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Myostatin Is Associated With Cognitive Decline in an Animal Model of Alzheimer's Disease

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Abstract

With aging, there are progressive functional declines in multiple organ systems. One of the major physiological problems observed in aged people is skeletal muscle loss. This age-related muscle loss causes muscle weakness and disability, which in turn might reduce the quality of life in older adults and lead to the progression of several diseases, particularly Alzheimer's disease (AD). Some researchers have hypothesized that loss of muscle mass and strength is linked to the risk of developing AD. In addition, unintended weight loss often occurs in AD patients and might reflect dementia severity. However, the causal relationship between muscle atrophy and cognitive deficits in AD is unclear. We found that double transgenic amyloid precursor protein and presenilin 1 (APP/PS1) mice that co-express APP and PS1 at older ages exhibited lower body weight and lean tissue mass than sex- and age-matched wild-type (WT) mice. In addition, muscle atrophy and the extent of memory decline were strongly correlated in APP/PS1 mice. Myostatin levels in the gastrocnemius (GAS) muscle of 12-month-old APP/PS1 mice were elevated. We determined that the cellular and molecular mechanism of muscle atrophy was through the ubiquitin-proteasome pathway. Furthermore, myostatin knockdown in the GAS muscles increased grip strength and muscle mass, leading to memory improvement in myostatin short-hairpin RNA-treated APP/PS1 mice. We conclude that high-level myostatin expression might mediate or trigger muscle atrophy and cognitive deficits.

Keywords Alzheimer's disease · Memory impairment · APP/PS1 transgenic mice · Myostatin

Introduction

Alzheimer's disease (AD), a common form of dementia affecting the elderly, was discovered more than 100 years ago. Since then, researchers have found that AD starts with memory problems and eventually progresses to involve multiple cognitive deficits and neuropsychological alternations [1]. The disease brings a host of issues because the progressive cognitive decline from AD adversely affects the quality of life of patients with AD and their caregivers. Additionally, in economic evaluations and treatment opinions, AD is an important public health problem.

Among the many risk factors of AD, advancing age is the largest one. Loss of muscle mass and strength is common and related to various adverse health outcomes in the elderly [2], a condition known as sarcopenia. This gradual muscle loss leads to the progression of several chronic diseases, such as metabolic syndrome, cancer, and AD [3]. In the wasting condition, the rapid decline of muscle mass and strength contributes primarily to excessive protein breakdown and often accompanied by reduced protein synthesis, which leads to muscle weakness, reduced quality of life, and increased disability and mortality [4]. In addition, failing muscle power appears associated with the risk of developing AD [2]. Unintended weight loss is a prognostic factor for dementia severity and often occurs in patients with AD [5]. In a related study, researchers assessed body composition, scanned whole brain tissue with magnetic resonance imaging (MRI), and neuropsychological tested 70 patients ≥ 60 years old without dementia

Yung-Shuen Lin and Fang-Yu Lin contributed equally to this work.

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and 70 age-matched patients with early symptoms of AD. Their findings suggest that the loss of lean tissue mass hastens the clinical progression of AD, and it causes brain atrophy and cognitive impairment [6]. Additionally, previous studies suggest that muscle may act as a "sentinel tissue" affecting other tissues [7, 8]. Taking these findings together, it seems important to better understand the effects of muscle wasting on AD, because it leads to loss of muscle mass, reduced autonomy, a greater risk of falling, decubitus ulcers, and systemic infection, all of which increase the burden of the disease and worsen the quality of life for AD patients and for their caregivers [7]. However, no prior study has reported on the physiological regulation and the role of muscle atrophy in AD-related cognitive deficits, in part because the causal relationship between muscle loss and AD development remains unexplained.

Growing evidence indicates that skeletal muscle acts like an endocrine organ [9]. Muscle-secreted growth factors and cytokines, known as myokines, regulate systemic physiology through autocrine, paracrine, and endocrine pathways [10]. This might affect disease progression caused by intertissue communication during systemic damage [11]. Among myokines, myostatin is a member of the TGF-β family and a well-studied negative regulator of skeletal muscle mass [12]. We wanted to determine whether muscle atrophy affects AD-associated cognitive decline in APP/PS1 mice; thus, we investigated the function of myostatin in AD progression. Here, we provide the first evidence that the extent of muscle mass loss is associated with the severity of cognitive deficits in APP/PS1 mice, but *myostatin* (*Mstn*) shRNA treatment in the gastrocnemius (GAS) muscles of APP/PS1 mice inhibited muscle degradation via the ubiquitin-proteasome pathway and attenuated their memory impairment.

