國立臺灣師範大學生命科學系 碩士論文

評估海藻糖對第十七型脊髓小腦萎縮症小鼠及組織切片培養之作用

Evaluation of the efficacy of trehalose through the mouse cerebellar organotypic slice culture and SCA17 transgenic mice

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致　謝

說起自己實驗室生涯，真的是血淚交織。
大三毅然決然地放棄選修教育學程，而進入謝老師的實驗室，相信往後在人生的回眸裏，一定依然是一個關鍵的轉捩點。

我不後悔。相信未來的自己，亦不會對這個決定感到悔恨。

因為自己從大三、大四，乃至碩一。在謝老師的實驗室中，我學到相當紮實的實驗技巧，縝密的研究思維，最重要的，是我在這三年內，實現了我從小到大想進實驗室的夢想。

雖然之後的人生規劃，似乎不見得再與研究有很直接的接軌，但在這實驗室中點點滴滴的磨練，已儼然內化成我人格中的一部分。往後無論遇到甚麼樣的困境，相信自己亦會以碩士班中訓練的嚴謹態度，追根究柢的精神，迎刃而解。

俗話說：不經一番寒徹骨，焉得梅花撲鼻香？
碩士班的生涯裡，生活總是圍繞著實驗室，有時一天沒有走進動物房，就會覺得良心不安；有時要趕著生 Data 時，更常常得要在實驗室裡過夜。還記得當時寢室的室友最常與我問話便是：「你昨天真的有回寢室嗎？」
然其實我常常在他們已經睡熟了之後才回到宿舍，而在室友們還沒清醒的時就已經整裝回到實驗室實驗。

辛苦。這樣子的日子就算現在回想起來，都仍有點不寒而慄。然而，要不是經過這樣的磨練，下了這樣的決心，我想最後的果實勢必不會這般甜美。

而在甫屆踏入社會的年紀，我也不禁茫然：人世中的哪一樁情事，不也是如此呢？我們往往都只看到檯面上成功的光采，卻忽略了背後的辛勞。

感謝研究所的這些歲月，讓我明白，往後無論面對何種挑戰，都要全力以赴；無論碰上多大的困境，都要不屈不撓，屢敗屢戰。
孟子曾說：苦其心志，勞其筋骨，餓其體膚，空乏其身，行拂亂其所為，所以動心忍性，曾益其所不能。
而在我即將脫離學子身分的前夕，回眸自己一路走來，歪歪倒倒的斑斑足跡，不禁充滿感激的潸然。

感謝指導教授 謝老師，十分感念您當時在我大三時，不曾嫌棄我緩慢的實驗進度，甚至在學長姐交織的責備聲中仍不斷鼓勵我，使我得以慢慢成長茁壯；而在您這三年如沐春風的指導裡，您永遠是我心目中學者的典範。

感謝親身教導我實驗技巧的 陳偉毅 學長。我一直都不是一個聰明伶俐的好學弟，時常犯一些令你匪夷所思的離譜錯誤 …。感謝你最終還是願意扶持我，讓我得以扛起自己的實驗擔子。

感謝時常被我死纏著問問題的 張雅津 學姊。我沒有一點就通的資質，總是一個問題問了又問問了又問，最後還煩著您親自指導我實驗上的困境。沒有您，我的碩士生涯必然荊棘叢生。

感謝常常與我一起討論實驗和解決實驗問題的 許振銘 學長 和 陳薇琳 學姊。你們是我實驗的最好夥伴！與你們一同在無垠的研究汪洋裡披荊斬棘，衝鋒陷陣的日子該會是我實驗室生涯中一段美好的回憶！

也相當謝謝跟著我的兩個學弟：育晨與建民。我知道對於你們我總是比較嚴格，也相當囉嗦；一份 paper 總是要你們修了又修，講了又講。實驗的部分更是叮嚀又叮嚀，重做又重做。但希望你們都能理解，對於你們，我總是發自內心的希望你們實驗順利，研究生涯愉悅；最後，祝福你們都能如期畢業！

感謝所有 D309 實驗室的成員，沒有你們在這之中的點綴，我的實驗室生涯必然黯然失色！每個人，都是一副完美拼圖中不可或缺的一塊。你們都是！缺一不可。

感謝 Image Core 的 陳映玲 學姊。我的 data 大概有 3/4 都是從 confocal 出來的，實在很謝謝你總是不厭其煩地在非常時間把儀器借給我使用，還得不時地被我纏著詢問相關問題 ……。希望妳接下來的研究生涯也可以一直很順利！

當然，我的所有老鼠夥伴們！雖然你們最終都被我親手犧牲了，但希望你們在天之靈，都能了解沒有你們，我們對於自然界的認知，就會停滯不前。你們永遠是最高貴的靈魂，無可取代！

要感謝的人事物實在太多，族繁不及備載。然追憶卻似水年華。
我深知自己能走到今天的成就，絕非自己的功勞，而是來自於身旁大家不吝惜地伸出援手。且讓我，在往後能為各位祝福。

