

Trehalose Attenuates the Gait Ataxia and Gliosis of Spinocerebellar Ataxia Type 17 Mice

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Abstract Spinocerebellar ataxia type 17 (SCA17) is caused by CAG/CAA repeat expansion on the gene encoding a general transcription factor, TATA-box-binding protein (TBP). The CAG repeat expansion leads to the reduced solubility of polyglutamine TBP and induces aggregate formation. The TBP aggregation, mostly present in the cell nuclei, is distinct from that in most other neurodegenerative diseases, in which the aggregation is formed in cytosol or extracellular compartments. Trehalose is a disaccharide issued by the Food and Drug Administration with a Generally Recognized As Safe status. Lines of evidence suggest trehalose could prevent protein aggregate formation in several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease. In this study, we evaluated the therapeutic potential of trehalose on SCA17 using cerebellar primary and organotypic culture systems and a mouse model. Our results showed that TBP nuclear aggregation was significantly decreased in both the primary and slice cultures. Trehalose (4 %) was further supplied in the drinking water of SCA17 transgenic mice. We found both the gait behavior in the footprint analysis and motor coordination in the rotarod task were significantly

improved in the trehalose-treated SCA17 mice. The cerebellar weight was increased and the astrocyte gliosis was reduced in SCA17 mice after trehalose treatment. These data suggest that trehalose could be a potential nontoxic treatment for SCA17.

Keywords Spinocerebellar ataxia · Trehalose · TBP · Intracellular aggregation

Abbreviations

AD	Alzheimer's disease
ANOVA	Analysis of variance
DIV	Days in vitro
DRPLA	Dentatorubral–pallidoluysian atrophy
FDA	Food and Drug Administration
GFAP	Glial fibrillary acidic protein
GRAS	Generally Recognized As Safe
HD	Huntington's disease
LSD	Least significant difference
PD	Parkinson's disease
polyQ	Polyglutamine
SBMA	Spinal and bulbar muscular atrophy
SCA	Spinocerebellar ataxias
SCA17	Spinocerebellar ataxia type 17
TBP	TATA-box-binding protein
TBS	Tris buffered saline
TG	Transgenic
WT	Wild-type

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Introduction

Polyglutamine (polyQ) diseases are hereditary neurodegenerative diseases caused by CAG/CAA repeat sequence

excessive expansion. Huntington's disease (HD), dentatorubral–pallidolusian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA), and spinocerebellar ataxias (SCA) are subtypes of polyQ diseases [1]. The abnormally expanded CAG/CAA sequence causes the polyQ overextended mutant protein to be misfolded and dysfunctional, a state believed to result in the diseases' pathology. In addition, these abnormal polyQ proteins are insoluble, and they accumulate and cause cell degeneration [2, 3].

SCA17 is a subtype of SCA caused by the polyQ expansion in the TATA-box-binding protein (TBP) [4], a transcription factor playing an essential role in three types of RNA polymerase [5]. The length of polyQ in a normal individual is between 25 and 42 repeats [6], and an individual suffering from SCA17 has polyQ repeated beyond this number [7]. The polyQ expanded mutant TBP usually forms aggregates in the nuclei. This is different from most other neurodegenerative diseases in which aggregates are mainly in the cytosolic (AD, PD and HD) or extracellular (AD) compartments [8]. Cerebellar atrophy and Purkinje cell degeneration are the major clinical pathology of SCA17 [9]. Furthermore, gliosis in the brain is also a striking feature of SCA17 patients [10].

Trehalose is a disaccharide that generally exists in yeast, bacteria and invertebrates. It has been reported that trehalose can help cells to resist stress such as hydration and to avoid oxidation by protecting the protein from denaturing [11]. Using trehalose in drinking water significantly reduced the chance of the mutant Huntington protein becoming toxic in the body and saved the motor function of the HD transgenic mouse model [12, 13]. Recent study showed that trehalose ameliorates the neurotoxicity in fibroblasts of patients with HD [14] and C-terminal HSP70-interacting protein (CHIP)-mutation related ataxia [15]. A study also pointed out that treating the SCA14 Purkinje primary cells with trehalose may serve as a chemical chaperone to ameliorate toxic protein aggregation and prevent the disease pathology [16]. In AD studies, it has been reported that trehalose can reduce the tau protein hyperphosphorylation and neuron inflammation. Furthermore, the trehalose treatment can diminish the abnormal amyloid β protein aggregation and ameliorate the AD pathology in the AD transgenic mouse model [17]. Recent study has also indicated that trehalose therapy slows the pathology in a chronic MPTP-induced Parkinson's disease mouse model [18]. We presume the anti-aggregation ability of trehalose will be a potential treatment for SCA17, another polyQ neurodegenerative disease.

Materials and Methods

Mouse Breeding and Genotyping

The animal experiments were all conducted in accordance with the guidelines and were approved by the National Taiwan Normal University Research Committee. SCA17 transgenic mice with human TBP (hTBP)-109 poly-glutamine (109Q) were established in our laboratory [19]. Transgenic line 69 was used in this study and was maintained by breeding heterozygous male mice with FVB/N wild-type female mice from the National Laboratory Animal Center (Taipei, Taiwan). The transgenic mice showed ataxia and poor rotarod performance around 6 weeks of age; while Purkinje cell degeneration occurred before the mice were 4 weeks old [19]. The mice were kept in individually ventilated cages (Lasco, Taipei, Taiwan) for a 12-h light/dark cycle.

