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Phenotypic Characterization of MPS IIIA (*Sgshmps*^{3a}/*Sgshmps*^{3a}) Mouse Model

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Mucopolysaccharidosis IIIA is a heritable neurodegenerative disorder resulting from the dysfunction of the lysosomal hydrolase sulphamidase. This leads to the primary accumulation of the complex carbohydrate heparan sulphate in a wide range of tissues and CNS degeneration. Characterization of animal model is the beginning point of the therapeutic clinical trial. Mouse model has a limitation in that it is not a human and does not have all of the disease phenotypes. Therefore, delineate of the phenotypic characteristics of MPS IIIA mouse model prerequisite for the enzyme replace treatment for the diseases. We designed 6-month duration of phenotypic characterization of MPS IIIA mouse biochemically, behaviorally and histologically. We compared height and weight of MPS IIIA mouse with wild type from 4 weeks to 6 months in both male and female. At 6 months, we measured GAG storage in urine kidney, heart, liver, lung and spleen. The brain GAG storage is presented with Alcian blue staining, immunohistochemistry, and electron-microscopy. The neurologic phenotype is evaluated by brain MRI and behavioral study including open field test, fear conditioning, T-maze test and Y-maze test. Especially behavioral tests were done serially at 4month and 6month. This study will show the result of the MPS IIIA mouse model phenotypic characterization. The MPS IIIA mouse provides an excellent model for evaluating pathogenic mechanisms of disease and for testing treatment strategies, including enzyme or cell replacement and gene therapy.

Keywords: MPS IIIA, Mouse model, Phenotypic characterization

Introduction

The mucopolysaccharidoses (MPS) are a family of heritable disorders caused by deficiencies of lysosomal enzymes required for the degradation of glycosaminoglycans (GAG). Mucopolysaccharidosis type III (MPS IIIA, Sanfilippo syndrome) results from the absence of one of four lysosomal enzymes that are required for the sequential degradation of the GAG heparan sulfate; sulfamidase (MPS IIIA), α-N-acetylglucosaminidase (MPS IIIB), acetyl CoA:α-glucosaminide-N-acetyl trans-ferase (MPS IIIC), or N-acetylglucosamine 6-sulfatase (MPS IIID)[1]. Estimates of incidence range of MPS IIIA or Sanfilippo syndrome is from 1:24,000 in The Netherland[2] to 1:66,000 in Australia[3] to approximately 1:324,000 in British Columbia[4]. MPS IIIA is the most common subtype in Northern Europe, whereas MPS IIIB is more prevalent in Italy and Greece[5,6]. MPS IIIA is the most common of the MPS and is characterized by severe degenerative CNS disease, resulting in progressive mental retardation. After a period of normal development, patients exhibit a range of symptoms, including rapid loss of social skills with hyperactivity and aggressive behavior, loss of learning ability, disturbed sleep patterns, hirsutism, coarse faces and diarrhea. Death occurs in severely affected children in the mid- to late-teenage years usually as a result of respiratory infection[7]. A mouse model of MPS IIIA exists, arising from a spontaneous missense mutation in the sulfamidase gene that dramatically reduces sulfamidase activity to 3% of wild-type (WT)[8,9].

MPS IIIA mice have been reported to closely reproduce the human disease, presenting with hepato-splenomegalia, neurodegeneration, neuroinflammation, and shortened lifespan[9-11] Likely due to the residual enzymatic activity MPS IIIA mice have a milder phenotype than other MPS III mouse models[12]. Mouse

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characterization is important to confirm the mouse is suitable as a non-clinical animal used for the purpose of developing and validation of therapeutic agent.

The aim of this study is to know the suitable biomarker and behavioral assessment according to mouse growth and characterize MPS IIIA mouse biochemically, histologically and behaviorally.

Materials and Methods

We designed 6-month duration of phenotypic characterization of MPS IIIA mouse biochemically, behaviorally and histologically in both male and female mice (Fig. 1).

1. Animals

Both male and female mice were used from 4-week-old to 24-week-old in this study. B6.Cg-Sgsh^{mps3a}/PstJ (Stock Number:003780) were purchased from the Jackson Laboratory. Briefly, the MPS IIIA mice contain a novel sulfamidase mutation, D31N that has not previously been observed among human patients. This is a point mutation (G to A) at nucleotide position 91 with a corresponding amino acid change from aspartic acid to asparagine (D to N) at position 31[8], which affects the function of the catalytic site. Consequently, residual sulfamidase activity is present, which better models the condition existing in humans, compared with total absence of enzyme activity in knockout mouse models (http://jaxmice.jax.org/strain/003780.html)[8,10]. The C57BL6 strain was used for the WT control mice.

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee, Laboratory Animal Research Center, Samsung Biomedical Research Institute (Seoul, Korea). All procedures conformed to the international guidelines for the ethical use of laboratory animals and efforts were made to minimize the number of animals used and to avoid any unnecessary suffering.

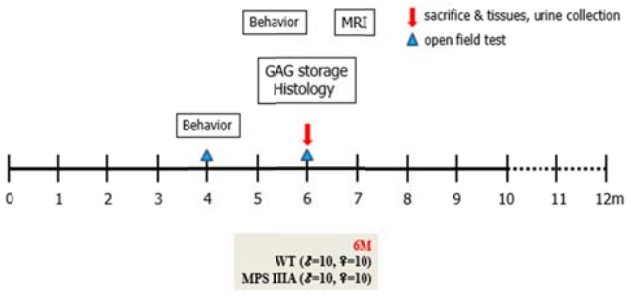

Fig. 1. This study was designed as 6-month duration of phenotypic characterization of MPS IIIA mouse biochemically, behaviorally and histologically in both male and female mice.

