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Corresponding Author: Dr. Masayuki Nakamura, M.D., Ph.D.

Corresponding Author's Institution: Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences

First Author: Hitoshi Sakimoto, M.D.

Order of Authors: Hitoshi Sakimoto, M.D.; Masayuki Nakamura, M.D., Ph.D.; Omi Nagata, M.D.; Izumi Yokoyama; Akira Sano, M.D., Ph.D.

Department of Psychiatry, Kagoshima University
Graduate School of Medical and Dental Sciences
8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
Phone: +81-99-275-5346
FAX: +81-99-265-7089
e-mail: nakamu36@m.kufm.kagoshima-u.ac.jp

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27 January 2016

Dear Prof. Katsuhiko Mikoshiba

Please find enclosed our manuscript titled "**Phenotypic abnormalities in a chorea-acanthocytosis mouse model are modulated by strain background**", which we submit for consideration as a research article in *Biochemical And Biophysical Research Communications*.

Chorea-acanthocytosis (ChAc) is a rare hereditary neurodegenerative disorder characterized clinically by adult-onset hyper kinetic movement, chorea, various psychiatric symptoms, and erythrocyte acanthocytosis. We have previously identified *VPS13A* as the gene responsible for ChAc (Nat. Genet. 28, 121–122, 2001) and have developed a mouse ChAc model using gene-targeting techniques (J. Neurochem. 92, 759–766, 2005). The mutant mice, which have a hybrid C57BL/6J and a 129/Sv genetic background, displayed variable phenotypes, strongly suggesting the existence of modifier genes.

In this study, we backcrossed the model mice, and created four strains carrying the *Vps13a* mutation on C57BL/6, 129/S6, Balb/c, and FVB genetic backgrounds. We investigated the effects of the genetic background on the phenotypic variation of ChAc model mice using a number of behavioral analyses. ChAc-model mice backcrossed to the different inbred strains exhibited differences in symptoms. We suggest that this is a result of symptom-modifying factors of ChAc in the genetic background.

This paper has been reviewed by an experienced medical editor whose first language is

Department of Psychiatry, Kagoshima University
Graduate School of Medical and Dental Sciences
8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
Phone: +81-99-275-5346
FAX: +81-99-265-7089
e-mail: nakamu36@m.kufm.kagoshima-u.ac.jp

English and who specializes in editing papers written by physicians and scientists whose native language is not English.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to *Biochemical And Biophysical Research Communications*.

Please address all correspondence to: Masayuki Nakamura, M.D., Ph.D., Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan. Tel.: +81-99-275-5346; Fax: +81-99-265-7089; E-mail: nakamu36@m.kufm.kagoshima-u.ac.jp.

Thank you for your time and consideration. I look forward to receiving your response.

Yours sincerely,

Masayuki Nakamura, M.D., Ph.D.

Highlights

- ChAc model mice were backcrossed to four different strains.
- The phenotypes varied according to strains.
- Genetic background affects ChAc pathogenesis.

Phenotypic abnormalities in a chorea-acanthocytosis mouse model are modulated by strain background

Hitoshi Sakimoto, Masayuki Nakamura*, Omi Nagata, Izumi Yokoyama, Akira Sano

Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

*Corresponding author. Masayuki Nakamura, Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

Tel.: +81-99-2755346; Fax: +81-99-2657089

Abstract

Chorea-acanthocytosis (ChAc) is an autosomal recessive hereditary disease characterized by neurodegeneration in the striatum and acanthocytosis that is caused by mutations in the *VPS13A* gene. We previously produced a ChAc model mice encoding a human disease mutation with deletion of exons 60–61 in the *VPS13A* gene . The behavioral and pathological phenotypes of the model mice varied a good deal from individual to individual, indicating that differences between individuals may be caused by the content of a genetic hybrid 129/Sv and C57BL/6J strain background. To establish the effect of the genetic background on phenotype, we backcrossed the ChAc-model mice to different inbred strains: C57BL/6J and 129S6/Sv. Although no significant difference between ChAc-mutant mice and wild-type mice on the C57BL/6J background was observed, the ChAc-mutant mice on the 129S6/Sv showed abnormal motor function and behavior. Furthermore, we produced ChAc-mutant mice on two different inbred strains: BALB/c and FVB. Significant reduction in weight was observed in ChAc mutant mice on the FVB and 129S6 backgrounds. We found a marked increase in the osmotic fragility of red blood cells in the ChAc mutant mice

backcrossed to 129S6/Sv and FVB. The phenotypes varied according to strain, with ChAc mutant mice on the FVB and 129S6 backgrounds showing remarkably abnormal motor function and behavior. These results indicate that there are modifying genetic factors of ChAc symptoms.