學者陳之藩曾說：得之於人者太多 出之於己者太少。
願，所有於我有恩的各位，都能平安，喜樂。

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Abstract

Spinocerebellar ataxia (SCA) is an autosomal dominant and progressive neurodegenerative disease chartered by ataxia, parkinsonism, dementia and seizures. Although there remains lots unclarified mechanism in SCA17, it is believed that the mutation on the TATA box binding protein (TBP) is responsible for the disease. The CAG repeat expansion of *TBP* gene leads to the reduced solubility of polyglutamine (polyQ) TBP and induces aggregate formation. For TBP plays an important role in transcription initiation, the abnormal aggregate is believed to cause neuron degeneration especially in the cerebellar Purkinje cells. Cerebellar organotypic culture is a system which could provide research evidence on tissue level. In addition, the cerebellar organotypic culture could provide the normal interaction between Purkinje cells and the other cells *in vitro*. We have established this system to study and monitor the cerebellar cell development, neuron survival, Purkinje cell aggregate forming and death and for a drug screening platform. Trehalose is reported to prevent protein degradation and aggregate formation in several disease models, including Huntington’s diseases, Alzheimer's disease, SCA14 and some other neurodegenerative diseases caused by polyQ expansion. In this study, we evaluated the therapeutic effect of trehalose using SCA17 cerebellar organotypic culture system. Our results showed that TBP aggregation formed in the Purkinje cells at in vitro day 3 (DIV3) and became more obvious at DIV7 in the SCA17 cerebellar slice culture. Furthermore, we found that the TBP aggregation significant decreased in our slice culture

at DIV7 after treatment with trehalose. To identify the effect of trehalose in vivo, trehalose supplied in the drinking water of SCA17 transgenic mice was conducted. In the behavior test, we found that mice drinking trehalose ameliorated their hyperactivity and improved their coordination in rotarod test. Furthermore, we confirmed that the calbindin expression level was upregulated in the trehalose treatment mouse cerebellum. In addition, the cerebellum size in trehalose treatment mouse is bigger than that of vehicle treatment mouse. In our 4% trehalose treatment study, we found the gait behavior and motor coordination of SCA17 mice were rescued in the footprint and rotarod task, respectively. We also could observe the astrocyte gliosis performance was downregulated after trehalose treatment. However, the microglia cell was activated especially in transgenic trehalose treatment group. Furthermore, the MnSOD was also upregulated after trehalose treatment. These data suggest that trehalose could be a potential non-toxic treatment for SCA17.

Key words：neurodegenerative diseases, spinocerebellar ataxia, trehalose, purkinje cell

Introduction

Polyglutamine Diseases (PolyQ diseases)

Polyglutamine diseases are hereditary neurodegenerative diseases caused by CAA/CAG repeat sequence excessive expansion. The abnormal expanded sequence will cause the polyglutamine (polyQ) overextended in the diseases coding gene and this abnormal protein is believed to result in disease's pathology. Huntington's disease (HD), Dentatorubral Pallidoluysian atrophy (DRPLA), Spinal and bulbar muscular atrophy (SBMA), and Spinocerebellar ataxias (SCA) are the subtypes of polyQ diseases (Orr and Zoghbi, 2000).

Neuron cell degeneration is the major symptoms of polyQ diseases, but no exact pathogen and reason is identified until now. However, the abnormal expanded polyQ will make the protein misfolded and deregulated is believed to be one of the possible reasons. Besides, these abnormal polyQ proteins will be insoluble and accumulate in cell and make the cell died (Schaffar et al., 2004, Nagai et al., 2007). On the other hand, the polyQ-expanded protein will be cleaved by proteases, and the truncated protein will form the insoluble inclusion body in cell even easier than full-length protein. The inclusion body with mutant protein will compete with the normal protein and result in cell degeneration (Raspe et al., 2009). Furthermore, the polyQ-expanded protein was reported to enhance the abnormal interaction with transcription factor and

cause the neuron death (Okazawa, 2003). The imbalanced protein kinase function and mitochondrial efficiency lose are also reported to participate in the polyQ diseases pathology (Bence et al., 2001, Lin and Beal, 2006).

Spinocerebellar Ataxia

Spinocerebellar ataxia (SCA) is an autosomal dominant hereditary disease and mostly caused by abnormal polyQ-expansion and result in neurodegeneration, mainly in cerebellum and spinal cord. Furthermore, severe brain atrophy is also found in the progressive pathology of patients.

Although the diseases-causing genes are different, gait instability and dystonia are the collective phenotype within patients. The neuron degeneration in cerebellum and spinal cord are the major reason for these apparent symptoms in SCA. However, the detail pathological progress and molecular cell signaling transduction pathway about SCA are still unclear. There are many patient family fall in the disease progress in various age and have diverse pathological phenotype. However, some people with abnormal extended polyQ sequence but never suffered from SCA (Schelhaas et al., 2000). Therefore, more effort is need to uncover the pathogenesis of these polyQ diseases.