2. Clinical observations

Body weight (g) was evaluated by total body weight containing tail of the mouse. Animals were weighed once a week at the same time, using an electronic balance (Balance, Daejong instrument Co., Ltd) from 4-week-old to 24-week-old.

At the same time, after evaluating the body weight, measure the length of the mouse was done using electronic digital caliper (Monotaro AD-5763-150) A&D company and recorded the value within 0.1cm accuracy. To measure the entire length of the mouse, the length was evaluated starting from the nose (0 cm) to the beginning of the tail and tail to end of tail.

3. Preparation of tissue extracts for GAG analysis and quantitative analysis of GAG accumulation

Brain, heart, liver, spleen and lung tissues were extracted for GAG analysis. Urinary GAG was also analyzed. Tissue extracts were prepared by homogenizing tissues in phosphate buffer saline (PBS) using a tissue homogenizer. Homogenates were centrifuged at 20,000 g for 30 min, and supernatants were collected. The total protein concentration (mg/ml) was assayed using a bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). GAG concentrations in tissue homogenates and urine were quantified via colorimetric assay (Kamiya Biomedical Co., USA) according to the manufacturer's instructions, and absorbance was measured at 620 nm using a chondroitin 6-sulfate standard curve. The GAG levels in tissue extracts were adjusted for protein concentration, determined using the BCA assay, and expressed as μg GAG per mg protein. GAG levels in urine were normalized to the creatinine content.

4. Behavioral assessments

A total of four behavioral evaluations which were open field test, Y-maze test, T-maze test and fear conditioning test were performed at 4 months and 6 months of age in both male and female mice.

The method of implementation is as follows.

1) Open field test

The open field test is a common measure of exploratory behavior and general activity in both mice and rats, where both the

quality and quantity of the activity can be measured[13]. Mice were allowed to explore in an open-top arena (44.5 cm×44.5 cm) that is sided with opaque walls (15.0 cm height) and video-recorded for 20 min. Recorded videos were analyzed for exploration distance, moving speed, resting time, retention time of each subject mouse in central and peripheral zone, respectively, and number of entries into each zone. Central zone was defined as enclosed area (31.0 cm×31.0 cm) at center of the arena and peripheral zone was rest of the arena excluding central zone.

2) Y-maze test

The Y-maze is a modification of the T-maze which evaluates memory and special learning in rodents though quantification of spontaneous alternation (a measure of spatial cognition). It is well known as being useful for measuring short-term memory, general locomotor activity, and stereotypical behavior patterns[14,15]. Mice were placed in one-arm and allowed to freely enter other arms for 5 min under dim light. Their movements were video-recorded and analyzed for spontaneous alternations or same arm returns. For example, if a mouse was placed at arm A and traveled to arm B and C accordingly, the movement was counted as one spontaneous alternation. On the other hand if a mouse was placed at arm A and returned to arm A after traveled to arm B or C, the movement was counted as one same-arm return. Number of spontaneous alternations and same-arm returns were divided by number of possible alternations in order to determine spontaneous alternation rate and same-arm return rate, respectively. The maze's arm was made by opaque acrylic plastic (35 cm×5 cm×10 cm) and arms were separated by 120 degrees.

3) T-maze test

In addition to Y-maze test, T-maze test was also conducted to evaluate mouse alternative working memory[16] using modified T-maze that consisted of two goal arms (left and right; 12.0 cm×31.4 cm×20.0 cm), running arm (52.0 cm×12.0 cm×20.0 cm), and starting arm (24.0 cm×12.0 cm×20.0 cm) made by plexiglass. The maze's walls were transparent whereas each arm's bottom and gate were stained in black. Gates were selectively closed or open in order to control itinerary of mouse subjects during forced-choice trial and following free-choice trial for T-CAT test. During forced-choice trial, one of goal arms was closed and mice were not allowed to make alternations but complete only one itinerary (starting – running – open goal – starting arm). On the other hand, two goal arms were both open and mice were allowed to make alternations until completed 14 itineraries during free-choice trial. Number of alternations was divided by number of total itineraries and in order to compute alternation rate of mouse subjects.

4) Fear conditioning

Fear conditioning is a behavioral paradigm in which organisms learn to predict aversive event[17]. It is a form of learning in which an aversive stimulus (e.g., an electrical shock) is associated with a particular neutral context (e.g., a room) or neutral stimulus (e.g., a tone), resulting in the expression of fear responses to the originally neutral stimulus or context. Mouse fear memory was evaluated using fear conditioning system (Coulbourn instruments) as follows: On day 1, mice were stimulated by auditory cue for 30 sec (80 dB, 3,600 Hz tone) and foot-shock for 2 sec (0.6 mA direct-current) in fear conditioning chamber. On day 2, the mice were placed back in the same chamber and video-recorded at 40 Hz frequency (30 frames per 0.75 sec) for 300 sec. Recorded videos were post hoc analyzed using Freeze Frame Software Ver 3.32 that automatically calculated motion index and relative absence of motion. Freezing duration was counted when mice were in the relative absence of motion for longer than 0.75 sec or 2 sec bouts and divided by total duration.