Keywords

Chorea-acanthocytosis; ChAc-model mouse; Behavior experiments; Genetic modifiers

1. Introduction

Chorea-acanthocytosis (ChAc; MIM 200150) is a human, autosomal recessive neurodegenerative disorder. Clinically, ChAc is characterized by adult-onset chorea, acanthocytosis in erythrocytes, and Huntington's disease-like neuropsychiatric symptoms. The main neuropathological feature of ChAc is degeneration of the striatum. In addition, other neuropsychiatric deficits are observed, including oral dyskinesia and dystonia, frequently with self-mutilation, myopathy, and peripheral neuropathy. Cognitive impairment and personality and behavioral changes are also common. Psychiatric symptoms can include anxiety, paranoia, depression, obsessive behavior, and marked emotional instability [1]. ChAc is caused by loss-of-function mutations in the 250-kb *VPS13A* gene located on human chromosome 9q21. The gene encodes a 360-kDa protein named chorein [2,3]. Previously, we identified the sequence for mouse *Vps13a* cDNA and determined the exon–intron structure of the gene. We produced a ChAc model mouse of hybrid genetic background (129/Sv and C57BL/6J) by deleting exons 60–61 of *Vps13a*, which mimics a human disease mutation, and performed phenotypic analysis [4]. Behavioral and hematological abnormalities were observed in

ChAc-model mice after 70 weeks of age; mice exhibited locomotor dysfunction and reduced social interactions. However, not all ChAc-model mice presented with ChAc-like phenotypes, and the onset and symptoms of the disease were found to cover a wide range of ages [4]. Humans with ChAc also exhibit a range of ages of onset and symptoms, even in the same family lineage. As the mice tested were on a hybrid genetic background, it is probable that genetic modifiers affect the penetrance of the disease phenotypes. In the present study, to establish the effect of genetic modifiers on ChAc, we compared phenotypes of the ChAc-model mice with different inbred strains.

2. Materials and methods

2.1. Animals

ChAc-model mice (*Vps13a*^{tm1asan} mice), with a homozygous deletion of exons 60–61 in *Vps13a* and corresponding to a human disease mutation, were produced by gene targeting, as previously described [3,4]. This study was carried out in accordance with the guidelines for Animal Experimentation (MD14017) and Gene Recombination Experiment (24054) of Kagoshima University Graduate School of Medical and Dental

Sciences. In this study, we backcrossed heterozygous *Vps13a*^{tm1asan} mutation mice with each wild-type strain for more than 10 generations onto four inbred strains of mice, breeding with wild-type male and female mice each more than once. Four strains of mice with the mutation were produced: C57BL/6JJcl-*Vps13a*^{tm1asan} (C57BL/6-ChAc) (CLEA Japan Inc., Tokyo, Japan), 129S6/SvEv-*Vps13a*^{tm1asan} (129-ChAc) (Taconic Labs, Hudson, NY, USA), BALB/cbyJJcl-*Vps13a*^{tm1asan} (Balb/c-ChAc) (CLEA Japan Inc.), and FVB/NJcl-*Vps13a*^{tm1asan} (FVB-ChAc) (CLEA Japan Inc.) [5]. Male mice homozygous for the *Vps13a*^{tm1asan} mutation (Strain-ChAc) were compared with male mice homozygous wild-type littermates for each test. Mice were group housed with a normal light–dark cycle (lights on at 7:00 AM, lights off at 7:00 PM) in a clean facility and given free access to food and water. Mice were observed closely and body weight checked once a week. Osmotic fragility of red blood cells was tested in all strains. Behavioral analyses were performed at 10 and 40 weeks of age for 20 C57BL (10 wild-type mice, 10 ChAc$^{Del/Del}$ mice), 21 129S6 (10 wild-type mice, 11 ChAc$^{Del/Del}$ mice), 15 BALB (7 wild-type, 8 ChAc$^{Del/Del}$ mice), and 17 FVB (9 wild-type mice, 8 ChAc$^{Del/Del}$ mice), and at 70 weeks of age for 17 C57BL (8 wild-type mice, 9 ChAc$^{Del/Del}$ mice), 19

129S6 (9 wild-type mice, 10 $ChAc^{Del/Del}$ mice), 15 BALB (7 wild-type, 8 $ChAc^{Del/Del}$ mice), and 17 FVB (9 wild-type mice, 8 $ChAc^{Del/Del}$ mice). All mice received the behavioral analyses three times in total (10, 40, and 70 weeks of age). All four strains were assessed for prepulse inhibition (PPI), balance beam, rotarod, and wire hanging tests. No significant abnormal phenotype in these tests was observed in C57BL/6-ChAc and Balb/c-ChAc (data not shown). Additional analyses were performed for the FVB-ChAc and 129-ChAc lines, whose mutant mice showed both significantly lower weight and abnormal osmotic fragility of erythrocytes. Behavioral testing was performed during the light cycle on all strains according to the following schedule: Day 1, wire hanging; Day 3, open fields; Day 7, rotarod; Day 14, balance beam; Day 17, prepulse inhibition (PPI); and Day 21, Novel objects recognition test (NORT).

2.2. Light microscopy of red blood cells

Blood samples without EDTA (5 μL) were immediately mixed with 5 μL of isotonic sodium chloride solution containing 10 units of heparin per milliliter, and blood was mounted between a glass object slide and a glass cover slip [6].

2.3. Osmotic fragility test of red blood cells

We performed the Osmotic fragility test according to protocols described previously [4]. The hemolytic ratio was calculated by determining the absorbance at 540 nm and estimating the concentration of NaCl (%) to cause 50% hemolysis (C50) [7]. Results are expressed as C50 ± SD.

2.4. Rotarod

Mice were placed onto the rotating rod (Rotarod; O'Hara & Co., Ltd, Tokyo, Japan) that rotates at an accelerating speed from an initial speed of 3 rpm to the final speed of 30 rpm in 5 min. Mice received four trials per day for 3 consecutive days to attain a steady baseline level of performance [8]. Each animal was given a single test session consisting of two trials. Latency to fall from the top of the rotating barrel was recorded by the rotarod timer.

