Additionally, another study hypothesized that onset of a social behavior decline in middle-aged mice may precede cognitive deficits during aging [16]. However, further investigation is required because we did not
assess cognitive function in middle-aged mice in our study. Furthermore, a recent study showed that high adiposity in middle-aged mice may be accompanied by cognitive impairment [54]. In this regard, verification of the association of obesity status and cognitive function with a NAD-dependent metabolic disturbance could clarify this point in future, especially in middle-aged or older CD157 KO mice.

Declarations

Ethics approval and consent to participate

All animal experiments were carried out in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan and were approved by the Kanazawa University Committee on Animal Experimentation.

Consent for publication

Not applicable

Availability of data and materials

Please contact author for data request

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Competing interests

The authors declare that they have no competing interests

Authors’ contributions

M.G., O.L., Y.Y. and H.H. designed the experiments. M.G., O.L., A.A.S and S.M.C. performed the behavioral experiments. H.G., H.O., A.B.S., S.Y., K.I. and Y.Y. contributed to data interpretation and revise manuscript. H.H., Y.Y., O.L. and M.G. wrote the manuscript.

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References

1. Higashida H, Hashii M, Tanaka Y, Matsukawa S, Higuchi Y, Gabata R, et al. CD38, CD157, and RAGE as Molecular Determinants for Social Behavior. Cells [Internet]. 2019 [cited 2020 Jul 1];9:62. Available from: https://www.mdpi.com/2073-4409/9/1/62

2. Kim U-H. Multiple Enzymatic Activities of CD38 for Ca ²⁺ Signaling Messengers. Messenger [Internet]. 2014 [cited 2020 Jul 1];3:6–14. Available from: http://www.ingentaconnect.com/content/10.1166/msr.2014.1030

3. Lee HC. Cyclic ADP-ribose and Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP) as Messengers for Calcium Mobilization. Journal of Biological Chemistry [Internet]. 2012 [cited 2020 Jul 1];287:31633–40. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.R112.349464

4. Quarona V, Zaccarello G, Chillemi A, Brunetti E, Singh VK, Ferrero E, et al. CD38 and CD157: A long journey from activation markers to multifunctional molecules: CD38 and CD157. Cytometry Part B: Clinical Cytometry [Internet]. 2013 [cited 2020 Jul 1];84B:207–17. Available from: http://doi.wiley.com/10.1002/cyto.b.21092

5. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, et al. Evolution and Function of the ADP Ribosyl Cyclase/CD38 Gene Family in Physiology and Pathology. Physiological Reviews [Internet]. 2008 [cited 2020 Jul 1];88:841–86. Available from: https://www.physiology.org/doi/10.1152/physrev.00035.2007

6. Jin D, Liu H-X, Hirai H, Torashima T, Nagai T, Lopatina O, et al. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature [Internet]. 2007 [cited 2020 Jul 1];446:41–5. Available from: http://www.nature.com/articles/nature05526

7. Zhong J, Amina S, Liang M, Akther S, Yuhi T, Nishimura T, et al. Cyclic ADP-Ribose and Heat Regulate Oxytocin Release via CD38 and TRPM2 in the Hypothalamus during Social or Psychological Stress in Mice. Frontiers in Neuroscience [Internet]. 2016 [cited 2020 Jul 1];10. Available from: http://journal.frontiersin.org/Article/10.3389/fnins.2016.00304/abstract

8. Higashida H, Yokoyama S, Munesue T, Kikuchi M, Minabe Y, Lopatina O. Cd38 Gene Knockout Juvenile Mice: A Model of Oxytocin Signal Defects in Autism. Biological & Pharmaceutical Bulletin [Internet]. 2011 [cited 2020 Jul 1];34:1369–72. Available from: http://joi.jlc.jst.go.jp/JST.JSTAGE/bpb/34.1369?from=CrossRef

9. Liu H-X, Lopatina O, Higashida C, Tsuji T, Kato I, Takasawa S, et al. Locomotor activity, ultrasonic vocalization and oxytocin levels in infant CD38 knockout mice. Neuroscience Letters [Internet]. 2008 [cited 2020 Jul 1];448:67–70. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304394008013359