5. Neuropathology

1) Brain volume

Brain MRI used a 7.0-Tesla MRI system (Bruker-Biospin, Fa¨llanden, Switzerland) under inhaled anesthesia with 1.5–2% isoflurane into O2-enriched air. MRI was with a 20-cm gradient set capable of a rising time of 400 mTm^{-1}. Nine slices with 1.0-mm thickness were obtained using the following parameters: for T2-weighted (T2W) images, TR=3,000 ms, TE=70 ms, FOV=19.2 mm×19.2 mm, matrix size=192×192, NEX=6. After MRI, rat pups were allowed to recover.

Assessment of the percent volume ratio of brain, ventricles, cerebellum to whole brain from MRI, nine coronal MRIs per mouse brain were obtained, and two examiners calculated the percent volume ratio manually by outlining serial images in a blinded manner using ParaVision 2.0.2 software (Bruker, BioSpin, Karlsruhe, Germany). The boundaries of whole brains, ventricles or cerebellum were outlined on each image, and the corresponding area was calculated using the software.

2) Histology analysis

After the perfusion of the mice with ice-cold normal saline, their tissues were collected and fixed with 4% paraformaldehyde overnight at 4°C. The next day, the tissues were embedded in

paraffin (Sigma-Aldrich) after dehydration through a 70–100% ethanol gradient. Finally, the paraffin blocks were cut to a thickness of 4 μm. Lysosomal-associated membrane protein-2 (Lamp-2) immunoreactivity is a lysosomal protein marker used for the detection of lysosomal storage disorders, and is an indicator of disease state, in the surface cerebral cortex, caudate nucleus, thalamus, cerebellum, and white matter. For the immunohistochemical detection of Lamp-2, sections were treated with 10% normal goat serum (Dako) for 20 min at room temperature to block nonspecific binding. Subsequently, sections were incubated with rat anti-Lamp-2 monoclonal antibody (1/100; Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min at room temperature. After washing in PBS, the sections were incubated for 30 min at room temperature with HRP-labeled, polymer-conjugated secondary antibodies against rat IgG (Lamp-2). A color reaction was developed using ready-to-use DAB (3,30-diaminobenzidine) substrate-chromogen solution (Dako) for 5 min, and then washing with distilled water. Finally, the nuclei were lightly counterstained with Mayer's hematoxylin for 1 min. For alcian blue staining, brains were immersion-fixed in cold 2% paraformaldehyde with 2% glutaraldehyde in PBS, and the parietal neocortex was cut in 1mm slices. Tissues were post fixed in 1% osmium tetrox-