2.5. Wire hanging test

The test begins with the mouse hanging by its forelimbs in the middle of an elevated wire placed on top of a metallic container. Three-hundred seconds was the timeout period. The latency to fall was recorded [9].

2.6. Balance beam

Mice walked along a 28-mm-wide beam elevated 50 cm above the bench by metal supports to reach an enclosed goal box. Following training, mice were placed on the beam at one end and allowed to traverse the beam to reach the goal box. Foot slips were scored when one or both hind limbs slipped from the beam.

2.7. Open field analysis

Animals were moved to the new cage for 1 h before the test to accustom them to the novel environment and then placed in a box (50 × 50 cm). Traces were drawn for 10 min by an image-analyzing program (Image OF4; O'Hara & Co., Ltd) and the total moving distance (cm) and total moving duration (s) were calculated [4].

2.8. Novel objects recognition test (NORT)

This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The choice to explore the novel object reflects the use of learning and recognition memory [11]. We performed the experiment according to protocols described previously [12]. The time spent investigating the novel object was recorded.

2.9. Prepulse inhibition (PPI)

Acoustic startle responses for PPI experiments were measured in a startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA). We performed the experiment according to protocols described previously with some modifications [13]. We set the background noise to 70 dB and tried pre-pulses at 12 dB. In addition, each previous or the last five trials were single 40-ms, 120-dB, pulse-alone startle stimuli [14].

2.10. GFAP immunohistochemistry

The animals were anesthetized with ether to minimize pain and the brains were removed. The brains were fixed in 4% PFA, then dehydrated by 30% sucrose for cryosection into 40 μm thick slices. Immunohistochemical staining was performed according to the manufacturer's protocol with the VECTASTAIN ABC (Avidin Biotinylated enzyme Complex) kit (Vector Laboratories, Burlingame, CA, USA). The coronal sections were incubated with the primary antiserum for the glial fibrillary acidic protein (GFAP) (1/500 rabbit polyclonal; Abcam, Cambridge, MA, USA) and diluted with phosphate-buffered saline containing 0.1% Triton X-100, overnight at 4 °C [15]. Positive cells were counted at a magnification of ×200. Five visual fields of the stratum and hippocampus (CA1 region) were randomly selected from each section. Positive cells in five random visual fields were counted in each sample and the number of GFAP-expressing cells was determined in each subgroup [16] (FVB: $n = 3$, ChAc-model mice and $n = 3$, wild-type mice; 129S6: $n = 3$, ChAc-model mice and $n = 3$, wild-type mice).

2.11. Statistical analysis

All statistical analyses were performed using the Welch's t-test for comparisons between wild-type mice and ChAc-model mice. All data are shown as mean ± standard error (SE) for normally distributed continuous variables. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Body weight

Mouse weight was measured weekly over 70 weeks. FVB-ChAc$^{Del/Del}$ ($t_{(15)} = 3.060$, $P < 0.01$) and 129-ChAc$^{Del/Del}$ ($t_{(15.21)} = 2.469$, $P < 0.05$) mice showed significantly reduced body weight when compared with wild-type controls at 70 weeks of age (Fig. 1A).

3.2. Hematological analysis

3.2.1. Osmotic fragility analysis of red blood cells

The red blood cells from ChAc-model mice showed an increase in their *in vitro* osmotic fragility when exposed to hypotonic NaCl solutions. The NaCl concentration

that produced 50% hemolysis (C50) was significantly lower (Fig. 1B) in 129-ChAc$^{Del/Del}$ ($t_{(15)} = 3.060$, $P < 0.01$) and FVB-ChAc$^{Del/Del}$ ($t_{(13.12)} = 7.378$, $P < 0.01$) mice when compared with the controls.

3.2.2. Light microscopy of red blood cells

Light microscopic observation of peripheral blood smears of 129-ChAc and FVB-ChAc mice showed heterogeneity in the sizes of erythrocytes, including definite acanthocytes, and these cells also showed abnormal shapes (Fig. 1C). The percentage of acanthocytosis in both mutant mice increased as the mice aged (10% at 20 weeks, > 80% at 60 weeks; data not shown).

3.3. Neuropsychological symptoms

3.3.1. Open field test

In the open field test, the FVB-ChAc$^{Del/Del}$ mice exhibited a significant increase in total distance ($t_{(11.44)} = 2.273$, $P < 0.05$, Fig. 2A) and total moving duration ($t_{(16.98)} = 2.444$, $P < 0.05$, Fig. 2B) when compared with wild-type mice of the same strain at 70

weeks of age. There was no significant difference at 40 weeks, but a similar trend was observed ($p = 0.07$). Furthermore, the patterns of motion observed for FVB-ChAc$^{Del/Del}$ mice were quite different from those observed for the wild-type control mice. Wild-type mice were generally observed to stay close to the walls, whereas the mutant mice tended to move around in a more complex manner (Fig. 2C). No significant difference was observed between mutant and wild-type mice in total distance traveled for the other strains.