Materials and Methods

Animals

The APP/PS1 mutant mice used in this study were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and express both chimeric mouse and human amyloid precursor protein (Mo/HuAPP695swe) and mutant human presenilin 1 (PS1dE9). Male mice containing APP/PS1 double transgenes and wild-type (WT) mice (non-transgenic littermates of APP/PS1 mice) were used and housed in 27 × 16 × 12-cm^3 plastic cages with free access to food and water. They were maintained under controlled conditions at a 12-h light/dark cycle, temperature of 23 ± 1 °C, and humidity of 50 ± 10%. All animal experimental procedures and animal handling adhered to the guidelines established by the ethics committee for the use of experimental animals at the National Cheng Kung University, College of Medicine.

Measuring Grip Strength

The grip strength of forelimbs and hindlimbs was measured using a grip strength meter (GS3; BIOSEB, Chaville, France). Mice were placed on a 100 × 80-mm metal mesh and allowed to grasp the wire gauze by the forelimbs or hindlimbs. The mouse's tail was then pulled backward horizontally until the grip released the gauze, at which point the maximal force was recorded as grip strength. Each mouse underwent five such trials. Grip strength was normalized based on body weight.

Body Composition

Mouse body composition was measured using a micro-CT scanner (SkyScan 1076; Bruker AXS, Karlsruhe, Germany). The settings of scan parameters were 1-mm thickness of aluminum filter, 50-kV voltage, 35 μm of pixel size, and 0.6° rotation step. The micro-CT data were translated into two-dimensional (2D) cross-sectional gray-scale images using NRecon (Bruker AXS). From these 2D images, the volume of interest was calculated using CT-An (Bruker AXS).

Contextual Fear Conditioning Test

Mice were trained in a specially designed chamber 30 × 24 × 21-cm chamber (Med Associates, Fairfax, VT, USA) with a house light, a speaker mounted on the wall through which tones were played, and a shock-grid floor that was connected to a shocking apparatus (Shock-Grid Scrambler, Med Associates). The chamber was cleaned with 75% ethanol before each new session. On the training day, each mouse was carried to the training chamber for 2 min. A 20-s pure 3-kHz tone (white noise) was then played for each mouse. The last 3 s of the white noise was paired with a 0.7-mA foot shock. This training procedure was repeated four times with a 40-s intertrial interval (ITI). After another 2 min, the mouse was returned to its home cage. Twenty-four hours after they had been trained, the mice were tested for 3 min for contextual fear without being subsequently exposed to either the tone or foot shock to assess their contextual freezing. Mouse behavior was recorded using video camera and freezing data were analyzed using FreezeScan software (CleverSys, Reston, VA, USA). Freezing levels were measured as a percentage of total time spent motionless.

Object-Place Recognition Task

The experimental apparatus consisted of a 30 × 30 × 30-cm white rectangular open field. Mice were habituated to the experimental apparatus for 10 min/day for 3 consecutive days before the training. On the training day, the mice were placed in the same box, which then contained two new identical objects, and were allowed to freely explore the environment

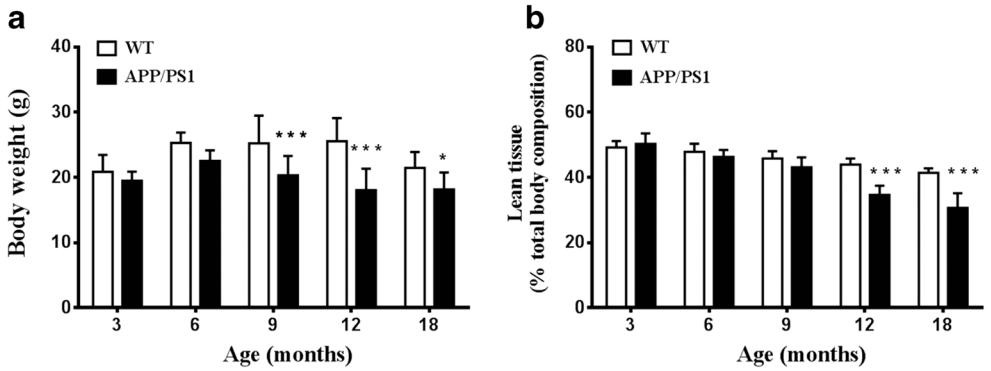

Fig. 1 APP/PS1 mice lose weight at 9, 12, and 18 months old and have less lean tissue mass at 12 and 18 months old. **a** The body weights of APP/PS1 and WT mice was measured (WT groups: $n = 12$ in each group; APP/PS1 groups: $n = 8$ at 3 and 6 months; $n = 12$ at 9, 12, and 18 months). **b** The body composition of 3-, 6-, 9-, 12-, and 18-month-old APP/PS1 and WT mice was measured using whole-body micro-CT ($n = 6$ in each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. age-matched WT mice