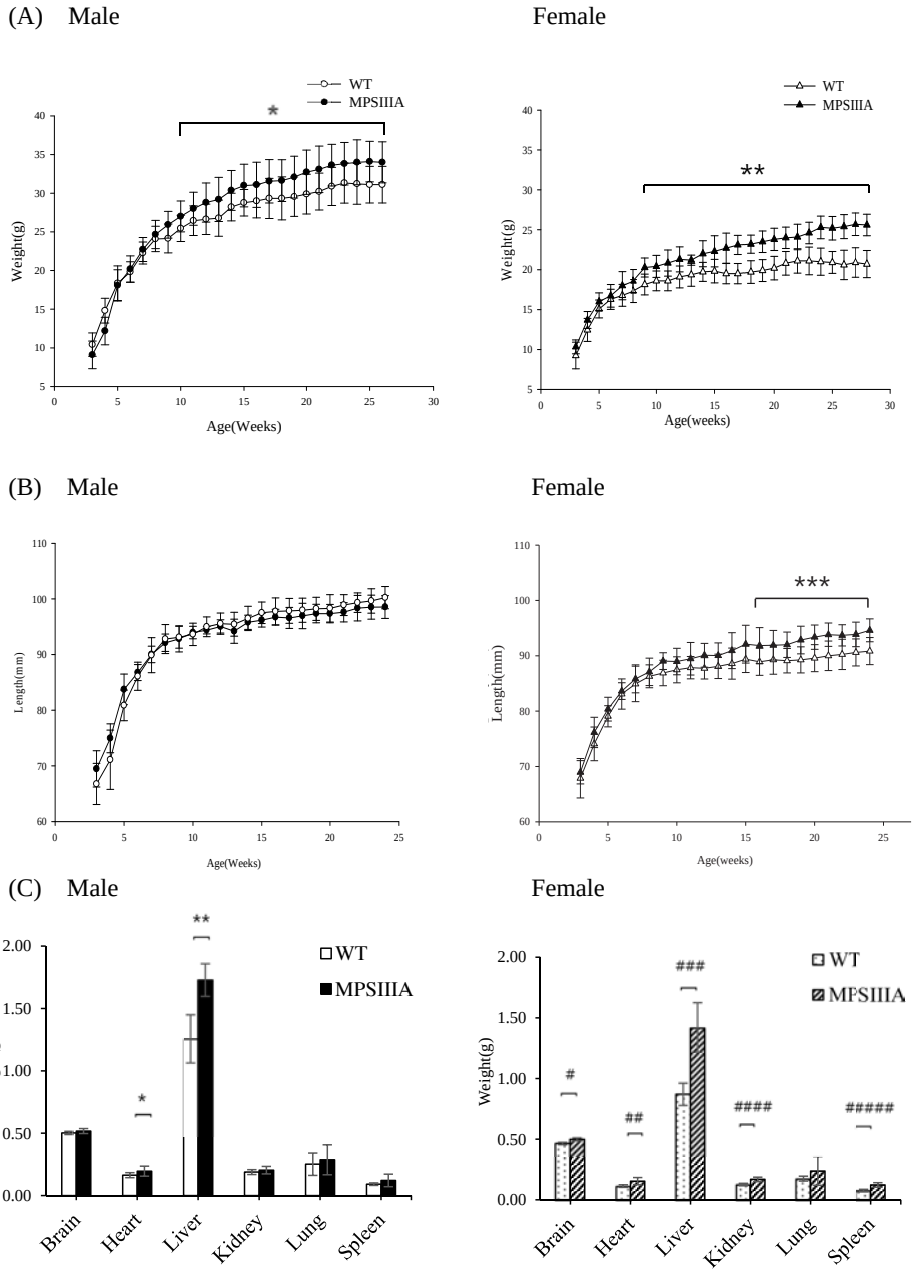

Fig. 2. (A) Mean body weights for male and female unaffected wild type (WT) and MPS IIIA mice. (B) Mean body length for male and female WT and MPS IIIA mice. $^{*}P$=0.024, $^{**}P$<0.002. (C) Mean organ weights for brain, heart, lever, kidney, lung, and spleen in WT and MPS IIIA mice. $^{\#}P$< 0.001, $^{\#\#}P$=0.001, $^{\#\#\#}P$<0.001, $^{\#\#\#\#}P$<0.001, $^{\#\#\#\#\#}P$<0.001. All data expressed as means± standard deviation and n=10 per group.

ide and embedded in Spurr's resin (Polysciences, Warrington, PA, USA). Sections were stained with alcian blue and assessed using light microscopy for lysosomal storage, indicated by cytoplasmic vacuolization. The abnormal morphology of neurons, especially Purkinje cells in the cerebellum, was assessed with hematoxylin and eosin staining. For hematoxylin and eosin staining, after tissue processing, fixed brains were embedded in paraffin wax. Sections of 4 mm were cut from paraffin blocks and stained with hematoxylin and eosin. Images of each section were captured with a magnifier digital camera using a Nikon ECLIPS 80i FL Upright Microscope (Nikon Melville, NY) and saved as JPEG files.

6. Statistical analysis

All results are expressed as mean±standard deviation. Statistical significance was determined for the compared measurements via one-way analysis of variance (ANOVA) tests, followed by Bonferroni's correction. *P*-values <0.05 were considered significant. Stata software (ver. 11.0, Stata Corp LP, College Station, TX, USA) was used for all analyses.

Results

1. Clinical observations

Both male (*n*=10) and female (*n*=10) MPS IIIA mice were heavier than their age-matched WT mice (male; *n*=10, female; *n*=10), with a highly significant disease group by age (both *P*<0.001). The pattern of body weight gain was similar MPS IIIA mice and WT mice for both male and females. Body weight was