10. Lopatina O, Inzhutova A, Pichugina YA, Okamoto H, Salmina AB, Higashida H. Reproductive Experience Affects Parental Retrieval Behaviour Associated with Increased Plasma Oxytocin Levels in Wild-Type and Cd38-Knockout Mice: Reproductive experience on maternal behaviour in mice. Journal of Neuroendocrinology [Internet]. 2011 [cited 2020 Jul 1];23:1125–33. Available from: http://doi.wiley.com/10.1111/j.1365-2826.2011.02136.x
11. Gerasimenko M, Cherepanov SM, Furuhara K, Lopatina O, Salmina AB, Shabalova AA, et al. Nicotinamide riboside supplementation corrects deficits in oxytocin, sociability and anxiety of CD157 mutants in a mouse model of autism spectrum disorder. Scientific Reports [Internet]. 2020 [cited 2020 Jul 1];10. Available from: http://www.nature.com/articles/s41598-019-57236-7

12. Higashida H, Liang M, Yoshihara T, Akther S, Fakhrul A, Stanislav C, et al. An immunohistochemical, enzymatic, and behavioral study of CD157/BST-1 as a neuroregulator. BMC Neuroscience [Internet]. 2017 [cited 2020 Jul 1];18. Available from: http://bmcneurosci.biomedcentral.com/articles/10.1186/s12868-017-0350-7

13. Kasai S, Yoshihara T, Lopatina O, Ishihara K, Higashida H. Selegiline Ameliorates Depression-Like Behavior in Mice Lacking the CD157/BST1 Gene, a Risk Factor for Parkinson's Disease. Frontiers in Behavioral Neuroscience [Internet]. 2017 [cited 2020 Jul 1];11. Available from: http://journal.frontiersin.org/article/10.3389/fnbeh.2017.00075/full

14. Lopatina O, Yoshihara T, Nishimura T, Zhong J, Akther S, Fakhrul AAKM, et al. Anxiety- and depression-like behavior in mice lacking the CD157/BST1 gene, a risk factor for Parkinson's disease. Frontiers in Behavioral Neuroscience [Internet]. 2014 [cited 2020 Jul 1];8. Available from: http://journal.frontiersin.org/article/10.3389/fnbeh.2014.00133/abstract

15. Lopatina OL, Furuhara K, Ishihara K, Salmina AB, Higashida H. Communication Impairment in Ultrasonic Vocal Repertoire during the Suckling Period of Cd157 Knockout Mice: Transient Improvement by Oxytocin. Frontiers in Neuroscience [Internet]. 2017 [cited 2020 Jul 1];11. Available from: http://journal.frontiersin.org/article/10.3389/fnins.2017.00266/full

16. Boyer F, Jaouen F, Ibrahim EC, Gascon E. Deficits in Social Behavior Precede Cognitive Decline in Middle-Aged Mice. Frontiers in Behavioral Neuroscience [Internet]. 2019 [cited 2020 Jul 1];13. Available from: https://www.frontiersin.org/article/10.3389/fnbeh.2019.00055/full

17. Garaschuk O, Semchyshyn HM, Lushchak VI. Healthy brain aging: Interplay between reactive species, inflammation and energy supply. Ageing Research Reviews [Internet]. 2018 [cited 2020 Jul 1];43:26–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568163717302155

18. Harada CN, Natelson Love MC, Triebel KL. Normal Cognitive Aging. Clinics in Geriatric Medicine [Internet]. 2013 [cited 2020 Jul 1];29:737–52. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0749069013000591

19. McQuail JA, Frazier CJ, Bizon JL. Molecular aspects of age-related cognitive decline: the role of GABA signaling. Trends in Molecular Medicine [Internet]. 2015 [cited 2020 Jul 1];21:450–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1471491415000994

20. Oizumi H, Kuriyama N, Imamura S, Tabuchi M, Omiya Y, Mizoguchi K, et al. Influence of aging on the behavioral phenotypes of C57BL/6J mice after social defeat. Ryabinin AE, editor. PLOS ONE [Internet]. 2019 [cited 2020 Jul 1];14:e0222076. Available from: https://dx.plos.org/10.1371/journal.pone.0222076