and the objects for 10 min. After 24 h, the mice were placed back in the same environment for 5 min to test retention memory. The same two objects were there, but one was in a new location. The objects and the experimental apparatus were cleaned with 75% ethanol between trials to remove olfactory cues. Mouse behavior was recorded using a video camera mounted above the apparatus, and the time that the mice spent exploring the two objects was measured. The discrimination index (DI) was calculated as the difference between the time spent exploring each object divided by the time used to explore both objects:

$$\mathrm{DI} = (t_{\mathrm{novel}} - t_{\mathrm{familiar}})/(t_{\mathrm{novel}} + t_{\mathrm{familiar}}) \times 100.$$

Western Blotting

Frozen muscle was dissected from the GAS muscle of mice and then homogenized in lysis buffer (1% Triton X-100, 0.1% SDS, 50 mM Tris-HCl, pH 7.5, 0.3 M sucrose, 5 mM EDTA, 2 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl fluoride) plus protease and phosphatase inhibitors, using the manufacturer's instructions. Primary antibodies acted against myostatin (Abcam, Cambridge, UK) and actin (Millipore, Burlington, MA, USA). The antibodies were reacted overnight at 4 °C, then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. Immunoreactive proteins were detected using a detection reagent (ECL Plus; PerkinElmer, Boston, MA, USA). Actin was an internal control. Protein levels were first normalized to internal control levels for each sample, after which each signal was calculated as a percentage of the corresponding control.

Measuring Messenger RNA

Muscle tissue samples were dissected from the mice. Total RNA was extracted using a total RNA spin kit (Bioman, Taipei, Taiwan). We used a reverse transcription (RT) system (ImProm-II; Promega, Madison, WI, USA) to synthesize cDNA, and a real-time quantitative RT polymerase chain reaction (qRT-PCR) to calculate messenger RNA (mRNA) levels using the StepOnePlus™ Real-Time PCR System (Life Technologies, Carlsbad, CA). The myostatin (*Mstn*)

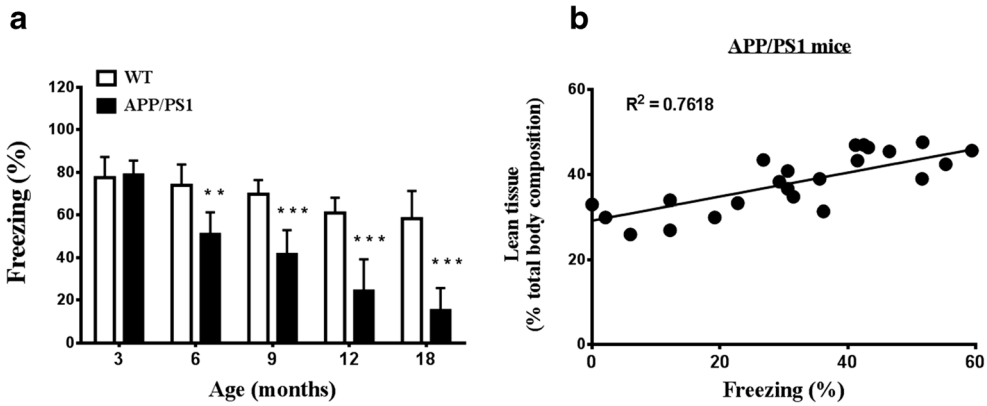

Fig. 2 Lean tissue mass is linked to freezing levels. **a** Age-dependent impairment of contextual fear memory in APP/PS1 mice ($n = 6$ in each group). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. age-matched WT controls. **b** Plotting the percentage of lean tissue vs. freezing responses showed a linear relationship in APP/PS1 mice ($R^2 = 0.7618$; $n = 30$)