Spinocerebellar Ataxia type 17

Spinocerebellar ataxia type 17 (SCA17) is a subtype of SCA. Ataxia, dementia, psychiatric symptoms, cognitive dysfunctions, spasticity,

dystonia, chorea and parkinsonism are the clinical symptoms of SCA17 (Friedman et al., 2007). The TATA-box-binding-protein (TBP) gene localized on autosomal chromosome 6q27 (Koide et al., 1999), is a transcription factor and playing an essential role in three types of RNA polymerase (Gill and Tjian, 1992). SCA17 is reported to be caused by the CAG sequence repeat expanded in TBP and induce the neurodegeneration (Koide et al., 1999). Alter the length of N-terminal polyQ is reported to change the TBP normal function (Friedman et al., 2007). The length of polyQ in normal person was between 25~42 repeats (Gostout et al., 1993), and the clinical research has found that the person will suffer from the SCA17 if the polyQ repeats beyond the number (Nakamura et al., 2001).

The onset of SCA17 patient is mostly after their middle age, however the truly beginning of SCA17 is depend on the length of polyQ and the sensitivity to the toxic protein (Fujigasaki et al., 2001). Cerebellar atrophy and Purkinje cell degeneration is the major clinical pathology of SCA17 (Friedman et al., 2007). Besides, there is gliosis in the brain of SCA17 patients (Toyoshima et al., 2004).

Although it is still unclear about the pathogenesis of SCA17, it is reported that the abnormal expanded polyQ in TBP will enhance the interaction between the transcription factor like TF Ⅱ B and TBP. The irregular interaction will compete with the interaction with normal TBP to induce the cell toxicity and neuron to degenerate (Friedman et al., 2007).

Organotypic Cerebellar Slice Culture

Organotypic slice culture has been established in various regions like striatum, hippocampus, cortex, spinal cord and cerebellum for many years (Newell et al., 1995, Ostergaard et al., 1995, Krassioukov et al., 2002, Rytter et al., 2003, Birgbauer et al., 2004). To maintain the slice long-term survival and sufficient supply of oxygen, many experimental methods like “roller tube ” and “membrane interface” are tested. In “roller tube”, the slice is glued onto a glass coverslip and placed in a glass tube, the tube will rotated slowly. The slice will be exposed in medium for half of the time and exposed in oxygen in the other time (Gahwiler, 1981a, b). The “membrane interface” culture method is cheaper and more convenient than the “roller tube” (Gahwiler, 1981a, Stoppini et al., 1991). The “membrane interface” culture used the semi-permeable porous membrane to maintain the organotypic slice absorb the nutrient from the medium, in the other hand, the membrane will keep the slice between atmosphere and medium to maintain the slice well survived (Bergold and Casaccia-Bonnefil, 1997). This arrangement allows the slices can be maintained in 6-well plates without any special rotation incubator. Therefore, the “membrane interface” slice culture method replaced the “roller-tube” method in recent years.

During the cerebellum development, Bergmann glia cells play an important role for granule cells (Spacek, 1985, Grosche et al., 1999) and Purkinje cells to be the scaffold (Yamada et al., 2000, Lordkipanidze and

Dunaevsky, 2005). Furthermore, in Bergmann glia cell development, the glia morphogenesis will enhance the Purkinje cell synaptogenesis (Lippman et al., 2008). However, the oligodendrocytes are reported to be the central role in cerebellar circuits' formation (Doretto et al., 2011). It is reported that the NG2-positive oligodendrocytes will participate in Purkinje cell and climbing fiber synapses formation (Lin et al., 2005). In addition, the cerebellar slice size was smaller and the Purkinje cell dendritic tree was also stunted in an oligodendrocyte-depleted condition. In addition, the glia cells were also reduced in this model (Mathis et al., 2003, Collin et al., 2004).Therefore, to maintain the normal Purkinje cell development and functioning, it is necessary to keep the different types of cerebellar cells in correct interaction.

Organotypic slice culture is a good semi-*in vitro* system to study the intracellular pathway leading neuron degeneration (Dusart et al., 1997, Ghoumari et al., 2000). In addition, nervous system slice culture was believed to be a powerful tool for the study of neural disorders as the slices maintain the neuron complex cell relationships and network that make the system closely represented the *in vivo* environment (Gahwiler, 1981b, Stoppini et al., 1991). It is reported that organotypic slice culture is also a good system to study the cell interaction and functional change in neuron diseases such like multiple sclerosis (Davids et al., 2002, Birgbauer et al., 2004, Mulholland et al., 2005).

Furthermore, to find the potential candidate drug to rescue the disease,

slice culture has also been set up for a drug screen platform because of the advantage of the well keeping 3D morphology(Meng et al., 2007). In recent study, the organotypic cerebellar slice culture has been used to study the interaction between the neuron stem cell and Purkinje cell in SCA model (Lu et al., 2011). There is also study reported that the slice culture is a good model to research the relationship between Purkinje cell and the exogenous compounds (Hill et al., 2009). Taking together, the organotypic slice culture is a nice system to be utilized turnover the SCA17 cell degeneration pathogenesis and find the potential drug to rescue the phenotype.