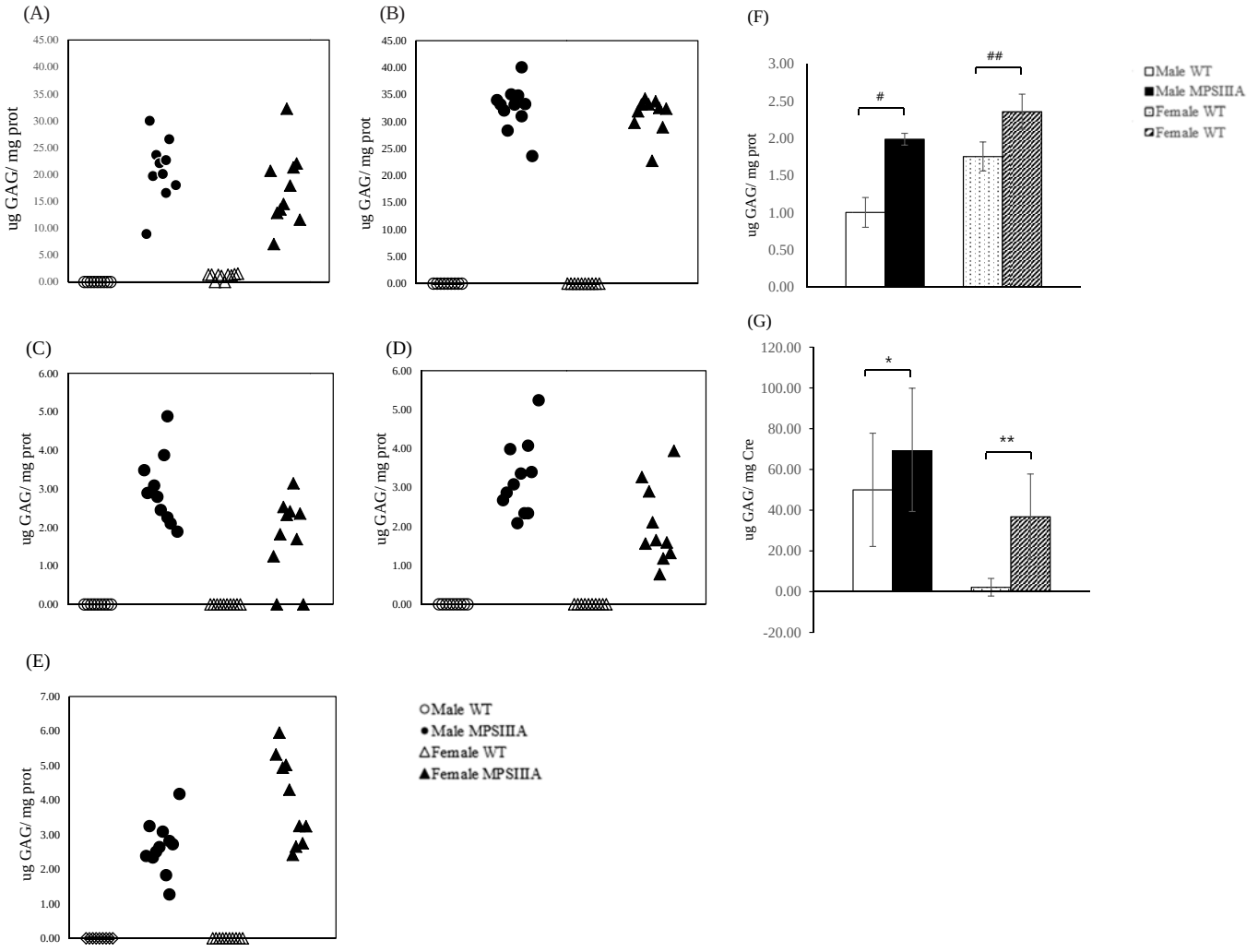

Fig. 3. Glycosaminoglycans accumulation in various organs and urine. (A) liver, (B) kidney, (C) lung, (D) spleen, (E) heart, (F) brain, (G) urine. Each group *n*=10 and (F), (G) data expressed as means±standard deviation. **P*=0.003, ***P*=0.034, #*P*=0.001, ##*P*<0.001.

similar between MPS IIIA and WT mice up to 9 weeks of age in male and 8 weeks of age in female, with MPS IIIA mice then tending to gain greater body weight. At each age suggested body weight was significantly greater (P<0.05) in MPS IIIA mice at 9, 12, 14, 15, 19, 20 to 22 and 24 weeks for males and at 4 and 8 to 24 weeks for females (Fig. 2A). Body length was similar between MPS IIIA and WT mice in male. In female mice, the length of MPSIIIA mice were longer than WT mice after 16 weeks of age (Fig. 2B). In MPS IIIA male mice, increased body weights were due at least in part to significantly increased liver weights (P=0.002) and heart weight (P=0.024) in male (Fig. 2C). In female mice, brain (P<0.001), heart (P=0.001), liver (P<0.001), kidney (P<0.001), spleen (P<0.001) weights were all significantly increased (Fig. 2C).

2. GAG accumulation in tissues and urine

The measured GAG concentrations in the brain, heart, liver, kidney, lung, spleen tissues of each group are presented in Fig. 3. In MPS IIIA mice, the brain, heart, liver, kidney, lung, spleen tissues showed significantly increased GAG concentrations compared to those of the WT mice in both male and female (P=0.003, $^{**}P$=0.034). In MPS IIIA mice, the urine GAG concentrations were also significantly increased compared to the WT mice (P=0.001, $^{\#\#}P$<0.001).

3. Behavioral assessments

In the open field test of 4-month-age male mice, the total movement distance was not statistically significant. However, the MPS IIIA group showed a significant shift in the central migration distance and the WT was significantly longer in the peripheral migration distance (Fig. 4A). The same results were obtained for the comparison of the retention time at the center and edge and the resting time during the entire movement (Fig. 4B). Likewise, it looks the same in the number of times it enters the center and the time it stays on the edge (Fig. 4C). In the T-maze and Y-maze tests, the spontaneous alternation behavior percentage was significantly higher in WT mice (Fig. 4D, E). In the fear conditioning test, when freezing time was evaluated with stimulation at intervals of 0.75 seconds or 2 seconds, the freezing time in WT was significantly higher in both cases (Fig. 4F).

In the open field test of 4-month-age female mice, there is a significant difference in the number of arrivals in the central region (Fig. 5A). In the fear conditioning test, when the stimulation was given at intervals of 0.75 seconds, the freezing time on the 2nd