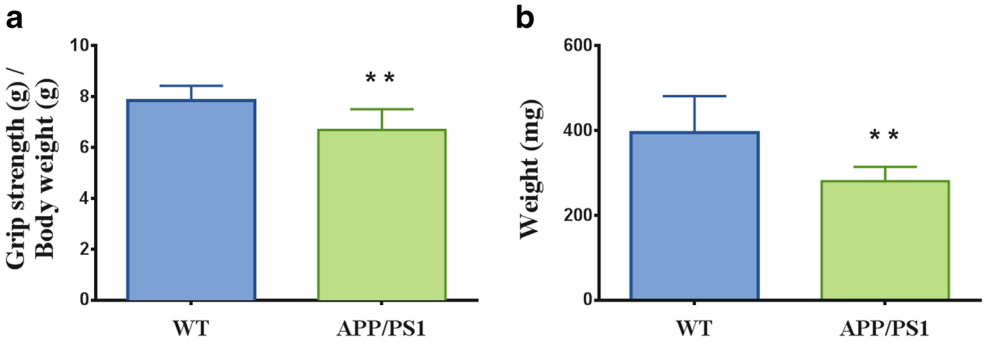

Fig. 3 Grip strength and mass of GAS muscles are lower in 12-month-old APP/PS1 mice. **a** Grip strength data were the maximum force of five pulls per mouse normalized to body weight (weighed in pairs; $n = 9$ in each group). $^{**}p < 0.01$ vs. age-matched WT controls. **b** GAS muscle mass was lower in APP/PS1 mice ($n = 9$ in each group). $^{**}p < 0.01$ vs. age-matched WT controls

probe (Mm01254559_m1), MuRF1 (*Trim63*) probe (Mm01185221_m1), atrogin1/MAFbx1 (*Fbxo32*) probe (Mm00499523_m1), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) probe (Mm99999915_g1) were purchased from Life Technologies. The RT reaction profile was as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 1 min at 60 °C. The levels of mRNA were first normalized to endogenous control *Gapdh* mRNA for each sample, and then measured as fold changes based on control values.

In Vivo Delivery of Myostatin Plasmid

The *Mstn* shRNA (TRCN0000030558) and scrambled shRNA conjugated on the pLKO.1 vector within the puromycin-resistant region were provided by the National RNAi Core Facility, Academia Sinica, Taiwan. *Mstn* shRNA was mixed with in vivo jetPEI (Polyplus Transfection, Illkirch, France) according to the manufacturer's instructions. We then intramuscularly injected 50 μl of the jetPEI/shRNA solution into each APP/PS1 mouse GAS muscle. *Mstn* gene knockdown was confirmed using Western blotting.

Statistical Analysis

Prism 6 (GraphPad Software, La Jolla, CA, USA) was used for all statistical analyses. All values are expressed as mean ± standard error of the mean (SEM). Linear correlations between two variables of interest were determined using Pearson's correlation coefficient. Significance was set at $p < 0.05$.

Results

Older APP/PS1 Mice Weigh Less and Have Less Lean Tissue

Previous studies show that weight loss is a warning sign for AD progression [2]. Unintended weight loss is a prognostic factor for dementia severity and often occurs in patients with AD [5]. Thus, we measured the body weight of male APP/PS1 mice at 3, 6, 9, 12, and 18 months old. A two-way ANOVA revealed the main effects to be of genotype (WT vs. APP/PS1 mice, $F_{(1,102)} = 53.03$, $p < 0.0001$), age