21. Peters R. Ageing and the brain. Postgraduate Medical Journal [Internet]. 2006 [cited 2020 Jul 1];82:84–8. Available from: http://pmj.bmj.com/cgi/doi/10.1136/pgmj.2005.036665
22. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. Nature [Internet]. 2016 [cited 2020 Jul 1];539:180–6. Available from: http://www.nature.com/articles/nature20411

23. Dutta S, Sengupta P. Men and mice: Relating their ages. Life Sciences [Internet]. 2016 [cited 2020 Jul 1];152:244–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0024320515300527

24. Flurkey K, Joanne MJ, Harrison DE. The mouse in aging research. The Mouse in Biomedical Research. 2nd Edition. 2007. p. 637–72.

25. Fahlström A, Yu Q, Ulfhake B. Behavioral changes in aging female C57BL/6 mice. Neurobiology of Aging [Internet]. 2011 [cited 2020 Jul 1];32:1868–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0197458009003625

26. Sheth KA, Iyer CC, Wier CG, Crum AE, Bratasz A, Kolb SJ, et al. Muscle strength and size are associated with motor unit connectivity in aged mice. Neurobiology of Aging [Internet]. 2018 [cited 2020 Jul 1];67:128–36. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0197458018300940

27. Shoji H, Miyakawa T. Age-related behavioral changes from young to old age in male mice of a C57BL/6J strain maintained under a genetic stability program. Neuropsychopharmacology Reports [Internet]. 2019 [cited 2020 Jul 1];39:100–18. Available from: http://doi.wiley.com/10.1002/npr2.12052

28. Singhal G, Morgan J, Jawahar MC, Corrigan F, Jaehne EJ, Toben C, et al. Effects of aging on the motor, cognitive and affective behaviors, neuroimmune responses and hippocampal gene expression. Behavioural Brain Research [Internet]. 2020 [cited 2020 Jul 1];383:112501. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0166432819314354

29. Gorina YaV, Komleva YuK, Lopatina OL, Volkova VV, Chernykh Al, Shabalova AA, et al. The battery of tests for experimental behavioral phenotyping of aging animals. Advances in Gerontology [Internet]. 2017 [cited 2020 Jul 1];7:137–42. Available from: http://link.springer.com/10.1134/S2079057017020060

30. Lamberty Y, Gower AJ. Age-related changes in spontaneous behavior and learning in NMRI mice from middle to old age. Physiology & Behavior [Internet]. 1992 [cited 2020 Jul 1];51:81–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/003193849290206H

31. Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. Molecular Brain [Internet]. 2016 [cited 2020 Jul 1];9. Available from: http://www.molecularbrain.com/content/9/1/11

32. Weber M, Wu T, Hanson JE, Alam NM, Solanoy H, Ng H, et al. Cognitive Deficits, Changes in Synaptic Function, and Brain Pathology in a Mouse Model of Normal Aging. eneuro [Internet]. 2015 [cited 2020 Jul 1];2:ENEURO.0047-15.2015. Available from: http://eneuro.org/lookup/doi/10.1523/ENEURO.0047-15.2015

33. Bach ME, Barad M, Son H, Zhuo M, Lu Y-F, Shih R, et al. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. Proceedings of the National
34. Frick KM, Baxter MG, Markowska AL, Olton DS, Price DL. Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiology of Aging [Internet]. 1995 [cited 2020 Jul 1];16:149–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/0197458094001553

35. Magnusson KR, Scruggs B, Aniya J, Wright KC, Ontl T, Xing Y, et al. Age-related deficits in mice performing working memory tasks in a water maze. Behavioral Neuroscience [Internet]. 2003 [cited 2020 Jul 1];117:485–95. Available from: http://doi.apa.org/getdoi.cfm?doi=10.1037/0735-7044.117.3.485

36. Camacho-Pereira J, Tarragó MG, Chini CCS, Nin V, Escande C, Warner GM, et al. CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. Cell Metab. 2016;23:1127–39.