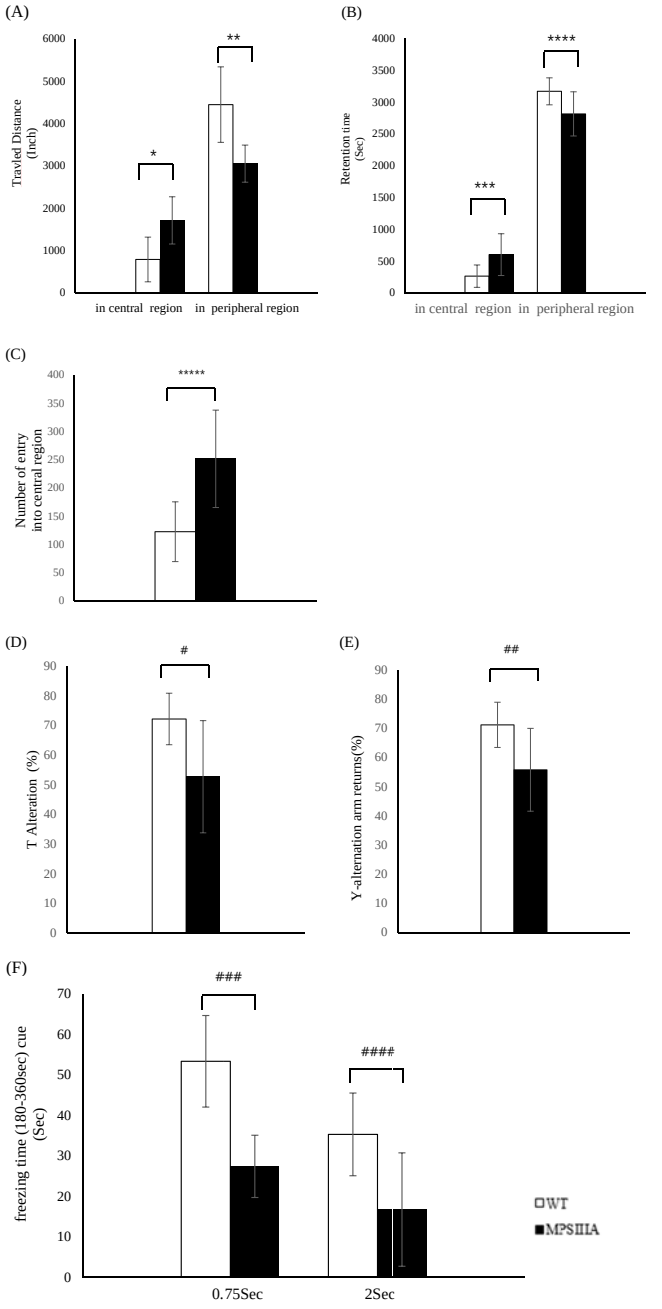

Fig. 4. In behavioral test of 4-month-age male mice, the MPS IIIA group showed a significant shift in the central migration distance and the WT was significantly longer in the peripheral migration distance in open field test (A). The same results were obtained for the comparison of the retention time at the center and edge and the resting time during the entire movement (B). Likewise, it looks the same in the number of times it enters the center and the time it stays on the edge (C). In the T-maze and Y-maze tests, the spontaneous alternation behavior percentage was significantly higher in WT mice (D, E). In the fear conditioning test, when freezing time was evaluated with stimulation at intervals of 0.75 seconds or 2 seconds, the freezing time in WT was significantly higher in both cases (F). All data expressed as means±standard deviation and n=10 per group. $^{*}P$=0.006, $^{**}P$=0.005, $^{***}P$=0.016, $^{****}P$=0.025, $^{*****}P$=0.005, $^{\#}P$=0.018, $^{\#\#}P$=0.025, $^{\#\#\#}P$=0.016, $^{\#\#\#\#}P$=0.007.

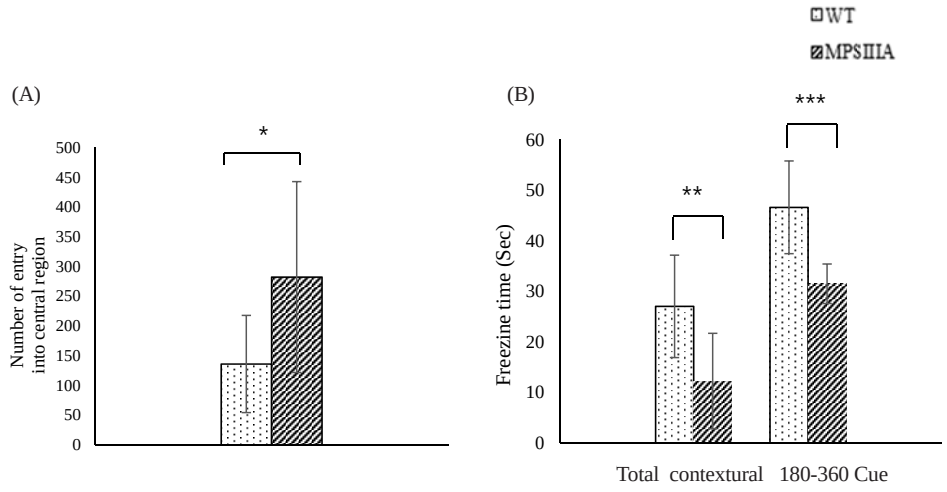

Fig. 5. In the open field test of 4-month-age female mice, there is a significant difference in the number of arrivals in the central region (A). In the fear conditioning test, when the stimulation was given at intervals of 0.75 seconds, the freezing time on the 2nd day and the freezing time after the stimulation on the 3rd day were significantly higher in the WT group (B). All data expressed as means±standard deviation and n=10 per group. **P*=0.032, ***P*=0.026, ****P*=0.004.

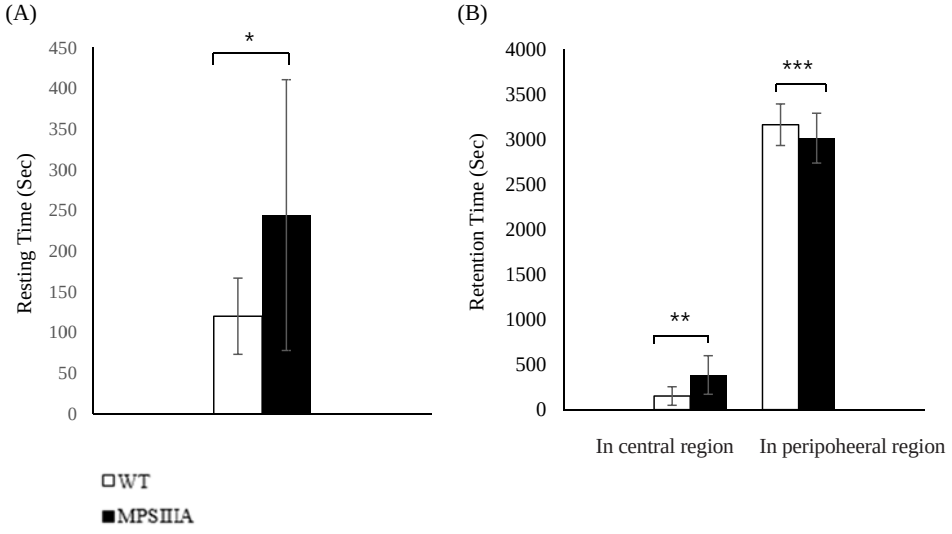

Fig. 6. The open filed test at the 6 months of age in male mice, we observed that the resting time and the retention time of the MPS IIIA still increased and confirming that the brain function of the MPS IIIA has decreased (A, B). All data expressed as means±standard deviation and n=10 per group. **P*=0.003, ***P*=0.013, ****P*=0.048, *****P*=0.019.

day and the freezing time after the stimulation on the 3rd day were significantly higher in the WT group (Fig. 5A). In the T-maze and Y-maze tests, there was no statistically significant difference between the two groups in female mice.