Trehalose

There are lots of neuron degeneration are attribute to misfolded protein which forming the aggregation, such as Alzheimer's diseases (AD), Huntington's diseases (HD), Parkinson's diseases (PD) and Prion diseases (Beranger et al., 2008). In addition to abnormal aggregation, neuron inflammation is also reported a progressing clinical pathology in cancer, chronic diseases, nerve diseases and psychological diseases (Coussens et al., 2002, Laake et al., 2004, Mantovani, 2005).Therefore, choose the candidate drug which can anti-inflammation and inhibit the abnormal protein aggregation would be potential for rescue the chronic neurodegeneration diseases.

Trehalose is a disaccharide which general exist in yeast, bacteria and invertebrate. There is reported that trehalose can help cell to resist the

stress like hydration, and oxidation by protecting the protein from denaturing (Chen and Haddad, 2004).

In AD study, there is reported that trehalose can reduce the tau protein hyper -phosphorylation and neuron inflammation. Furthermore, the trehalose treatment can diminish the abnormal amyloid β (Aβ) protein forming aggregation and ameliorate the AD pathology in AD transgenic mouse model (Beranger et al., 2008). In addition, adding trehalose in drinking water significantly reduced the mutant huntingtin protein becoming the toxic inclusion body and rescued the motor function of HD transgenic mouse model (Tanaka et al., 2004).The study also point out treating the trehalose in SCA14 Purkinje primary cell helped the cell resume the normal function and morphology (Seki et al., 2010). Trehalose may play the role of chemical chaperone to ameliorate the toxic protein aggregating and rescue the disease pathology (Seki et al., 2010).We presume the ability of anti-aggregation and anti-inflammation of trehalose will be a positive candidate to cure SCA17, another polyQ neurodegeneration diseases.

Material and Method

SCA17 transgenic mice

The FVB/N transgenic mice with 109 poly-glutamine (109Q) expanded in human TBP (hTBP) driven by PCP2/L7 promoter, was established in our laboratory (Chang et al., 2011).Transgenic line 69 was utilized in this study and maintained by breeding heterozygous with FVB/N wild type mice (National Laboratory Animal Center, Taipei, Taiwan). The transgenic mice showed the phenotype of ataxia and Purkinje cell progress degeneration. In addition, the mice have the poor rotarod performance (Chang et al., 2011). We keep the mice in Individually Ventilated Cages (IVC) system under 12hours/12hours light and dark cycle. All the animal experiment was carried out in accordance with the guideline and approved by the National Taiwan Normal University Research Committee.

Tail DNA extraction

Mouse tail about 2 mm was chopped and incubated in Direct PCR buffer (Viagen Biotech, Los Angeles, USA) with 50μg/ml proteinase K at 55℃for 5.5 hours for digestion of the protein. After the incubation, we extracted the DNA from the supernatant and stored in -20℃ for further genotyping analysis.

Genotyping analysis

For identifying the mice's genotype, we use PCR analysis with primers, PL7-F (5'-TAT GGT GAG AGC AGA GAT GG-3'), TBP-3R (5'-CTGCTGGGACGT TGACTGCTG-3'), SRY-F'(5'-GAATA TTCCC GCTCT CCGGAG-3') and SRY-R'(5'-ACCTG TTGTC CAGTT GCACT-3'). Under the condition of 95°C 1 minutes for denaturing, 68℃ 1 minutes with -0.1℃ touch-down in each cycle for annealing and 72℃ 1.5 minutes for elongation for 35 cycles. After the PCR amplification, we load the sample in 2%agarose (Genetek Biosciences, Maharashtra, India) for checking the product. The 456 bps fragment product would be the amplified transgenic DNA. In addition, the male mice would also have the 294 bps DNA fragment amplification product of SRY gene.

Organotypic Cerebellar Slice Culture

The cerebellar slice culture protocol was modified from a previous report (Birgbauer et al., 2004). Briefly, the postnatal day 7 SCA17 transgenic and wild type littermate mice were decapitated. The whole brain was taken out and transferred to ice-cold culture medium containing 50% Basal Medium Eagle (Invitrogen, Grand Island, USA), 25% Hank's Buffered Salt Solution (Invitrogen), 25% Horse Serum (Invitrogen), 0.5%D-(+)-Glucose (Sigma, Saint Louis, U.S.A), 1mM GlutaMAX Ⅰ (Invitrogen), and 1mM Penicillin/Streptomycin (Invitrogen). We separate the cerebellum from the other brain regions in ice-cold medium, and wrapped the hemisphere with low melting point agarose (Bio Basic,

Markham Ontario, Canada)in 1X D-PBS (Invitrogen). The cerebellum was then cut into 400μm parasagittal sections with Vibratome (VT1200S, Leica, Germany). To improve the survival rate of cerebellar slices, we also supplied 95% O_2 injecting in buffer during the sectioning. We then transferred the slice to membrane of 30 mm culture plate inserts with 0.4 μm pore size (Millipore, Billerica, USA) in six-well plates. The cerebellar slices were maintained in membranes with 1 ml culture medium at 37℃ in an atmosphere of humidified 5%CO_2.