3.3.2. Prepulse inhibition (PPI)

In the PPI test, 129-ChAc$^{Del/Del}$ mice displayed decreased PPI when compared with wild-type littermates at 70 weeks of age ($t_{(12.93)} = 2.608, P < 0.05$, Fig. 2D). This observation was not seen in other strains-ChAc^{Del} mice. None of the strains tested showed a significant difference in PPI between wild-type and mutant mice at 40 weeks of age (data not shown).

3.4. Cognitive function

3.4.1. Novel object recognition test (NORT)

During the sample phase, the total time spent in exploration did not differ significantly between groups (data not shown). After a 24-h interval between the sample and the test phase, 129-ChAc^{Del} mice spent a significantly shorter amount of time exploring the novel object ($t_{(8.67)} = 2.380$, $P < 0.05$, Fig. 2E).

3.5. Motor function

3.5.1. Rotarod test

Although the mean latency to fall off the rotarod was significantly shorter in 129-ChAc$^{Del/Del}$ mice (220.68 ± 82.73) compared with wild-type controls (268.90 ± 50.18) ($t_{(35.13)} = 2.251$, $P < 0.05$, Fig. 3A) at 40 weeks of age, there was no significant difference observed at 70 weeks of age. No significant difference was observed between wild-type and mutant mice of the other strains.

3.5.2. Wire hanging test

The average hanging times are shown in Fig. 3B. The wire hanging time was

significantly shorter for FVB-ChAc^{Del} mice when compared with wild-type controls ($t_{(9.99)} = 2.845, P < 0.05$) , but there was no significant difference between wild-type and mutant 129-ChAc mice in other strain. FVB-ChAc mice were tested at 70 weeks; however, results were inconclusive because of the hyperactivity of the mice.

3.5.3. Balanced beam

FVB-ChAc^{Del} ($t_{(7.88)} = 2.497, P < 0.05$) and 129-ChAc^{Del} mice ($t_{(10.51)} = 2.213, P <$ 0.05) exhibited a greater number of hind limb foot slips than the wild-type controls (Fig. 3C) at 40 weeks of age. Mice from these strains could not be tested at 70 weeks of age because of their locomotor disorder.

3.6. Immunohistochemical analysis of GFAP

Significantly more GFAP-positive cells were observed in the striatum of FVB-ChAc^{Del} mice when compared with the results of wild-type mice ($t_{(4.526)} = 2.624, P$ < 0.05, Fig. 4A,C). Conversely, in 129-ChAc^{Del} mice, significantly more GFAP-positive cells were observed in the hippocampus ($t_{(4.108)} = 3.024, P < 0.05$, Fig. 4B,C).

4. Discussion

In our previous study, we created a mouse model of ChAc by deleting exons 60–61 of the *VPS13A* gene. Phenotypic analysis of these mice demonstrated a range of phenotypes commonly associated with human ChAc; however, considerable variation in each phenotype was observed [4]. We hypothesize that the phenotypic variations observed in the ChAc-model mice are owing to the hybrid genetic background (129/Sv and C57BL/6J) in which the mutation was created. In this study, we investigated the effects of the genetic background for the phenotypic variations of ChAc-model mice by backcrossing the ChAc-model mice with four inbred strains: C57BL/6, 129/S6, Balb/c, and FVB.

4.1. Phenotypic analyses

The phenotypic abnormalities were observed in FVB-ChAc and 129S6-ChAc mice at 40 weeks of age. It is conceivable that mutant FVB-ChAc and 129-ChAc mice were affected by poor motor coordination. Despite this apparent decrease in motor

coordination, mutant FVB-ChAc mice traveled a greater total distance than wild-type mice in the open field test at 70 weeks of age. Moreover, mutant FVB-ChAc mice did not go around the edge of the open field, but moved at random, as shown in Fig. 2C. These results suggest that mice carrying the ChAc mutation on an FVB genetic background develop symptoms of hyperactivity [17]. This could possibly be attributed to neuropsychological hyperactivity. Furthermore, although there was no statistically significant hyperactivity observed in mutant 129-ChAc mice, there was a trend towards this phenotype. Various explanations have been suggested for the cause of hyperactivity, including a reduction in gamma-aminobutylic acid (GABA) [18] [19]. Upregulation of GABRG2 in ChAc model mice has been reported by our group as a decrease in GABA [20]. The decrease in GABA has also been noted in the mouse model of Huntington disease (HD) (YAC128 mice). HD mice also showed a trend towards hyperactivity, but it is understood to be attributed to chorea-like movement and not neuropsychiatric hyperactivity [21]. Interestingly, mutant 129-ChAc mice exhibited definite neuropsychological deficiencies, including a poor performance in the PPI test and reduced contact time with novel objects in NORT. Thus, the ChAc mutation on the

129S6 genetic background appears to lead to cognitive dysfunction, because NORT is a method to measure the recognition function of memory. General neuropathology in human ChAc is accompanied by a strong reactive gliosis and microglial activation, even more distinctive than that found in Huntington's disease patients [22]. Similarly, an immunohistochemical analysis showed that significantly more GFAP-positive cells were observed in the striatum of mutant FVB-ChAc mice and in the hippocampus of mutant 129-ChAc mice when compared with wild-type mice. Hyperactivity has been reported to be related to the neurotransmitter dopamine and the striatum [23]. The hippocampus is involved in the regulation of PPI [24] and there are close relationships between cognitive dysfunction and PPI [25]. GFAP, the major intermediate filament protein in astrocytes, is an excellent marker for fibrous astrocytes [26]. The difference in the phenotypes between ChAc mutants on the 129S6 and FVB backgrounds appears to be related to the difference of such neurodegenerative sites.