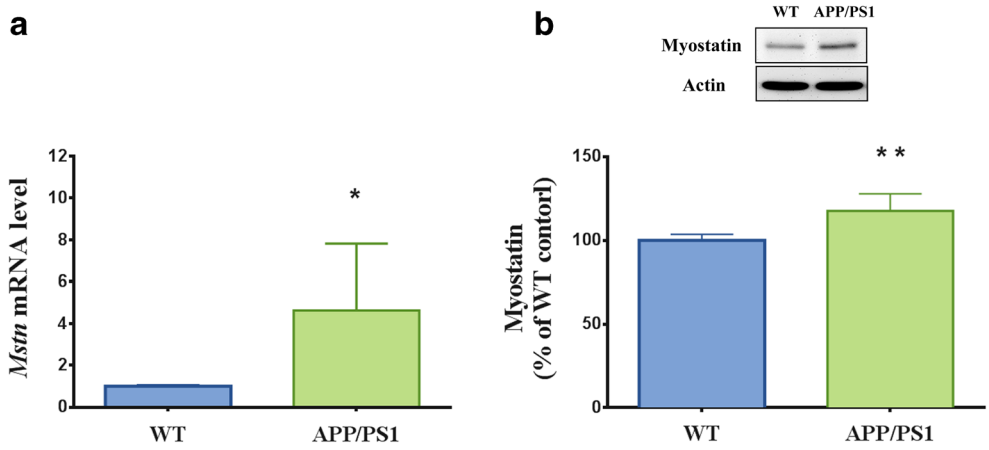

Fig. 4 mRNA and protein expressions of myostatin are elevated in 12-month-old APP/PS1 mice. **a** *Mstn* mRNA expression levels of the GAS muscles were detected by qRT-PCR ($n = 6$ in each group). **b** The protein expression levels of myostatin in the GAS muscles were measured by Western blotting ($n = 6$ in each group). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. WT mice of the same age

($F_{(4,102)} = 7.859$, $p < 0.0001$), and a significant genotype-by-age interaction ($F_{(4,102)} = 3.696$, $p = 0.0075$) (Fig. 1a). We found that 9- to 18-month-old APP/PS1 mice weighed significantly less than their age- and sex-matched WT mice.

To determine whether the loss of lean mass was associated with faster clinical progression of AD and causing brain atrophy and cognitive impairment, we used whole-body micro-computed tomography (micro-CT) and two-way ANOVA to calculate the percentage of lean tissue in APP/PS1 mice at 3, 6, 9, 12, and 18 months old. A two-way ANOVA revealed the main effects of genotype (WT vs. APP/PS1 mice, $F_{(1,50)} = 44.41$, $p < 0.0001$), age ($F_{(4,50)} = 52.65$, $p < 0.0001$), and a significant genotype-by-age interaction ($F_{(4,50)} = 10.69$, $p < 0.0001$) (Fig. 1b). Post hoc analysis showed that the percentage of lean tissue was significantly lower in 12- and 18-month-old APP/PS1 mice than in their age- and sex-matched WT mice.

Lean Mass Loss and Memory Impairment Severity in APP/PS1 Mice

Cognitive deficit is a defining feature of AD [13]. It can be detected before the appearance of significant neuropathological changes [14]. Thus, we used contextual fear conditioning and two-way ANOVA to analyze the cognitive function of APP/PS1 mice. A two-way ANOVA revealed the main effects of genotype (WT vs. APP/PS1 mice, $F_{(1,50)} = 94.49$, $p < 0.0001$), age ($F_{(4,50)} = 30.67$, $p < 0.0001$), and a significant stimulus type-by-age interaction ($F_{(4,50)} = 8.337$, $p < 0.0001$) (Fig. 2a). Post hoc analysis showed that the 6- to 18-month-old APP/PS1 mice had significantly lower freezing responses than did age-matched WT mice. We also confirmed an association between lean tissue mass and contextual fear responses. The data showed a linear relationship between the lean tissue percentages and the freezing responses of APP/PS1 mice ($R^2 = 0.7618$, $p < 0.0001$; Fig. 2b). The results suggest that lean tissue content is associated with levels of contextual fear memory in APP/PS1 mice.

Muscle Power Is Lower in 12-Month-Old APP/PS1 Mice

The lean tissue percentages of 12-month-old APP/PS1 mice were lower than those of WT controls (Fig. 1b). Thus, we used the grip strength meter to calculate forelimb and hindlimb muscle strength. Grip strength was lower in 12-month-old APP/PS1 mice than in age- and sex-matched WT mice ($t_{(16)} = 3.453$, $p = 0.0033$) (Fig. 3a). Evidence shows that the fast twitch muscle, such as gastrocnemius (GAS), is the major region damaged by the muscle atrophy process [15]. The data showed that the GAS muscle wet weight of 12-month-old APP/PS1 mice was significantly lower than that of age-matched WT mice (GAS: $t_{(16)} = 3.7$, $p = 0.0019$) (Fig. 3b). Our analysis of another fast-twitch muscle, the tibialis anterior (TA), showed that 12-month-old APP/PS1 mice had significantly less TA muscle mass than did age-matched WT mice