Transgenic mouse genotyping was conducted by PCR analysis of tail DNA. The mouse tail biopsy was lysed in Direct PCR buffer (Viagen Biotech, CA, USA) with 50 μ g/ml proteinase K at 55 °C for 5.5 h. PCR primers forward (5'-TATGGTGAGAGCAGA GATGG-3') and reverse (5'-CTGCTGGGACGTTGACTGCTG-3') were used to identify the transgene fragment 765 bp; primers forward (5'-GAA TATCCC GCTCTCCGGAG-3') and reverse (5'-ACCTGT TGTCCAGTTGCACT-3') were used to identify the male mice 294 bp *sry* fragment. The PCR was conducted for 30 cycles under the conditions of 1 min at 95 °C for denaturing, 1 min at 68 °C with -0.1 °C touchdown in each cycle for annealing, and 1 min at 72 °C for elongation.

Cerebellar Primary Culture and Immunostaining

The culture protocol was modified from several previous reports [20–22]. Culture medium based on NEUROBASAL[®] medium (Invitrogen) with supplements of 2 % B-27 (v/v, Invitrogen), 1 mM adenine (Sigma), 2 mM GlutaMax-I (Invitrogen), 3 mM KCl (Sigma), 5 μ g/ml gentamicin (Invitrogen), 100 U/ml penicillin and 100 μ g/ml streptomycin (Invitrogen). Cerebella were isolated from postnatal day 0–1 (P0–P1) SCA17 mice [19], cut into small pieces and incubated with culture medium containing 0.05 % trypsin/EDTA (Invitrogen) and 20 U/ml DNaseI (Sigma) for 15 min at 37 °C. To stop the proteolytic reaction, the medium was replaced with 10 % fetal bovine serum (Invitrogen) and 20 U/ml DNaseI (Sigma). After centrifugation, cells were resuspended in medium with 1 % fetal bovine serum. Finally, cells were seeded into a 96-well culture plate coated with 100 μ g/ml poly-L-lysine (Sigma). Trehalose (Gemfont Corporation, Taipei, Taiwan) was applied to cells with

medium containing 4 μ M cytosine arabinoside at day 5 (DIV5). After 18 days in culture (DIV18), the cells were fixed with 4 % paraformaldehyde and immunostained with primary antibodies [IP3R-1(for Purkinje cells), 1:1,000, Santa Cruz; 1TBP18 (for aggregation), 1:30,000, QED Bioscience], fluorescence-conjugated secondary antibodies (1:500, Invitrogen) and DAPI (1:10,000, Sigma). The staining results were observed using a live-cell image microscope (DMI 4000, Leica) and a high-content screening system (Molecular Devices). In each experiment, 120–200 Purkinje cells/mouse were analyzed; $n = 3$ per group.

Organotypic Cerebellar Slice Culture and Immunostaining

The cerebellar slice culture protocol was modified from a previous report [23]. Whole brains were isolated from post-natal day 7 (p7) SCA17 mice [19] and transferred to ice-cold culture medium containing 50 % Basal Medium Eagle (Invitrogen), 25 % Hank's Buffered Salt Solution (Invitrogen), 25 % horse serum (Invitrogen), 0.5 % D-Glucose (Sigma), 1 mM GlutaMAXI(Invitrogen), 100 U/ml penicillin (Invitrogen) and 100 μ g/ml streptomycin (Invitrogen). The cerebellum hemisphere was embedded with low-melting-point agarose (Bio Basic) in D-PBS (Invitrogen). The cerebellum was then cut into 350 μ m parasagittal sections with Vibratome (VT1200S, Leica). To improve the survival rate of the cerebellar slices, we continuously bubbled the buffer with 95 % O₂ and 5 % CO₂ during the sectioning. The slices were then cultured on 0.4 μ m pore size culture plate inserts (Millipore) in six-well plates. In each experiment, 4–6 slices per mouse were analyzed; $n = 4$ per group.

Trehalose treatments were applied to the slices on day 2. After culture for 7 days, slices were immunostained with primary antibody [IP3R-1 (for Purkinje cells), 1:1,000, Santa Cruz; 1TBP18 (for aggregation), 1:30,000, QED Bioscience], fluorescence-conjugated secondary antibody (1:500, Invitrogen) and DAPI (1:10,000, Sigma). The staining results were observed using a confocal microscope (TCS SP2, Leica).

Trehalose Treatment in SCA17 Mice

SCA17 transgenic mice (TG) and their wild-type littermates (WT) were randomly divided into four groups (WT-vehicle, WT-trehalose, TG-vehicle, and TG-trehalose, $n = 13$ in each group). Regular drinking water (vehicle) or drinking water with trehalose (2 or 4 %) was applied to the mice from 3 to 20 weeks old. We changed the regular or trehalose water once a week and monitored the mouse body weight, blood glucose, and drinking and eating amount every week. Behavioral analyses were performed during this period to evaluate the treatment effect.

Locomotor Activity Monitoring

The mice were placed in an open field black box (30 \times 30 \times 30 cm) and monitored under 2 Lux for 10 min. We used the EthoVision system (Noldus, Netherlands) to record and analyze the mouse total horizontal move distance, velocity and the path of movement ($n = 13$).

Rotarod Task

The mouse coordination and motor function were analyzed by the Rotarod (UGO BASILE, Italy). Before the rotarod test, mice were handled for 5 days to reduce their stress during the test. Mice were then tested using three trials per day at a speed fixed at 26 rpm for 200 s for 4 days. If the mouse fell down from the rod or spun around the rod without attempting to walk, we recorded the time as the running latency of the mouse. The latencies from the six trials of the last 2 days were averaged as the performance of each mouse ($n = 13$).

Footprint Analysis

Mouse footprints were monitored when the mice were 17 weeks old using the CatWalk XT system (Noldus). Each mouse was allowed to walk three times on the glass plate, and each mouse's paw prints were recorded and analyzed using the CatWalk XT 9.1 software (Noldus) ($n = 13$).