Wholemount Immunostaining

The cultured cerebellum slices were fixed in 4%paraformaldehyde (Sigma) in PBS containing 0.2% triton X-100 for 30 minutes at room temperature. After fixing the slices, we washed the slice for 3 times with the PBS containing 2%triton X-100 for 15 minutes. The cerebellar slices with membranes will be cut and put into the 24-well-plate and incubated overnight in 10%horse serum (Invitrogen)with the PBS containing 0.2% triton X-100 at 4℃. The primary antibodies (Table 1) were incubated in 5%horse serum with the PBS containing 0.1% tween-20 at 4℃ for 2 days. We further enhanced the primary antibody binding efficiency by 37℃incubation for 30 minutes, and then wash the slice with the PBS containing 0.1% tween-20 at room temperature 15 minutes for three times. The cerebellum sections were then incubated with secondary antibodies (Table 2) in PBS with 5%horse serum in the PBS containing 0.1% tween-20 at 37℃ for 2 hours. Afterwards, we washed the sections with PBS with 0.1% tween-20 at room temperature 15 minutes for three times.

Finally, the cerebellar slices were mounted on slides with mounting medium (SouthernBiotech, Los Angeles, U.S.A) for confocal (DMRE, TCS SP2, Leica) observation.

Western Blot

The mice were anaesthetized by 2.5%avertin (Sigma) intraperitonal injection (IP) and perfused with 0.9% sodium chloride(Sigma). The cerebellum was removed from the mouse brain and homogenized by sonicator (Microson XL 2000, Misonix, Newton, Australia) in RIPA solution[5 mM EDTA, 10 mM Tris (pH 7.4), 150mMNaCl, 0.1%SDS, 1%DOS, 1% NP40] containing proteinase inhibitor (Thermo, Waltham, USA) and phosphatase inhibitor (Sigma)on ice. After 30 minutes reaction on ice, the protein was collected in supernatant after 30 minutes centrifugation at 12000 rpm. For protein quantification, we used the BCA Protein Assay Kit (Thermo) to measure the protein concentration of each sample.

About 50 μg protein was loaded in each well for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in running buffer [192 mM Glycine (Sigma), 25 mM Tris Base (Bionovas, Ontario, Canada) in ddH2O] and transferred to polyvinylidenefluoride (PVDF, Millipore) membrane in transfer buffer [192 mM Glycine (Sigma), 25 mM Tris Base, 20% v/v methanol (GeneStar, Shanghai, China) in ddH_2O]. The polyvinylidene fluoride membrane was then incubated in 5% skim-milk for blocking the nonspecific epitopes binding for 2 hours. The primary antibodies (Table 1)were incubated in TBST contained 0.05% tween-20 at

4℃overnight. The membrane was washed with TBST contained 0.05% tween-20 five time for 15 minutes and then incubated with the horseradish peroxidase (HRP) labeling secondary antibodies (Table 2) in TBST contained 0.05% tween 20 for 2 hours. The membranes were finally washed by TBST contained 0.05% tween 20 before observing the signal images by LAS 3000 image system (LAS 3000, Fujifilm, Tokyo, Japan).

in vitro Trehalose treatment

At day *in-vitro* 1 (DIV1), we divided the cerebellar slices into6 groups based on the result of genotyping: Wildtype-ddH_2O, wildtype-trehalose (Gemfont Corporation, Taipei, Taiwan), wildtype-glucose (Sigma), SCA17-ddH_2O, SCA17-trehalose and SCA17-glucose. We tested the effect of 100μM and 500 μM trehalose or glucose and the medium was changed twice a week. At DIV7, we fixed and whole mountimmuno stained the sections. Counting the both IP3R1 and 1TBP18 double positive cells to estimate the trehalose efficiency in SCA17 cerebellar slice culture system.

in vivo Trehalose treatment

The 4% trehalose was added in ddH_2O for treatment and ddH_2O was used as vehicle. The treatment began at postnatal day 21 (P21) and finished at 20week old of mice. We changed the trehalose once a week and monitored the mouse body weight, drinking capacity and eating capacity every week.

Cryosections preparation

The mouse was anesthetized with avertin (0.4 g/kg body weight). After anesthetization, the mouse was perfused with 0.9% sodium chloride and 4% paraformaldehyde (Sigma) in PBS. We will take up the whole mouse brain and then postfix the brain in 4% paraformaldehyde for 4 hours at 4℃. The dehydration was performed in 10% sucrose (Bionovas) for 1 hour, 20% sucrose for 2 hours and 30% sucrose overnight. We removed the cerebellum from the whole brain and then the cerebellum was sectioned into 30 μm by LIECA CM3050 S cryostat-microtome (CM3050S , Leica)

Fluorescent Immunohistochemistry

The cerebellar sections were washed with TBST contain 2% triton X-100 10 minutes for three times and then the sections were incubated in 0.1M boric acid (Bionovas) for 5 minutes. The sections were washed with TBST contain 2% triton X-100 10 minutes and we transferred the cerebellarslices to retrival buffer(DAKO, San Antonio, USA) at 80℃for 30 minutes. After retrival, the slices were washed with TBST contain 2% triton X-100 10 minutes for three times. The slices were blocked with blocking buffer [10% horse serum and 1% BSA in TBST contain 0.2% triton X-100]for 2 hours at room temperature and then the cerebellum sections were incubated with primary antibodies (Table 1)in TBS contain 5% horse serum and 1% BSA at 4℃ overnight. After another wash with TBS three times for 10 minutes each time, the secondary antibodies (Table 2)in TBS was incubated with brain slices for 2 hours at 37℃. The

sections were washed with TBS 10 minutes for three times and then the DAPI was stained in TBS for 30 minutes at 37℃.Finally, the cerebellum sections were mounted on coating slide with mounting medium for confocal.