4.2. Modifier genes for ChAc

'Monogenic' diseases are known to be affected by factors other than a primary mutation.

Different mechanisms might explain the variability of monogenic disease expression, such as genetic heterogeneity, additional variant(s) in the disease gene in cis or trans position to the primary mutation(s), modifier genes, epigenetic changes (DNA methylation, histone modification), and environmental conditioning [27]. In this report, we consider that there are modifiers of ChAc symptoms in the genetic backgrounds of the various mouse strains we have tested. Jeremy *et al*. performed a similar study with a Huntington's disease model mouse, which shows ChAc-like neuropsychiatric symptoms, backcrossed to three inbred strains (FVB/N, 129, C57BL/6) [21]. Some phenotypes were penetrant on only the FVB/N and C57BL/6 strains, whereas others were only penetrant on the FVB/N and 129 strains. The study suggested that different genes might influence the presentation of different phenotypes [21]. Our study reaches similar conclusions with respect to the effect of the primary ChAc mutation. The variability in symptoms between mice carrying the ChAc mutation on different inbred backgrounds is likely to be a result of a genetic modifier(s) in the inbred background. In a previous study, the mutant mice, which have a hybrid C57BL/6J and 129/Sv genetic background, displayed inter-individual differences in the phenotypes, strongly

suggesting the existence of modifier genes [28]. This study showed that C57BL-ChAc showed no significant differences in the behavioral phenotypes when compared with C57BL-wild-type mice. However, 129S6-ChAc mice exhibited variable behavioral phenotypes. These results suggest that the 129S6 genetic background modified behavioral phenotypes of the hybrid C57BL/6J and 129/Sv ChAc mice. Although the magnitude of group differences and the variance with groups suggest relatively small effect sizes, which would necessitate large numbers of mice to uncover any putative genetic modifiers, identification of ChAc genetic modifiers will provide further insight into the molecular pathogenesis of this disease.

Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Fig. 1. Peripheral blood smear and osmotic fragility analysis and body weight of ChAc model mice. (A) Body weight in each strain background. Statistically significant low weight at 70 weeks of age was found in FVB (** $p < 0.01$) and 129S6 (* $p < 0.05$). (B) Osmotic fragility analysis of red blood cells was performed at 70 weeks of age for all four strains. The concentration of NaCl (%) to cause 50% hemolysis (C50) was estimated. Results (mean ± SE) are expressed as C50 ± SE. A significant difference between wild-type mice and FVB-ChAc (** $p < 0.01$) and 129S6-ChAc (* $p < 0.05$) model mice. (**C**) Many acanthocytes were observed in wet peripheral blood smears of 129-ChAc^{Del} and FVB-ChAc^{Del} mice. The ratio of acanthocytes increased as mice aged.

Fig. 2. Psychological symptoms in ChAc-model mice. (A) The total distance and (B) total moving durations in open field analysis. (C) Figures track the movement of FVB mice at 70 weeks of age. (D) PPI performed at 40 weeks and 70 weeks for all four strains. A statistically significant percentage of PPI was found in 129S6-ChAc (* $p < 0.05$). (E) Novel objects recognition test, NORT. 129-ChAc^{Del} mice spent a significantly shorter amount of time exploring the novel object (* $p < 0.05$). Data represent means ± SE.

Fig. 3. Motor function in ChAc-model mice. (A) Rotarod analysis at 40 weeks and 70

weeks (129S6 and FVB). The 129-ChAc^{Del} mice exhibited poorer performance on the rotarod than wild-type mice at 40 weeks of age ($* p < 0.05$). FVB-ChAc mice were not able to perform this test at 70 weeks because of motor dysfunction. (B) Wire hanging test. Wild-type mice on FVB lasted significantly longer when compared with FVB-ChAc^{Del} mice ($* p < 0.05$) at 40 weeks of age. (C) Balance beam. There were significant performance deficits in the FVB-ChAc^{Del} ($* p < 0.05$) and 129S6-ChAc^{Del} ($* p < 0.05$) mice at 40 weeks. Data of FVB-ChAc and 129-ChAc mice at 70 weeks are not shown. Mice were not able to perform this test at 70 weeks because of motor dysfunction. BALB/c-ChAc and C57-ChAc mice showed no significant difference in using the balance beam between wild-type and mutant mice at 40 and 70 weeks (data not shown). Data represent means ± SE.

Fig. 4. An immunohistochemical analysis of striatum and hippocampus using an anti-GFAP antibody. In FVB-ChAc and 129S6-ChAc mice, significantly more GFAP-positive cells were observed in the striatum of FVB-ChAc^{Del} ($* p < 0.05$) (A) and the hippocampus of 129S6-ChAc^{Del} ($* p < 0.05$) (B). (C) GFAP protein expression in the striatum of FVB-ChAc and the hippocampus of 129S6-ChAc at 70 weeks of age (DAB staining; positive cells, brown). Left: FVB group (striatum; magnification = ×200); Right: 129S6 group (hippocampus; magnification = ×200). Scale bars, 50 μm. Data represent means ± SE. GFAP: glial fibrillary acidic protein.