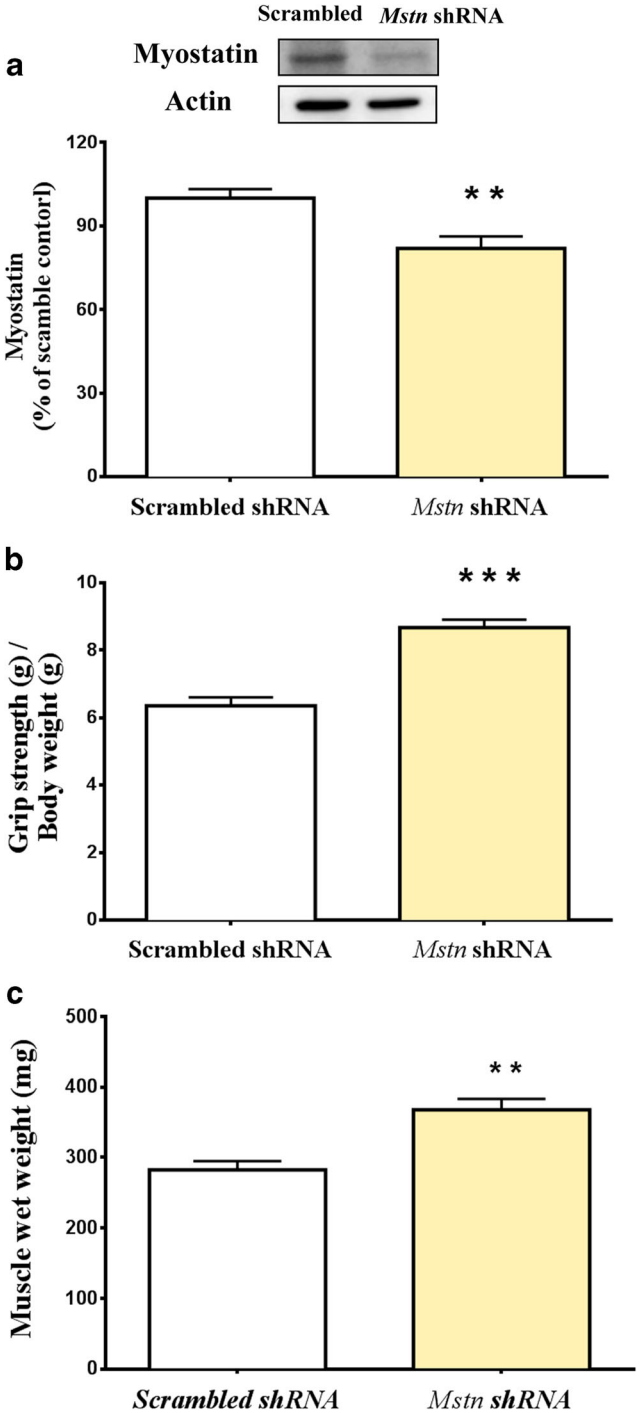

Fig. 5 Protein levels, grip strength, and muscle mass are calculated after myostatin knockdown in 12-month-old APP/PS1 mice. **a** Mice were injected with *Mstn* shRNA or scrambled control shRNA into the GAS muscles of 11-month-old APP/PS1 mice. After 1 month of injection, myostatin protein levels were measured using Western blotting analysis ($n = 6$ in each group). **b** Grip strength was measured in scrambled control shRNA- and *Mstn* shRNA-treated APP/PS1 mice ($n = 6$ in each group). **c** Weight (mg) of GAS muscles from both treatment groups ($n = 6$ in each group). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. scrambled shRNA-treated controls

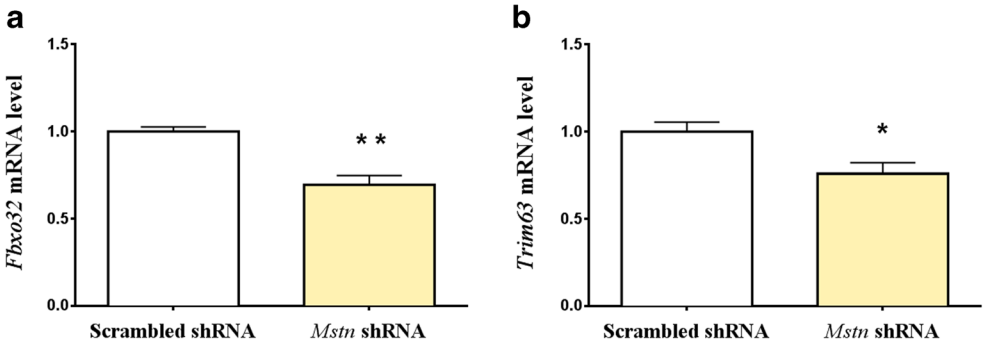

Fig. 6 *Fbxo32* and *Trim63* mRNA expression levels of GAS muscle are reduced in *Mstn* shRNA-treated APP/PS1 mice. We collected mouse GAS muscles to measure mRNA levels of *Fbxo32* **(a)** and *Trim63* **(b)** using qRT-PCR analysis ($n = 4$ in each group). *$p < 0.05$, **$p < 0.01$ vs. age-matched WT controls

($t_{(16)} = 4.549$, $p = 0.0003$) (Supplementary Fig. 1a); however, the triceps muscle wet weight was not affected ($t_{(16)} = 1812$, $p = 0.0888$) (Supplementary Fig. 1b). These data suggest that muscle strength and the muscle mass of GAS and TA were reduced in 12-month-old APP/PS1 mice compared with WT controls.