Immunofluorescent Staining of Mouse Cerebellar Cryosections

The mouse was perfused with 0.9 % NaCl followed by 4 % paraformaldehyde (Sigma) after being anesthetized with avertin (0.4 g/kg body weight). The whole mouse brain was then postfixed in 4 % paraformaldehyde for 4 h at 4 °C. The dehydration procedure was performed in 10 % sucrose for 1 h, 20 % sucrose for 2 h and 30 % sucrose overnight. The cerebellum was removed from the whole brain and sectioned into 30 μ m by cryostat-microtome (CM3050S, Leica).

The cerebellar sections were incubated with primary antibodies [anti-glial fibrillary acidic protein (GFAP) (1:1,000; Millipore), anti-IP3R1 (1:1,000; Santa Cruz), and anti-1TBP18 (1:30,000; QED Bioscience)] in Tris (TBS) containing 5 % horse serum and 1 % BSA at 4 °C overnight. After three 10-min washes with TBS, the cerebellar sections were incubated with secondary antibodies (1:500; Alexa Fluor dye-conjugated donkey anti-mouse, anti-rabbit or anti-goat IgG, Invitrogen, Carlsbad, CA, USA) in TBS for 2 h at 37 °C. After three 10-min washes with TBS,

sections were stained with DAPI (1:10,000) for 30 min at 37 °C. Finally, the cerebellar sections were mounted on gelatin-coated slides for observation under a confocal microscope (TCS SP2, Leica). In each experiment, 4–6 slices per mouse were analyzed; $n = 4$ per group.

Statistical Analysis

In vitro and in vivo data were presented as mean \pm SEM. One-way analysis of variance (ANOVA) and an independent t test were performed with SPSS software to evaluate the significance. Fisher's least significant difference (LSD) of the post hoc test was used to identify statistical significance between the two groups using one-way ANOVA tests. A p value cutoff of 0.05 was considered statistically significant.

Results

Trehalose Reduced the Purkinje Neuron Aggregation in Both Cerebellar Primary and Organotypic Slice Culture Platforms

Trehalose has been shown to have a positive curative effect and to prevent the abnormal protein forming aggregation in HD [12, 13] and SCA14 [16] models. To test whether trehalose is also effective on SCA17, we established the SCA17 mouse cerebellar primary culture as a quick evaluating platform (Fig. 1S). We analyzed the trehalose potential in neurite outgrowth promotion and aggregation reduction of Purkinje cells using this primary culture system. After treatment with trehalose (1–100 μ M) for 13 days, both the increase of neurite outgrowth (132 % at 100 μ M) and decrease of aggregation (63 % at 100 μ M) were significant (Fig. 1).

We further examined the aggregation-reducing activity of trehalose in an SCA17 cerebellar organotypic slice culture system (Fig. 2S), a platform more similar to the in vivo cerebellar environment than the primary culture. We applied different concentrations of trehalose to the culture media at DIV1 and analyzed the TBP aggregation level on the slice at DIV7 by immunostaining. We found that the TBP aggregation in the Purkinje cell nuclei was notably reduced after treatment with 10 μ M of trehalose (data not shown) and reached statistical significance after treatment with 100 μ M of trehalose (Fig. 2, $p < 0.001$).

Trehalose was Stable Under the Treatment Conditions

Since the trehalose treatment had an aggregation-preventing effect in both the SCA17 primary and slice culture, we applied trehalose to a small number of SCA17 mice

($n = 3$ –4 in each group) as a pilot study with the same protocol used for the HD mouse model [12]. We found 2 % trehalose applied in the mouse drinking water notably improved the rotarod performance and ameliorated the cerebellar atrophy of the SCA17 mice (data not shown). Unexpectedly, the abnormal TBP aggregation was not ameliorated by 2 % trehalose treatment (data not shown). To enhance the trehalose efficacy, we increased the concentration of trehalose to 4 % in the mouse drinking water for the treatment using a greater number of mice ($n = 13$ in each group). The drinking water with trehalose was refreshed every week. It has been indicated that given the combination of the molecular structure and physico-chemical properties, trehalose is a very stable disaccharide [24, 25]. The stability of 4 % trehalose within the water bottle for 1 week was monitored every other day by HPLC analysis. The consistency of the area of the trehalose peak from HPLC analysis revealed that the trehalose was stable for 1 week under the mouse culture conditions (Fig. 3a).

Trehalose Treatment had no Notable Effect on Body Weight and Blood Glucose in SCA17 Mice

To understand whether the high concentration of trehalose would harm the mice, we monitored the mouse eating and drinking amounts, body weight, and blood glucose level during the treatment. Each mouse ate about 3–6 g per day from 3 to 20 weeks old, and there was no notable difference among these four groups. Each mouse drank 4–6 ml of water (vehicle group) or 10–20 ml of the sweet 4 % trehalose water (treatment group) per day from 3 to 20 weeks old, which indicates the amount of trehalose intake by each mouse per day was about 0.4–0.8 g. The amount of trehalose consumption had no significant effect in body weight increase in the treatment groups as compared to the vehicle groups (Fig. 3b). Furthermore, the blood glucose levels were also maintained in the normal range (Fig. 3c), although the blood glucose level in the SCA17 mice was lower than in the wild-type mice at 5 weeks old, and lower than that at 20 weeks old. These data indicated the 4 % trehalose to be a safe dose for the mice.

Trehalose Treatment Ameliorated the Neurobehavior of SCA17 Mice

During the treatment, the motor coordination of animals was also analyzed with locomotor and rotarod tasks. SCA17 mice were identified as hyperactive in a 30 cm \times 30 cm open field in our previous study [19]. We found the trehalose could notably ameliorate the hyperactivity of SCA17 mice after a 14-week treatment (Fig. 4a). In addition, the rotarod performance was also improved in the treated SCA17 mice at 18 weeks old (Fig. 4b).