Figure

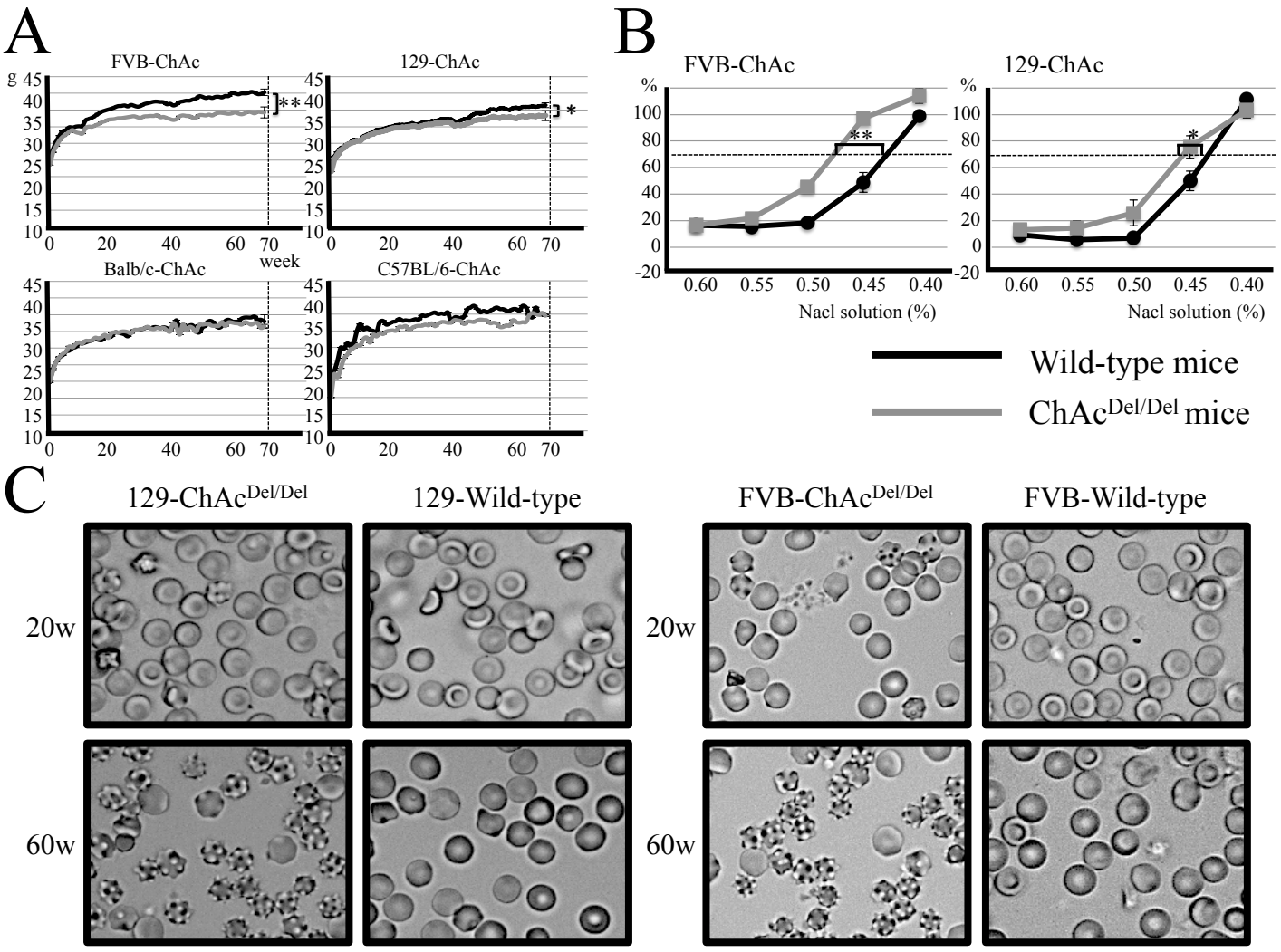

Figure. 1

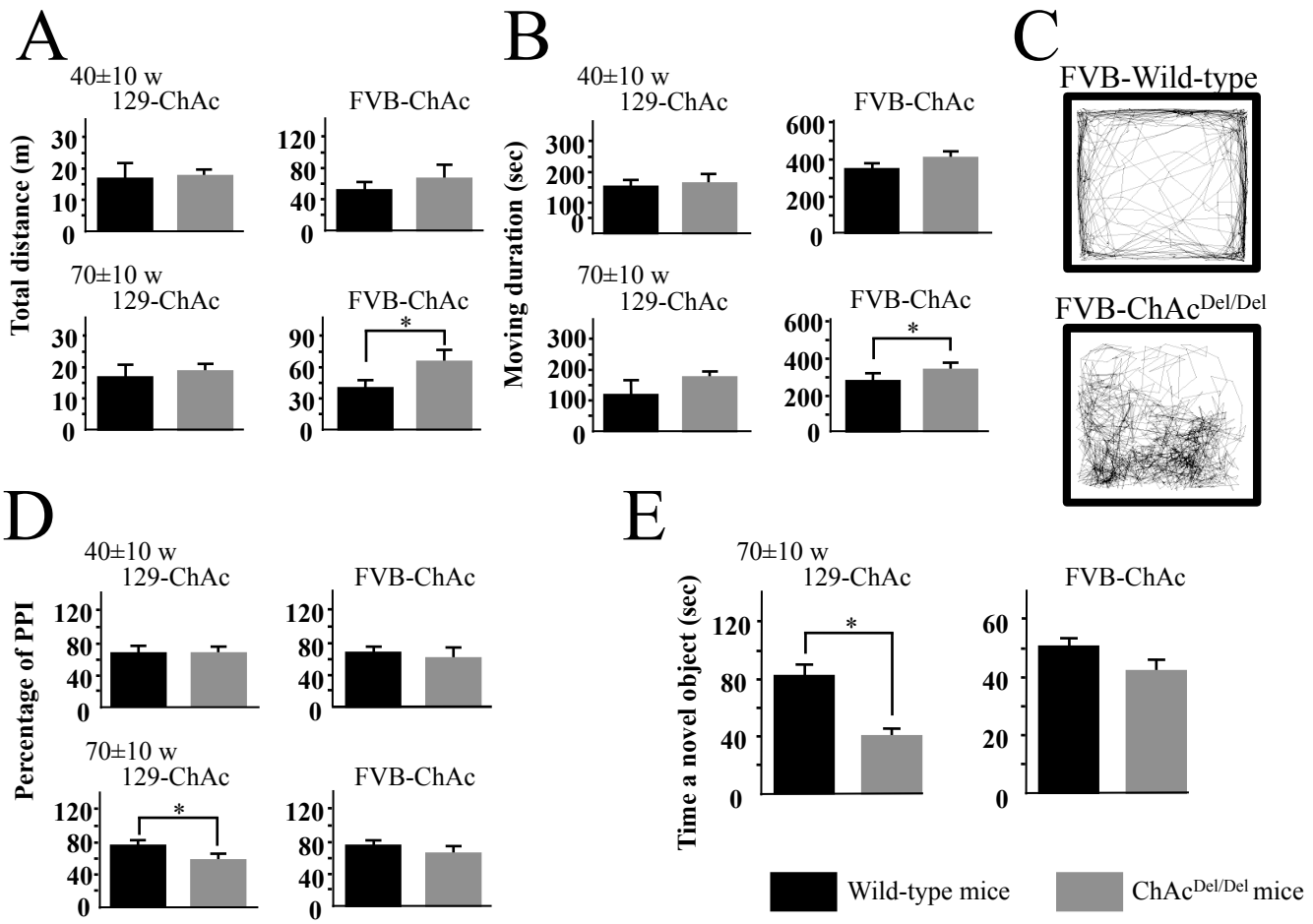
A
40±10 w
129-ChAc
FVB-ChAc
Total distance (m)
70±10 w
B
Moving duration (sec)
C
FVB-Wild-type
FVB-ChAcDel/Del
D
Percentage of PPI
E
Time a novel object (sec)