Myostatin Expression Is Higher in APP/PS1 Mice

Growing evidence has revealed that increased myostatin expression occurs in the pathogenesis of muscle atrophy [16]. Therefore, we used qRT-PCR and Western blotting to detect mRNA and protein expression of myostatin in 12-month-old APP/PS1 and WT mice. *Mstn* mRNA levels ($t_{(10)} = 2.788$, $p = 0.0192$) (Fig. 4a) and protein levels in the GAS muscles ($t_{(10)} = 3.965$, $p = 0.0027$) (Fig. 4b) were both higher in 12-month-old APP/PS1 mice than in age-matched WT mice.

To validate the effects of myostatin on APP/PS1 mice, we injected lentiviral-based *Mstn* shRNA into the GAS muscles of 11-month-old APP/PS1 mice. After 1 month of injections, GAS myostatin protein levels were lower in *Mstn* shRNA-treated mice than in scrambled shRNA-treated controls ($t_{(5)} = 4.353$, $p = 0.0073$) (Fig. 5a). The grip strength meter showed that forelimb and hindlimb muscle strength was significantly greater in *Mstn* shRNA-treated APP/PS1 mice than in scrambled shRNA-treated mice ($t_{(10)} = 6.703$, $p < 0.0001$) (Fig. 5b). Furthermore, we also calculated muscle wet weight of GAS muscle and found greater muscle wet weight in *Mstn* shRNA-treated APP/PS1 mice than that of scrambled-sequence control mice ($t_{(10)} = 4.304$, $p = 0.0016$; Fig. 5c).

Additionally, muscle atrophy is defined as a decrease in the strength and mass of muscle, which evidence suggests is due to cellular shrinkage caused by the loss of organelles, cytoplasm, and proteins [16]. In the cellular and molecular mechanisms of muscle atrophy, the ubiquitin-proteasome system (UPS) is believed to play a major role in mediating muscle atrophy through muscle protein shortfall [17]. Furthermore, the genes that encode two muscle atrophy-specific ubiquitin ligases, atrogin-1/MAFbx (Fbxo32), and MuRF1 (Trim63) have been discovered [18]. Myostatin-induced muscle atrophy may occur by activating the proteolytic systems in mature muscle fibers [19, 20]. Thus, we detected the mRNA expression of *Fbxo32* and *Trim63* in *Mstn* shRNA-treated APP/PS1

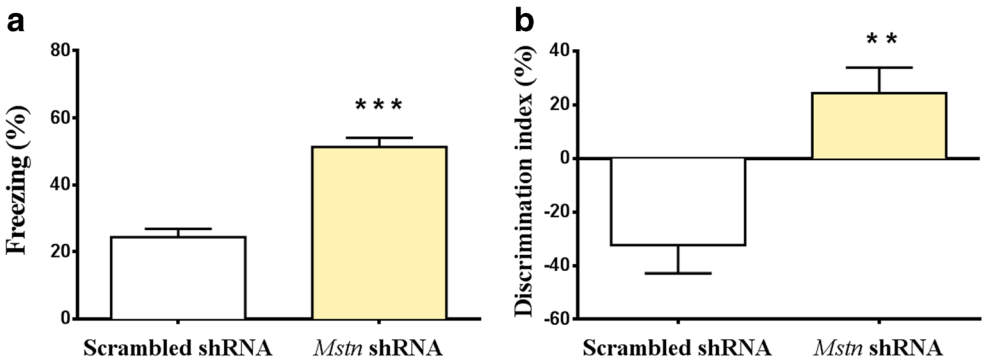

Fig. 7 Effects of myostatin knockdown in APP/PS1 mice on contextual fear and object location tests. **a** Cognitive function was assessed in scrambled shRNA- and *Mstn* shRNA-treated APP/PS1 mice using the contextual fear test ($n = 8$ in each group). **b** A spatial recognition memory test was done using an object-place recognition task. The discrimination index (DI) was measured as the time spent exploring each object divided by the time exploring both objects: $DI = (t_{novel} - t_{familiar}) / (t_{novel} + t_{familiar}) \times 100$ ($n = 8$ in each group). **$p < 0.01$, ***$p < 0.001$ vs. scrambled shRNA-treated controls