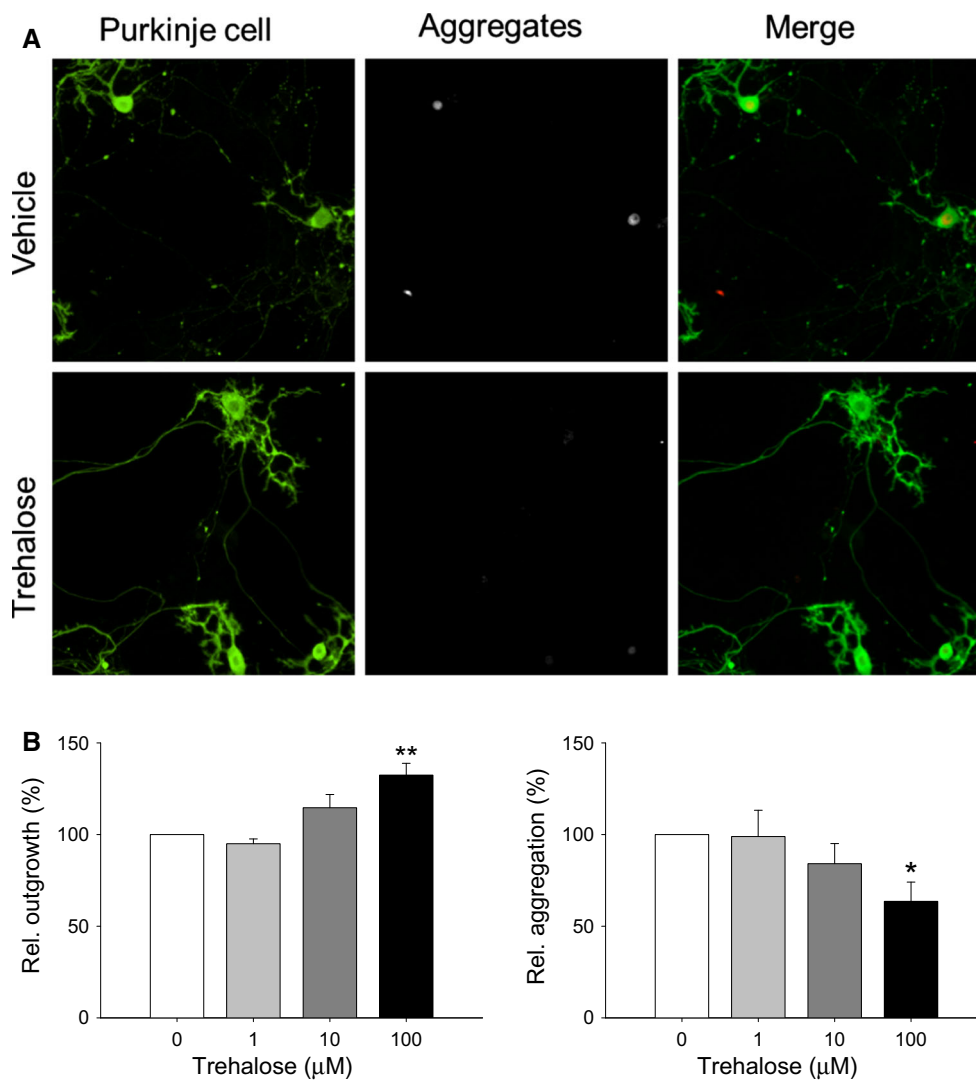


Fig. 1 Trehalose promoted neurite outgrowth and reduced aggregation of Purkinje cells in SCA17 mouse cerebellar primary culture. **a** The representative microscopic images of the primary culture treated with 100 μM of trehalose for 13 days. **b** Quantification of the relative Purkinje cell neurite outgrowth and aggregation after

treatment with 0–100 μM of trehalose (120–200 cells per mouse were analyzed; $n = 3$). To normalize, the relative neurite outgrowth length and aggregation level in vehicle-treated cells were set as 100 %

We further observed in the footprint analysis that the gait impairment of SCA17 transgenic mice was ameliorated by trehalose treatment (Fig. 5). The footprints of trehalose-treated mice resumed a more-regular pattern (Fig. 5a). The lengthened “Run duration” of SCA17 mice was reduced (Fig. 5b). The reduced “Step Sequence Regularity” was also partly recovered (Fig. 5c). The other altered footprint behaviors of SCA17 mice, including “Bass of Support” of the hind paw (Fig. 5d), “Print Position” (Fig. 5e), and “Stride Length” (Fig. 5f), were all significantly improved after trehalose treatment. These data indicate that the trehalose treatment ameliorated the impaired coordination resulting from the cerebellar atrophy in SCA17 transgenic mice.

Trehalose Treatment Ameliorated the Cerebellar Pathology of SCA17 Mice

After treatment for 4 months, we observed the SCA17 mouse cerebellum weight was found to be increased in trehalose-treated TG mice compared to vehicle treatment (Fig. 6a), indicating that the trehalose could ameliorate the SCA17 cerebellar atrophy. Immunostaining of the cerebellum was conducted to further understand the molecular and cellular effect of trehalose application in vivo. First, we found in both the wild-type and transgenic mice, the section sizes of the cerebella in the trehalose-treated groups were larger than in the control groups (Fig. 6b), indicating that the trehalose had a neuroprotective effect for SCA17