37. Chini C, Hogan KA, Warner GM, Tarragó MG, Peclat TR, Tchkonia T, et al. The NADase CD38 is induced by factors secreted from senescent cells providing a potential link between senescence and age-related cellular NAD+ decline. Biochemical and Biophysical Research Communications [Internet]. 2019 [cited 2020 Jul 1];513:486–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006291X19306060

38. Tarragó MG, Chini CCS, Kanamori KS, Warner GM, Caride A, de Oliveira GC, et al. A Potent and Specific CD38 Inhibitor Ameliorates Age-Related Metabolic Dysfunction by Reversing Tissue NAD+ Decline. Cell Metabolism [Internet]. 2018 [cited 2020 Jul 1];27:1081-1095.e10. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413118301943

39. Ishihara K, Hirano T. BST-1/CD157 regulates the humoral immune responses in vivo. Chem Immunol. 2000;75:235–55.

40. Kato I, Yamamoto Y, Fujimura M, Noguchi N, Takasawa S, Okamoto H. CD38 Disruption Impairs Glucose-induced Increases in Cyclic ADP-ribose, [Ca²⁺], and Insulin Secretion. Journal of Biological Chemistry [Internet]. 1999 [cited 2020 Jul 2];274:1869–72. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.274.4.1869

41. Mizuno A, Cherepanov S, Kikuchi Y, Fakhruil A, Akther S, Deguchi K, et al. Lipo-oxytocin-1, a Novel Oxytocin Analog Conjugated with Two Palmitoyl Groups, Has Long-Lasting Effects on Anxiety-Related Behavior and Social Avoidance in CD157 Knockout Mice. Brain Sciences [Internet]. 2015 [cited 2019 Sep 6];5:3–13. Available from: http://www.mdpi.com/2076-3425/5/1/3

42. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology [Internet]. 1985 [cited 2020 Jul 2];85:367–70. Available from: http://link.springer.com/10.1007/BF00428203

43. Lezak KR, Missig G, Carlezon WA. Behavioral methods to study anxiety in rodents. Dialogues Clin Neurosci. 2017;19:181–91.

44. Guerreiro S, Privat A-L, Bressac L, Toulorge D. CD38 in Neurodegeneration and Neuroinflammation. Cells [Internet]. 2020 [cited 2020 Jul 1];9:471. Available from: https://www.mdpi.com/2073-
45. Burke LK, Doslikova B, D'Agostino G, Garfield AS, Farooq G, Burdakov D, et al. 5-HT Obesity Medication Efficacy via POMC Activation is Maintained During Aging. Endocrinology [Internet]. 2014 [cited 2020 Jul 1];155:3732–8. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2014-1223

46. Pappas LE, Nagy TR. The translation of age-related body composition findings from rodents to humans. European Journal of Clinical Nutrition [Internet]. 2019 [cited 2020 Jul 1];73:172–8. Available from: http://www.nature.com/articles/s41430-018-0324-6

47. Lessard-Beaudoin M, Laroche M, Demers M-J, Grenier G, Graham RK. Characterization of age-associated changes in peripheral organ and brain region weights in C57BL/6 mice. Experimental Gerontology [Internet]. 2015 [cited 2020 Jul 1];63:27–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0531556515000078

48. Chiang S-H, Harrington WW, Luo G, Milliken NO, Ulrich JC, Chen J, et al. Genetic Ablation of CD38 Protects against Western Diet-Induced Exercise Intolerance and Metabolic Inflexibility. Kanzaki M, editor. PLOS ONE [Internet]. 2015 [cited 2020 Jul 1];10:e0134927. Available from: https://dx.plos.org/10.1371/journal.pone.0134927

49. Wang L-F, Miao L-J, Wang X-N, Huang C-C, Qian Y-S, Huang X, et al. CD38 deficiency suppresses adipogenesis and lipogenesis in adipose tissues through activating Sirt1/PPARγ signaling pathway. Journal of Cellular and Molecular Medicine [Internet]. 2018 [cited 2020 Jul 1];22:101–10. Available from: http://doi.wiley.com/10.1111/jcmm.13297