The open filed test at the 6 months of age in male mice, we observed that the resting time and the retention time of the MPS IIIA still increased and confirming that the brain function of the MPS IIIA has decreased (Fig. 6A, B).In female mice, the difference on retention time and travel distance of the central region between MPS IIIA mice and WT mice .

There was no significant difference in T-maze, Y-maze and fear conditioning test in both male and female mice at 6 months of age.

4. Neuropathology

Brain volume (*n*=3), ventricle volume (*n*=3), and cerebellum volume (*n*=3) were measured. The brain volume of the female MPS IIIA group was significantly larger than that of the female WT mice. The percent volume ratio of ventricle to whole brain, which can be suggested as an indicator of subcortical atrophy[18], increased significantly in MPS IIIA female mice compared with WT group. There was no difference in other parts (Fig. 4).

Fig. 7A displays a representative brain histology of the cerebral cortex, thalamus, and cerebellum stained with alcian blue for each group (*n*=3). Polysaccharide was bluishly stained and evaluated polysaccharide accumulation by using it.

Fig. 7B displays a representative brain histology of the cerebral cortex, thalamus, and cerebellum stained with hematoxylin and eosin for each group (*n*=3). The neuronal lysosomal storage of

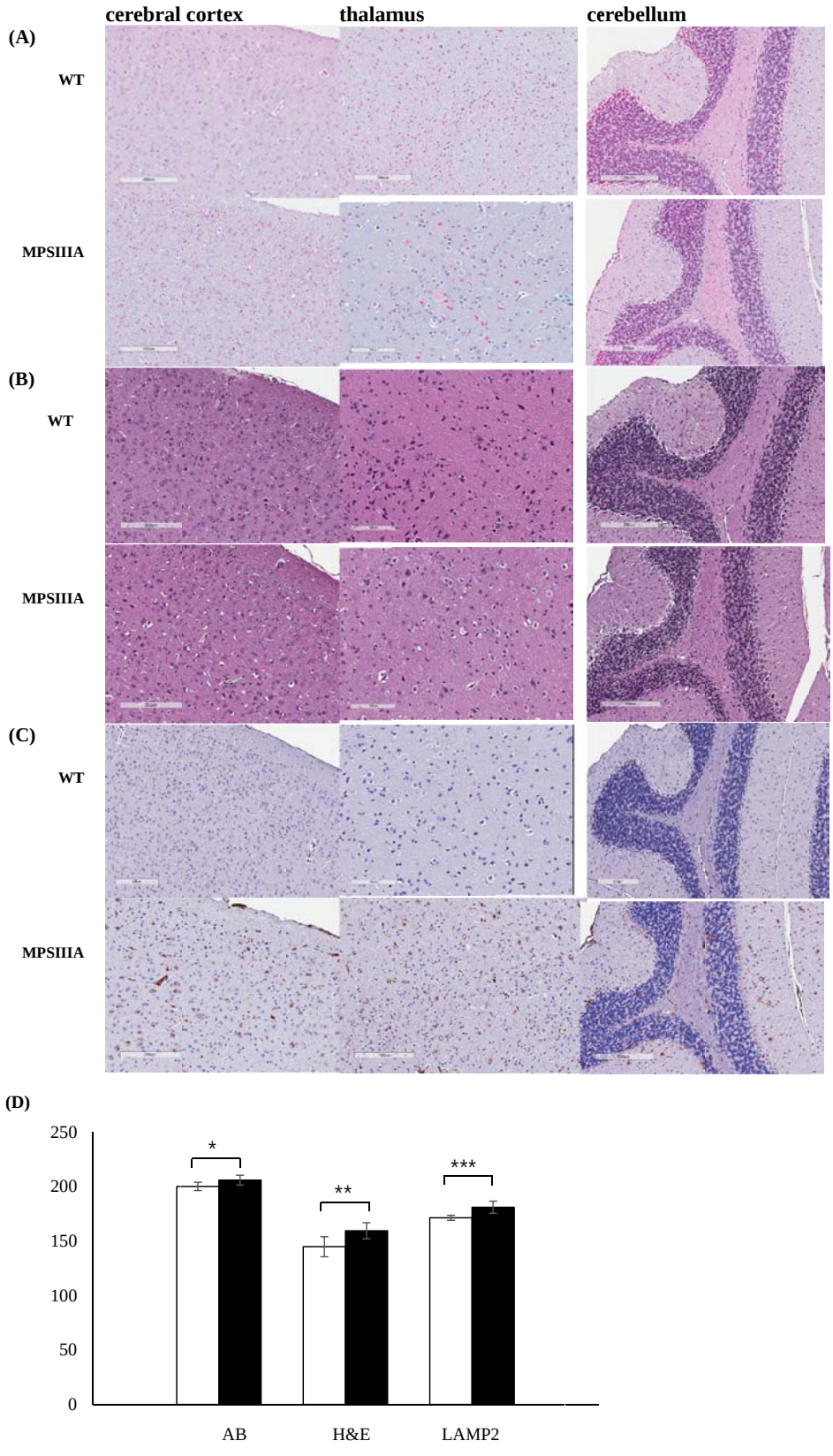

Fig. 7. In the AB stain, polysaccharide was bluishly stained (A). In H&E, the extent of increased lucent lysosomal storage was detected in MPS IIIA mice (B). In LAMP-2 staining, MPS IIIA mice showed increased anti-Lamp-2 immunostaining compared to the WT mice, which marks lysosomal storage (C).The differences between the images were quantified and compared (D). Quantified data expressed as means±standard deviation and n=3 per group. **P*=0.037, ***P*=0.013, ****P*=0.014 (D).