mice. qRT-PCR showed that the mRNA expression of *Fbxo32* and *Trim63* was significantly lower in the GAS muscles of *Mstn* shRNA-treated APP/PS1 mice than in scrambled shRNA-treated mice (Fbxo32: $t_{(3)} = 6.352$, $p = 0.0079$ (Fig. 6a); Trim63: $t_{(3)} = 3.760$, $p = 0.0329$ (Fig. 6b)).

AD-Related Memory Impairment Is Improved in Mice With Lower Myostatin Expression

To investigate whether myostatin affects the memory of mice, we injected *Mstn* shRNA into the GAS muscles of 11-month-old APP/PS1 mice. After 1 month, fear conditioning showed that *Mstn* shRNA-treated APP/PS1 mice had higher freezing responses than did scrambled shRNA-treated APP/PS1 mice ($t_{(14)} = 7.399$, $p < 0.0001$; Fig. 7a). In addition, we used the object location test to confirm that cognitive deficits were mitigated in myostatin knockdown in APP/PS1 mice ($t_{(14)} = 3.997$, $p = 0.0013$; Fig. 7b). This finding suggests that decreased myostatin expressions would retard cognitive deficits in APP/PS mice.

Discussion

We found initial evidence that the extent of muscle mass loss is associated with the severity of cognitive deficits in APP/PS1 mice. We also hypothesize that myostatin is a key mediator for regulating AD-triggered muscle atrophy and cognitive decline. AD is both a neurodegenerative disease and an aging-associated disease. Physical disabilities and cognitive declines are two major problems affecting the elderly. In addition, muscle atrophy is associated with a wide range of catabolic diseases. This debilitating loss of muscle mass reduces functional capacity and increases morbidity and mortality, which leads to a poor quality of life. Skeletal muscle is the largest organ of the body, and muscle-derived myokines, like myostatin, are now considered communicators between muscle and other organs. Myostatin is known to negatively regulate muscle mass and muscle mass loss is associated with cognitive impairment, but no prior study has reported the physiological regulation and the role of muscle wasting in AD-related cognitive deficits. Perhaps this is true because the causal relationship between muscle loss and the development of AD is unclear.

Weight loss is a common symptom of AD [11]. More than half of AD patients lose weight despite consuming more calories than do healthy controls [9]. We found that older APP/PS1 mice lost body weights and lean tissue mass: they weighed less than did their age-matched littermates and age- and sex-matched 9-, 12-, and 18-month-old WT mice. Moreover, at 12 months old, APP/PS1 mice began to lose lean tissue, the extent of which was strongly correlated with the severity of their cognitive deficits. In addition, more myostatin was expressed in the GAS and TA muscles of 12-month-old APP/PS1 mice. IGF1 is a modulator of greater muscle mass and strength in rodents and humans [21]. Thus, we further detected *Igf1* mRNA levels in the GAS muscles of 12-month-old APP/PS mice. The results show that *Igf1* mRNA levels were lower in GAS and TA muscles of 12-month-old APP/PS1 mice (data not shown). Furthermore, we found that the muscle atrophy mechanism was through the ubiquitin-proteasome pathway. To determine myostatin's role in regulating memory impairment, we injected *Mstn* shRNA into the GAS muscles of 11-month-old APP/PS1 mice. We found that memory was better in mice that had GAS muscles with lower myostatin levels, which suggests that the loss of muscle mass and strength is a risk factor that triggers a memory decline in APP/PS1 mice. However, how myostatin affects brain function requires clarification.

Accumulating evidence suggests that exercise is a useful way to build muscle mass and strength. Physical exercise mitigates AD and other age-associated neurodegenerative disorders [22, 23] and slows the onset of muscle atrophy in older animals [24, 25]. High myostatin expression is linked to obesity and insulin resistance [26, 27], whereas aerobic exercise can improve insulin resistance, which was mediated by decreased myostatin [26]. It is, therefore, possible that targeting myostatin expression will be an efficacious therapeutic intervention for muscle atrophy and AD-related memory problems.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflicts of interest.

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