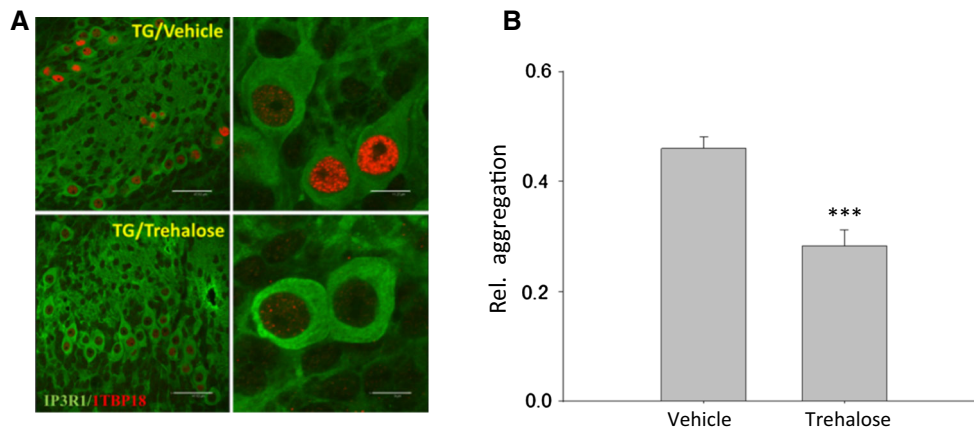


Fig. 2 Trehalose treatment reduced the TBP aggregation on the SCA17 cerebellar slice culture. Trehalose (100 μ M) was applied to the slice at DIV1, and the Purkinje cell nuclear aggregation was characterized by immunofluorescent staining at DIV7. **a** The

representative imaging of the slice culture on DIV7. **b** Quantification of the relative Purkinje cell aggregation after treatment (4–6 slices per mouse; $n = 4$). The Purkinje neuron TBP aggregation was significantly reduced by the trehalose treatment

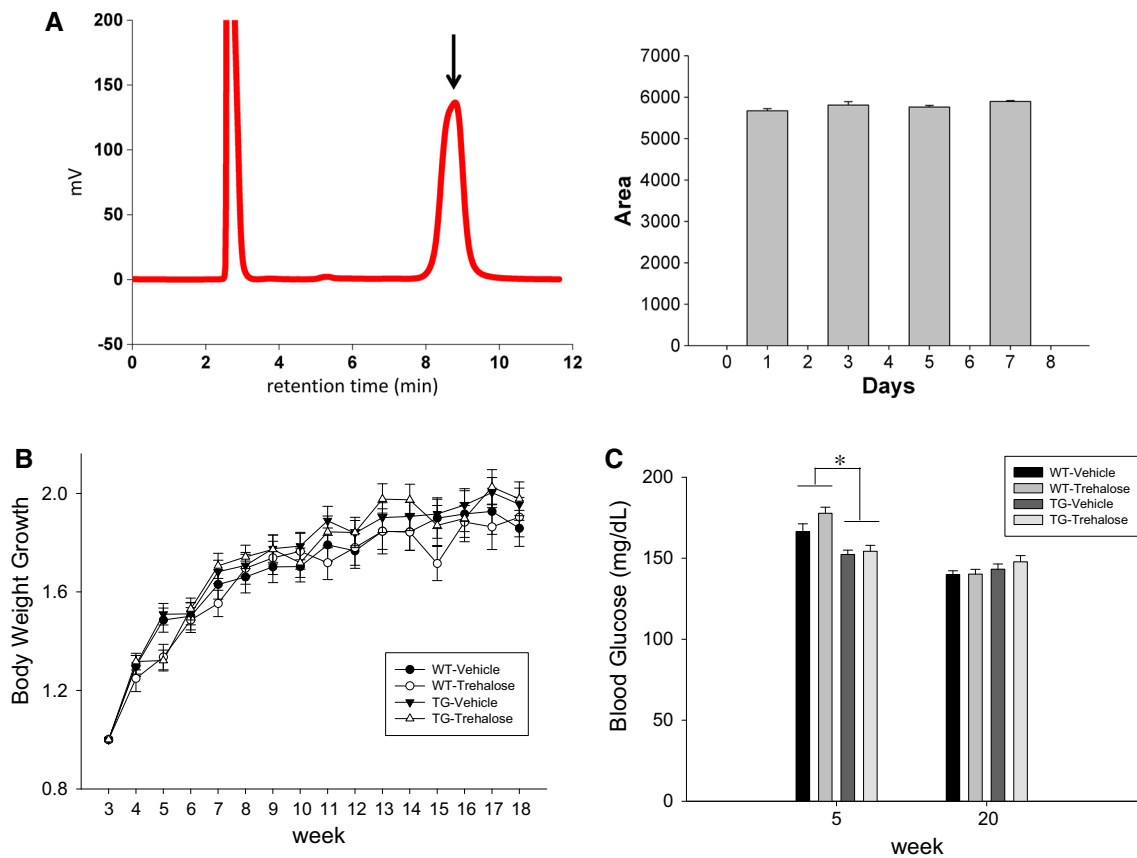


Fig. 3 Analysis of the 4 % trehalose stability and its effect on mouse body weight and blood glucose. **a** The 4 % trehalose solution was kept in the mouse water bottle for 1 week in the animal room and then characterized by HPLC. The quantification of the peak area (arrow) of trehalose indicated the high stability of trehalose during the treatment. **b** The mouse body weight was monitored during the

treatment period. The growth rate showed no differences between these four groups of animals. **c** The mouse blood glucose levels were not altered after trehalose treatment although the TG mouse blood glucose was lower than that of the WT mice at 5 weeks old, $*p < 0.05$ ($n = 13$)