50. Sjögren K, Hellberg N, Bohlooly-Y M, Savendahl L, Johansson MS, Berglindh T, et al. Body Fat Content Can Be Predicted In Vivo in Mice Using a Modified Dual-Energy X-Ray Absorptiometry Technique. The Journal of Nutrition [Internet]. 2001 [cited 2020 Jul 1];131:2963–6. Available from: https://academic.oup.com/jn/article/131/11/2963/4686741

51. Gargiulo S, Gramanzini M, Megna R, Greco A, Albanese S, Manfredi C, et al. Evaluation of Growth Patterns and Body Composition in C57Bl/6J Mice Using Dual Energy X-Ray Absorptiometry. BioMed Research International [Internet]. 2014 [cited 2020 Jul 1];2014:1–11. Available from: http://www.hindawi.com/journals/bmri/2014/253067/

52. Hamrick MW, Ding K-H, Pennington C, Chao YJ, Wu Y-D, Howard B, et al. Age-related loss of muscle mass and bone strength in mice is associated with a decline in physical activity and serum leptin. Bone [Internet]. 2006 [cited 2020 Jul 1];39:845–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S8756328206004169

53. Hogan KA, Chini CCS, Chini EN. The Multi-faceted Ecto-enzyme CD38: Roles in Immunomodulation, Cancer, Aging, and Metabolic Diseases. Frontiers in Immunology [Internet]. 2019 [cited 2020 Jul 1];10. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.01187/full

54. Pétrault O, Pétrault M, Ouk T, Bordet R, Bérézowski V, Bastide M. Visceral adiposity links cerebrovascular dysfunction to cognitive impairment in middle-aged mice. Neurobiology of Disease
Figure 1

The body weight and body mass index (BMI) of young adult (8-week-old) and middle-aged (1-year-old) mice. (A) Body weight of CD157 KO mice. (B) BMI of CD157 KO mice. (C) Body weight of CD38 KO mice. (D) BMI of CD38 KO mice. Unpaired t-test, * p < 0.05, **** p < 0.0001
Figure 2

Locomotor activity in the open field test during the habituation stage. Values indicate the distance travelled in the arena (A), average speed (B), time spent immobile (C), and time spent in the inner zone (D) in young adult and middle-aged CD157 KO mice. Values indicate the distance travelled in the arena (E), average speed (F), time spent immobile (G), and time spent in the inner zone (H) in young adult and middle-aged CD38 KO mice. (E) Distance of CD38 KO mice traveled in the arena. (F) Average speed of CD38 KO mice. Unpaired t-test, *p < 0.05, **p < 0.01, ***p < 0.001.
Figure 3

Behavior in the open field test when a non-social object was placed in the center. Values indicate the distance travelled in the arena (A), average speed (B), time spent immobile (C), and time spent in the inner zone (D) in young adult and middle-aged CD157 KO mice. Values indicate the distance travelled in the arena (F), average speed (F), time spent immobile (G), and time spent in the inner zone (H) in young adult and middle-aged CD38 KO mice. Unpaired t-test, *p < 0.05, **p < 0.01.
Figure 4

Behavior in the open field test when a social target was placed in the center. Values indicate the distance travelled in the arena (A), average speed (B), time spent immobile (C), and time spent in the inner zone (D) in young adult and middle-aged CD157 KO mice. Values indicate the distance travelled in the arena (F), average speed (F), time spent immobile (G), and time spent in the inner zone (H) in young adult and middle-aged CD38 KO mice. Unpaired t-test, *p < 0.05, **p < 0.01.
Figure 5

Social behavior in a three-chamber test in young adult and middle-aged CD157 KO mice (A–C) and CD38 KO mice (D–F). (A, D) The sociability stage. Time spent with a social (Str1) or a non-social object (Object). (B, E) The social novel preference stage. Time mice spent with a familiar mouse (Str1) or a novel mouse (Str2). (C, F) Total social time during which mice stayed in the arena with mouse targets. Bonferroni's post hoc comparison or Unpaired t-test, *p < 0.05, **p < 0.01, ****p < 0.0001.
Figure 6

A scheme depicting changes observed in young adult and middle-aged CD157 KO and CD38 KO mice.

Supplementary Files

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