Behavioral testing

Rotarod

We used the Rotarod (UGO BASILE, Italy) to measure the mouse coordination and motor function, The SCA17 transgenic mice and the wildtype littermates with trehalose and vehicle treatment were applied to the rotarod behavioral test every two weeks during the 4-to 20-week-old of age. Before the rotarod test, we handled the mouse for five days to let it be used to our hand to avoid the stress and then we trained the mouse for 4 days to learn and memorize the rotarod speed. After the training, we performed the handling for two days before each rotarod experiment. The rotarod condition is a fixed speed at 26 rpm for 200 seconds. If the mice fell down the machine or held the rod and spun around without attempt to walk anymore, we recorded the time of latency. The rotarod test performed with three trials per day for analysis.

Locomotor

We perform the locomotor experiment at the mouse age of 5,11and 17 week. The SCA17 transgenic mice and the wild type littermates with trehalose or control treatment (n=13) were placed and monitored in an

open field black box (30 x 30 x 30 cm) for 600 seconds. We used the EthoVision system (Noldus, Nederland) to record and analyze the mouse total horizontal move distance, velocity and the path of moving during the experiment.

Footprint

We performed the footprint experiment with the mouse age at 9 and 17 weeks, respectively. The SCA17 transgenic mice and their wild type littermates with trehalose or control treatment (n=13) were monitored by the CatWalk XT system (Noldus). First of all, we put the mouse on the glass plate of the CatWalk XT system to modify the intensity threshold for the correct footprint of the mouse. In the proceeding experiment, we let each mouse run for three times and record their footprint data. The data were analyzed by the CatWalk XT 9.1 software (Noldus).

Homecage

To analyze the common behavior of mouse, we performed the homecage behavior analysis with the mouse at age of 18 weeks. The SCA17 transgenic mice and their wild type littermates with trehalose or control treatment (n=4) were put in their home cage individually for overnight before the experiment. After the mouse got used to the cage they lived, we recorded the mouse behavior for 12 hours by the video camera at night. The video was analyzed and exported by the Home Cage Scan (Clever Sys. Inc., USA).

Statistical analysis

All values are expressed as mean and the error bar are expressed as SEM. We use the independent *T*-test to determine the significance of difference between groups by SPSS analysis software.

Results

No TBP aggregation was identified at postnatal day 7 transgenic mouse cerebellum

Our previous study has shown that polyQ-expanded TBP could form aggregation in transgenic mouse cerebellum (Chang et al., 2011). For TBP being an important transcription factor, it is believed the TBP abnormal aggregation would impair the transcriptional regulation and lead to neurodegeneration. Furthermore, we also detect the vacuoles in some Purkinje cell with aggregation (date not shown). The Purkinje cell vacuoles was believed to be a sign of degeneration (Vig et al., 2009).

To develop a drug screen platform from SCA17 mice, we first identified the aggregation onset of the transgenic mice. TBP aggregations in cerebellum at postnatal day 7 (Figure 1A and 1B) and 14 (Figure 1C and 1D) were examined. However, we could not detect any aggregation at postnatal day 7, indicated that the aggregation occurred after postnatal day 7. We also could not observe TBP aggregation at postnatal day 14; however, there were higher 1TBP18 signals in transgenic Purkinje cells (Figure 1D). These data reveal that the onset of TBP aggregation formation is between postnatal after 14.

TBP aggregation in SCA17 cerebellar organotypic slice culture is formed between DIV1 and DIV3, and the aggregation progress with time

We followed the condition for organotypic cerebellar slice culture protocol as previous reported (Zanjani et al., 2009). Briefly, we isolated the cerebella from the postnatal day 7 mice and sliced into 400 μm. After had cultured for 7 days, we fixed the slice and stained with antibodies (Figure 2A).

First of all, we observed the *day-in-vitro* (DIV) 7 organotypic cerebellar culture slice has normal phenotype. The dendritic tree and axon of Purkinje cells kept on the correct place similar to their *in vivo* condition (Figures 2B - 2D) The molecular, granular, and Purkinje layers were all at the correct place as same as the fresh dissected cerebellar slice from mice. We also examined the TBP aggregation in organotypic cerebellar slice culture. To identify when the TBP aggregation was formed, we examined the 1TBP18 puncta in Purkinje cell on slice culture. As shown in the previous study, we found there was no significant difference between wildtype and transgenic mouse cerebella at postnatal day 7 (Figure 2A and 2B).