GAG is presented as neuronal cytoplasmic vacuolization[19]. The number of distinct and enhanced neuronal vacuolizations observed in the cortical area was remarkably increased in the MPS IIIA and dyslamination and decreased cellular density were noted in the cortex area of MPS IIIA.

Fig. 7C displays the representative brain histologies of the cerebral cortex, thalamus, and cerebellum stained with Lamp-2 for each group (n=3). In MPS IIIA mice showed increased anti-Lamp-2 immunostaining compared to the WT mice, which marks lysosomal storage. The deposition of Lamp2 was clearly ameliorated in the cerebral cortex, thalamus, and cerebellum.

Discussion

The major goal of this study was to subsequently characterize the biochemistry, histology and behavior of the MPS IIIA (*Sgshmps*^{3a}/*Sgshmps*^{3a}) mouse. Characterization of animal model is the beginning point of the therapeutic clinical trial. Mouse model has a limitation in that it is not a human and does not have all of the disease phenotypes. Therefore, delineate of the phenotypic characteristics of MPS III A mouse model prerequisite for the enzyme replace treatment for the diseases.

Grossly, the MPS IIIA mice display coarse, unkempt fur by 20 to 25 weeks of age; at this age, a distinctly hunched posture and a broader, more flattened face is also observed. In this study, the pattern of body weight gain and length were similar MPS IIIA mice and WT mice for both male and females. But body weight was significantly greater (P<0.05) in MPS IIIA mice at 9, 12, 14, 15, 19, 20 to 22 and 24 weeks for males and at 4 and 8 to 24 weeks for females Increased body weights were due at least in part to significantly increased organ weight.

The MPS IIIA mice accumulate GAG in tissues and exhibit widespread and variable intracellular storage in a variety of cell types in peripheral organs including liver, heart, lung, spleen and kidney. Biochemically analysis of GAG in urine and pheripheral tissues are available for MPS IIIA mouse characterization.

Male MPS IIIA mice exhibited more behavioral abnormalities than MPS IIIA females, as observed in the previous study[20]. Our results also indicate behavioral abnormalities in both male and female MPS IIIA males at 4 and 6 months. But more about the various items in the male showed significant results, suggesting that behavioral test may be further available in females. Current study clearly demonstrates sex differences in some but not all behavioral tests in the congenic MPS IIIA strain, as was seen in the mixed MPS IIIA strain[20]. MPS IIIA mice showed increased frequency of entry, path length and speed in the central area whilst duration in the centre was unchanged. These increases can all be explained by increased hyperactivity causing the mice to move faster and so entering the centre more frequently and travelling further in the same amount of time. But in peripheral area, WT mice showed increased path length and retension time. Differences in behavior in the Central area and peripheral area may indicate that decreased the ability of the rodents to detect danger when compared to the instinct to go to the corner to avoid the danger.

The T-maze and Y-maze test are for the hippocampal-dependent task of spontaneous alternations.

Maze test is available for evaluate neurodegenerative dysfunction of MPS IIIA mice compared with WT mice.

Malinowska et al.[21] reported on treating MPS IIIB mice with genistein they found similar hyperactivity increases in male MPS IIIB mice, although differences were less marked than females. Although this would suggest that female mice may be more consistent for behavioral testing, Fu et al.[22]reported vertical activity changes only observed in males. In this work we have been able to determine several behavioral parameters that allow analysis of treatment effects on the neurological deterioration seen in MPS IIIA mice. In our study, male MPS IIIA mice may be better suitable than female mice and the behavioral assessment is appropriate at 4 months of age better than 6 months of age.

MPS IIIA mice also show a reduced sense of danger in some situations. Fear conditioning was normal in the contextual fear test but reduced in tone fear test[12] which could be caused by differences in sensory function, whilst no changes in the acoustic startle response have been observed[22]. A reduced sense of danger has been observed in the elevated plus maze test when performed in the dark[23] but not in the light[22]. In our study, decreased freezing time has been observed after stimuli and means that reduced sense of danger.

The percent volume ratio of ventricle to whole brain, which can be suggested as an indicator of subcortical atrophy[18], increased significantly in MPS IIIA female mice only compared with WT group. Many regions of the central nervous system (CNS) such as the cerebral cortex, cerebellum, brainstem, hippocampus and olfactory bulb contain neurons, glia and perivascular cells with lysosomal storage[10,24,25].

A wide variety of functions have been suggested for different GAG and for individual proteoglycans, particularly in the developing and aging brain[26]. In our study, quantification of the histology and comparison between the groups was possible. The MPS IIIA mice might be useful for investigations of the brain histological and neurological consequences as a therapeutic model.

The MPS IIIA mouse provides an excellent model for evaluating pathogenic mechanisms of disease and for testing treatment strategies, including enzyme or cell replacement and gene therapy. This study is meaningful because we designed 6-month duration of phenotypic characterization of MPS IIIA mouse biochemically, behaviorally and histologically in both male and female mice.

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