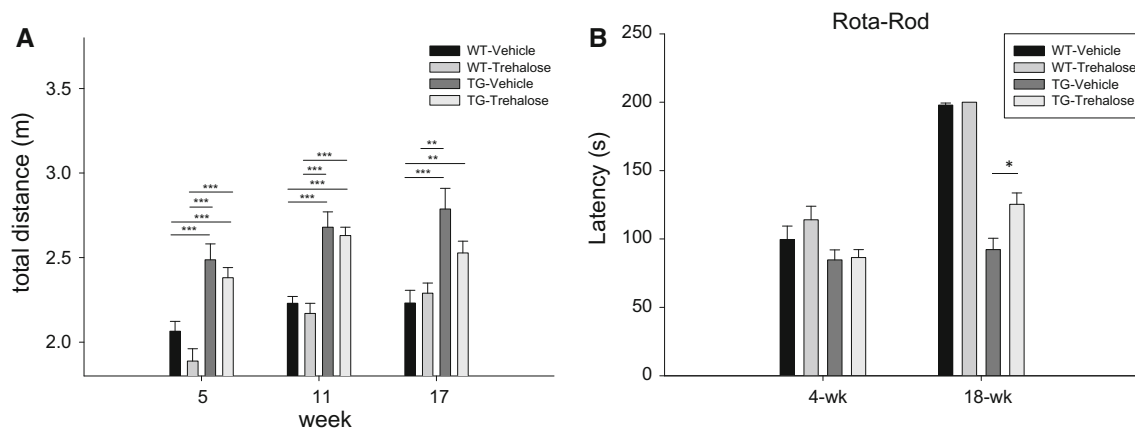


Fig. 4 Evaluation of the effect of 4 % trehalose treatment on motor behaviors. **a** The motor activity of mice was characterized by the total distance traveled in an open field (30 × 30 cm). The hyperactivity of TG mice was ameliorated when they were 17 weeks old after trehalose treatment, $**p < 0.005$; $***p < 0.001$. **b** The motor

coordination of the mice was analyzed by the rotarod task. The highly reduced latency on the rod of TG mice was ameliorated by trehalose treatment, and the motor coordination was significantly higher than in the vehicle-treated mice at 18 weeks of age, $*p < 0.05$ ($n = 13$)

transgenic mice. In addition, trehalose treatment of WT mice seems also enhanced IP3R1 signals over WT vehicle treated mice. Furthermore, astrocyte gliosis is well-known as one of the major neurodegenerative markers, as was also identified in our SCA17 mice [19]. Trehalose treatment reduced the astrocyte numbers in SCA17 mouse cerebellum by both immunofluorescent and immunohistochemical staining with GFAP antibody (Fig. 6c).

We also characterized the molecular effect of trehalose in SCA17 mice. The levels of MnSOD, p-ERK, pp38, β -catenin and GAD67 were not affected after trehalose treatment in the SCA17 mice by Western blot analysis (data not shown). HSP70, considered the major chaperone protein, played a neuron-protection role in neurodegenerative diseases [9, 26]. However, we could detect only a slight increase of HSP70 in the trehalose-treated transgenic cerebella (data not shown).

Discussion

In the present study, we demonstrated that the SCA17 mouse neurophenotypes, including behavior and pathology, were alleviated by the trehalose treatment. Although the molecular pathogenesis of SCA17 has not been fully clarified yet, cerebellar atrophy, Purkinje cell loss and TBP nuclear aggregation are significant features identified in SCA17 patients [7]. To identify potential treatments for polyQ-mediated SCA diseases, an *in vitro* Purkinje neuronal model could be used for a primary screening platform before the animal characterization [21, 27]. Therefore, both mouse cerebellar primary and organotypic slice cultures were established for this study.

From our experience, the cerebellar primary culture established from neonatal day 1 mice could be maintained for about 1 month, while TBP aggregation in the Purkinje cells was identified at DIV12. The neurite outgrowth retardation was correlated to the TBP aggregation within the Purkinje cells. For the cerebellar slice culture established from neonatal day 7 mice, a normal neuronal morphology within the cerebellar slice could be maintained for 2 weeks. TBP aggregation was identified in the Purkinje neuron of the SCA17 transgenic slice between DIV1 and DIV3. These results indicated that both the primary and slice cultures could be a suitable system to screen potential treatment for alleviating TBP aggregation.

Trehalose is an α -linked disaccharide synthesized by fungi, plants and invertebrates. Some reports suggested that the trehalose had low toxicity and could help the cell to protect against the stress threatening the cell's survival [11]. In addition, the trehalose had been reported to have potential in preventing neuron pathology and protecting against molecular dysfunction and abnormal behavior in many neurodegenerative diseases, such as AD [17], PD [18, 28] prion disease [29], HD [12–14], spinal and bulbar muscular atrophy (SBMA) and SCA14 [16]. In this study, trehalose administration in both SCA17 mouse cerebellar primary and slice culture revealed that trehalose treatment could prevent or alleviate the TBP aggregation formation *in vitro*.

To further understand whether trehalose could decrease TBP aggregation and prevent the SCA17 pathology *in vivo*, we added 2 % trehalose to the mouse drinking water. During the pilot study, we did not observe a significant protective effect of trehalose on SCA17 mice from the rotarod test in a condition with accelerated speed