On the DIV 1 culture, we also didn’t detect any positive 1TBP18

puncta in the Purkinje cells (Figures 2E-2F). However, the positive signal was observed at DIV 3 (Figures2G-2H). The results show that the TBP aggregation is serious at DIV 7 (Figures 2I-2J). In addition, we found there were more 1TBP18 puncta at DIV 7 than DIV 3 (Figures 2G-2J). These results indicate that the TBP aggregation is progressive in a time-dependent manner during the *in vitro* culture.

Evaluation of Chinese herbs/compounds through the mouse cerebellar organotypic slice culture

To identify the potential drugs to rescue the SCA17 pathology, we screened the Chinese herbs/compounds with our cerebellar organotypic slice culture. NH-005, NH-006, NH-016, NH-008-1, and trehalose were identified as potential herbs/compounds for which could reduce aggregation in polyQ SCA3/17 cell lines (personal communication). After treatment of NH-005 (100 μg/ml) or NH-006 (100 μg/ml), we found that the 1TBP18 puncta was significantly reduced in Purkinje cells (Figures3A-3B). For NH016 (100 μg/ml) and NH-008-1(100 and 500 nM), there was no significant improvement in ameliorating of the 1TBP18 puncta in Purkinje cells (Figures3C-3D). Trehalose has been shown to have a positive curative effect and prevented the abnormal protein forming aggregation in Huntington's diseases (Tanaka et al., 2004) and SCA14 (Seki et al., 2010) models. We also found that the TBP aggregation in Purkinje cells was significantly reduced after 100 μM treatment (Figure 3E).

Evaluation of 2% trehalose treatment efficacy through the SCA17 transgenic mice

To identify the trehalose efficacy *in vivo*, we applied 2% trehalose into mouse drinking water during the treatment. The mouse body weight was monitored every week. The results showed that there are no significant differences between treatment and vehicle (Figure 4A). The first rota-rod accelerating condition (4-30 rpm) couldn't distinguish the treatment and vehicle groups (Figure 4B), we changed the rota-rod condition to a fixed speed (26 rpm). We found that the trehalose treatment group performed better than vehicle treatment group in this condition (Figure 4C).

We sacrificed the mice for pathological examination after trehalose treatment for 17 weeks. First of all, we found that the size of cerebellum was larger in treatment group (Figure 4D), indicated that the trehalose treatment might rescue the SCA17 cerebellum atrophy phenotype. We also confirmed the trehalose positive effect in western blot analysis. A significant resume in calbindin expression was identified in trehalose treatment group (Figure 4E).The Purkinje cell morphology and IP3R1 intensity was improved after 2% trehalose treatment. We could observe better Purkinje cells dendritic tree morphology in treatment group than vehicle group (Figure 4F). However, we found that the SCA17 transgenic

mouse cerebellar Purkinje cells TBP aggregation was not significantly affected after trehalose treatment (Figure 4F). However, we could detect the astrocytes gliosis was reduced after 2% trehalose treatment (Figure 4F).

Evaluation of 4%trehalose treatment efficacy through the SCA17 transgenic mice

Since the 2% trehalose could not reduce the abnormal TBP aggregation *in vivo*, we performed the 4% trehalose treatment for SCA17 mice. First of all, we checked the 4% trehalose stability within our treatment condition by HPLC analysis (Figure 5A). To understand whether the high concentration trehalose would harm the mice, we monitor the blood glucose during treatment. The results showed that the growth of body weight had no significant difference between treatment and vehicle group (Figure 5B). Furthermore, the blood glucose were also keep in the normal level (Figure 5C), indicated that the 4% trehalose would not harm the mice. After 4% trehalose treatment for 4 month, we could observed the SCA17 mouse cerebellum weight was significantly rescued (Figure 5D), indicated that the trehalose could ameliorate the SCA17 cerebellum atrophy performance. To confirm this result, we perform the western blot with the antibody of Purkinje cell marker, calbindin. We could also observe the calbindin level was slightly rescued after 4% trehalose treatment (Figure 5E).

In our behavior test, we performed the rota-rod, locomotor, footprint and homecage analyses (Figure 6A). There was only slight improvement in the locomotor (Figure 6B) and rota-rod (Figure 6C) in the treated SCA17 mice, indicated that the trehalose treatment might resume ataxia performance in SCA17 transgenic mice.

In our footprint experiment, we could observe that the footprint of transgenic vehicle group was disordered. After drinking 4% trehalose, the footprint resume to a more regular pattern (Figure 6D). As reported in the previous study, *Lurcher* mice whose Purkinje cell degeneration caused by spontaneous mutation had altered footprint behaviors in “Run duration”, “Step Sequence Regularity Index”, “Bass of Support Hind Paw Mean”, “Print Position”, “Swing Speed” and “Stride Length” (Cendelin et al., 2010). We also examined the same parameter in our SCA17 transgenic mice and found the similar result (Figure 6). Figures 7E - 7N show the quantification of footprint analysis. The SCA17 transgenic mice showed a larger hind-paw distances than wildtype mice, which is consistent with the previous report that hind-paw distance would be enlarged in coordination deficient mice (Hamers et al., 2001, Cendelin et al., 2010). However, after 4% trehalose treatment, the SCA17 transgenic mouse hind paw distance was decreased to a similar level of wildtype mice. In addition, the print position measures the footprint distances between hind paw and front paw in the same side. We observed the transgenic mice had lager distances than that of wildtype group, indicating that the transgenic mice presented ataxia behavior. However, after trehalose treatment, we

found the print position was decreased. Furthermore, the swing speed and stride length were also rescued by 4% trehalose treatment.