Wild-type mice
ChAcDel/Del mice

Figure. 2

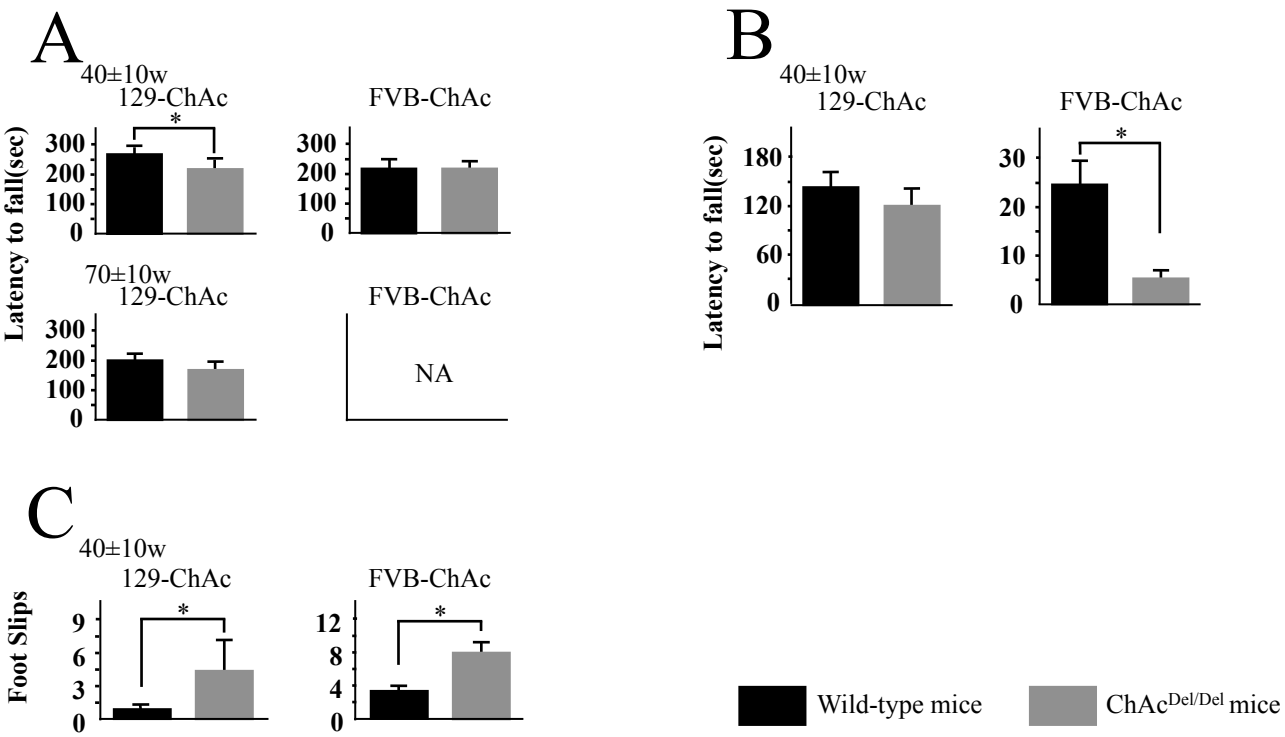
A
40±10w
129-ChAc
FVB-ChAc
Latency to fall(sec)
300
200
100
0
*
70±10w
NA
B
180
120
60
30
20
10
C
Foot Slips
9
6
3
12
8
4
Wild-type mice
ChAcDel/Del mice