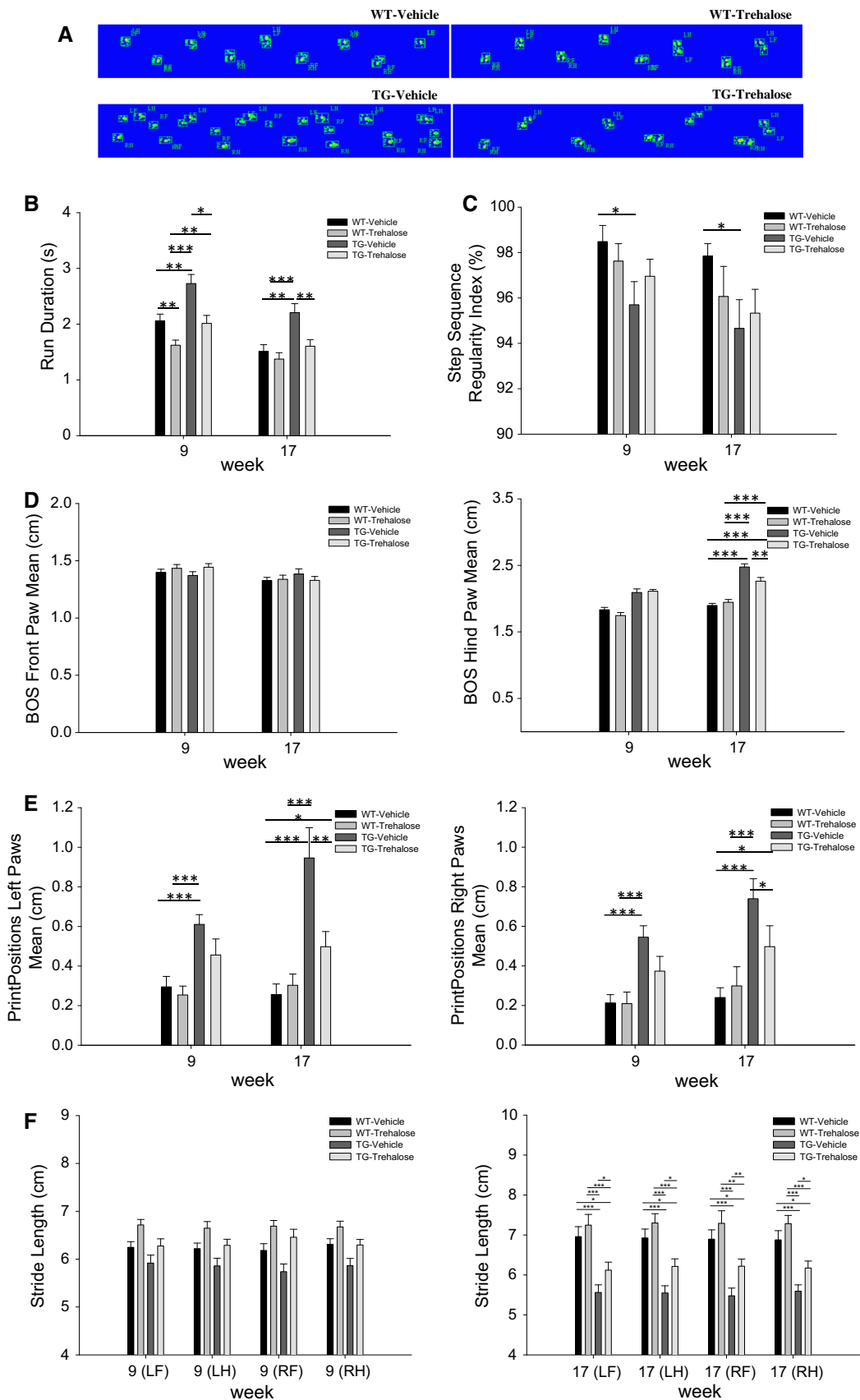


Fig. 5 The gait coordination of the mice was determined by a footprint behavior test during the treatment. **a** Footprint pattern, **b** run duration, **c** step sequence index, **d** BOS mean of both left and right paws, **e** print position of both left and right paws, **f** stride length of both left and right sides were all improved in the TG mice after trehalose treatment, $*p < 0.05$; $**p < 0.005$; $***p < 0.001$ ($n = 13$)

4–30 rpm in 5 min. We suspected that the condition was too rigid to distinguish the treatment group from the vehicle group. After modifying the rotarod condition to 26 rpm fixed speed, we observed the differences between

these two groups. This condition was further employed in the 4 % trehalose treatment study. Figure 4d shows the result and reveals that 4 % trehalose improved the SCA17 mouse motor coordination. Footprint analysis further confirmed that 4 % trehalose helped the mouse gait coordination. We also found the TG mouse cerebellar atrophy was ameliorated by trehalose, however, the TBP aggregations were not reduced by trehalose treatment as in the slice culture, which could be attributed that the TBP aggregation is localized in the nuclei which is less susceptible than the

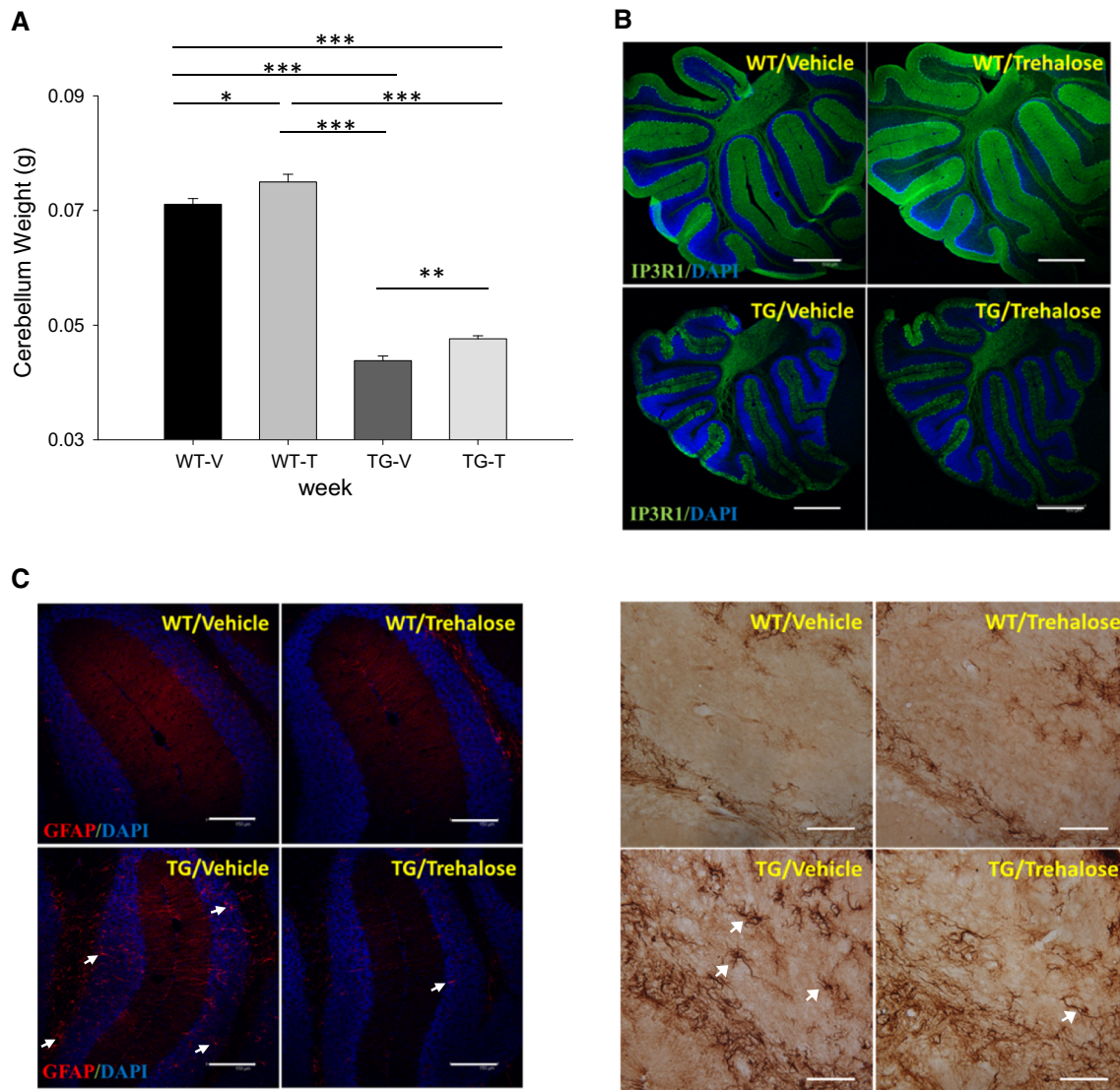


Fig. 6 Evaluation of the neuropathological effect of 4 % trehalose treatment on the 20-week-old mice. **a** The TG mouse cerebellar weight was severely reduced compared to the WT mice; this was significantly ameliorated after trehalose treatment, $*p < 0.05$; $**p < 0.005$; $***p < 0.001$ ($n = 13$). **b** The cerebellar morphology and Purkinje layers were identified by immunofluorescent staining with IP3R1 antibody. The cerebellar atrophy of TG mice was notably ameliorated after trehalose treatment as revealed by the enlarged

section areas of the cerebella (4–6 slices per mouse; $n = 3–5$). **c** The gliosis of the SCA17 mouse cerebellum was stained with GFAP antibody in immunofluorescent (*left*) and immunohistochemical (*right*) analysis. The trehalose treatment significantly reduced the gliosis in the cerebellar lobes of SCA17 mice (4–6 slices per mouse; $n = 3–5$). *Arrows*, astrocytes. *Scale bar* 600 μm (**a**), 75 μm (**b**, *c*, *left*), and 150 μm (**c** *right*)

cytoplasmic aggregation of other diseases [30, 31]. Furthermore, the penetration of trehalose in the intact brain is not as good as in the slice culture.

In our previous study, hyperactivity was reported to be one of the behavior markers in our SCA17 transgenic mice [19]. In the present study, we could observe that after 4 % trehalose treatment, the total distance analyzed by locomotor was notably reduced in the transgenic group, indicating that trehalose could ameliorate the hyperactivity of SCA17 mice. These data reveal that trehalose had a neuroprotective effect on SCA17 mice both in vitro and in vivo.

In this study, we also monitored the water-drinking level (data not shown). We found that the trehalose-treated mice drank more water than the vehicle group. We speculate the reason is because the sweet taste of the trehalose water; however, the trehalose consumption did not influence the mouse body weight. In addition, the trehalose water had no effect on mouse blood glucose, indicating that the trehalose administration did not raise the blood glucose, which could harm the mice. Interestingly, we found the blood glucose was significantly reduced in SCA17 transgenic mice at 5 weeks of age. Although no report pointed out that the blood glucose was affected in SCA patients, the hypometabolism phenomenon was observed by positron emission tomography (PET) with 2-[fluorine18]-fluoro-2-deoxy-D-glucose in the cerebellum of SCA patients [32], indicating that the dysfunction of energy metabolism might be another pathology of SCAs. However, at this moment, we do not know whether our SCA17 mice have a defect in energy metabolism or whether the trehalose treatment has any effect on the hypometabolism phenomenon of the cerebellum.

Gliosis is observed as a neuron degenerative marker in SCA17 mice [9, 19]. In this study, we could also detect that the astrocytes were highly activated in the transgenic mice. After trehalose treatment, the level of active astrocytes was reduced, suggesting that the trehalose attenuated the astrogliosis and neuron degeneration. It was reported that astrocytic hypertrophy induced by MPTP in a PD mouse model was greatly reduced by trehalose [28]. A similar effect was observed in the isoflurane-induced amyloidogenesis mice [33]. These reports and our results all indicate that trehalose is effective in protecting against neuroinflammation.

In sum, our data provide evidence that trehalose treatment has a neuroprotective effect on SCA17 transgenic mice. Together with the evidence from HD, SBMA and the SCA14 models, this natural disaccharide might have potential in treating polyQ diseases.

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Conflict of interest None.

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