Figure. 3

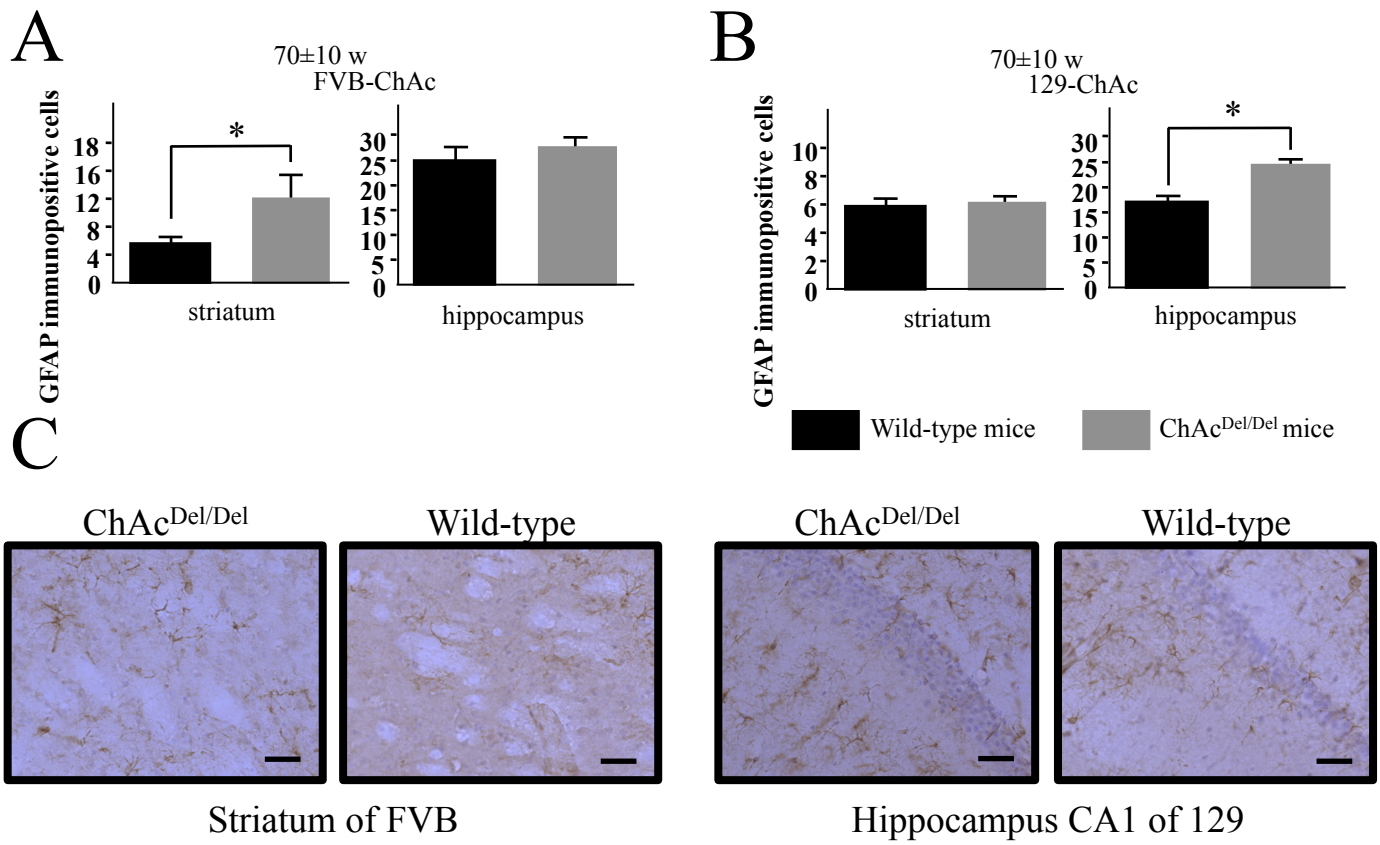
A
70±10 w
FVB-ChAc
GFAP immunopositive cells
striatum
hippocampus
B
70±10 w
129-ChAc
GFAP immunopositive cells
striatum
hippocampus
Wild-type mice
ChAcDel/Del mice
C
ChAcDel/Del
Wild-type
ChAcDel/Del
Wild-type
Striatum of FVB
Hippocampus CA1 of 129

Figure. 4

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