Evaluation the neuronal pathology on 4%trehalose treatment in SCA17 transgenic mice

After 4% trehalose treatment, we observed the transgenic cerebellar size was larger than that of vehicle treatment (Figure 7A), indicating the trehalose might protect against the cerebellar atrophy. Furthermore, the Purkinje cells showed the better dendritic morphology in the high magnification view (Figure 7B). We also confirm this result by immunohistochemical (IHC) staining with calbindin antibody. The data showed that the Purkinje cell not only had better dendritic performance, but also had larger cell soma (Figure 7C), which indicated that the trehalose had protective effect for SCA17 transgenic mice.

Astrocyte gliosis is well known as one of major neurodegenerative markers (Friedman et al., 2007), we also use GFAP antibody to evaluate the trehalose effect in SCA17 transgenic mice. As our anticipation, after 4% trehalose treatment, less astrocyte gliosis was identified no matter in Purkinje layer, granular layer (Figure 7D) or in deep cerebellar nuclei (DCN) (Figure 7E). The IHC analysis also confirmed this result. There was less astrocyte activated in the cerebellum (Figure 7F). Furthermore, less gliosis signal was also detected in the lobes (Figure 7G) and in DCN (Figure 7H). However, we observed that the microglia cells detected by

Iba1 antibody were significantly activated after 4% trehalose treatment in SCA17 transgenic mice (Figures7I-7J). The Bergman glia cell was also detected by S100 antibody, however we could only observe the S100 expression signal was reduced in transgenic mice no matter in treatment or in vehicle group (Figure 7K).

Evaluation the molecular effect of 4% trehalose treatment in SCA17 transgenic mice

We also checked the trehalose molecular effect in SCA17 transgenic mice. HSP70 is considered the major chaperon protein and played the important neuron protection role in neuron degenerative diseases (Friedman et al., 2007, Huang et al., 2011). However, we could not detect significant differences between in wildtype and transgenic groups (Figure 8A). Although the p-ERK was upregulated in transgenic mice, the trehalose could not affect the expression level (Figure 8A). The levels of GAD67, β-catenin and pp38 were also not effected after trehalose treatment (Figures 8B-8C). However, the MnSOD was resumed to the normal expression level after trehalose treatment, which indicated that the trehalose might ameliorate the oxidative stress in SCA17 transgenic mice. Furthermore, it is interesting that the p-JNK was downregulated in wildtype mice after trehalose treatment; nevertheless, trehalose treatment upregulated the p-JNK protein expression in SCA17 transgenic mice (Figure 8D).

Discussion

SCA17 is an autosomal dominant hereditary disease caused by abnormal amplification of CAG repeats in *TBP* gene and resulted in neuron degeneration. Although the molecular pathogenesis of SCA17 had not been clarified yet, cerebellar atrophy, Purkinje cell loss and TBP nuclear aggregation were obvious markers in SCA17 patient (Nakamura et al., 2001). In our previous study, we could also detect the TBP puncta, ataxia and neuronal degeneration in TBP-109Q transgenic mice (Chang et al., 2011). In this study, we further found the TBP puncta was co-localized with 1C2 and ubiquitin (data not show) which were reported to be detected in SCA17 patients. These results confirmed that the abnormal TBP puncta detected in the SCA17 transgenic mice was the aggregation. Furthermore, the presence of vacuoles was reported as a sign of degeneration of cells of SCA1 and *tottering* mice (Florez-McClure et al., 2004, Vig et al., 2006, Hoebeek et al., 2008, Vig et al., 2009). We could also found vacuoles within the Purkinje cells with intensive aggregation in our transgenic mice (data not show). These observations reveal that Our SCA17 transgenic mice represent anideal animal model in studying pathogenesis or screening potential treatments for polyQ mediated SCA diseases.

To identify potential treatments for SCA17, an *in vitro* model may be established for a screening platform. However, the Purkinje cell was difficult to be maintained in its normal function and morphology without co-culture with its surrounding glia cell. For example, the Bergmann glia

cell plays the role of scaffold in development stage of Purkinje cell (Lippman et al., 2008).Therefore, we tried to set up the organotypic cerebellar slice culture for the drug screen platform. The slice culture maintained the normal cerebellar neuronal morphology at DIV7, and we could observe the TBP aggregation was formed in SCA17 transgenic slice between DIV1 and DIV3, indicating that the slice culture could be a suitable system to screen the potential drugs for SCA17.

Trehalose is analpha-linked disaccharide synthesized by fungi, plants and invertebrates. There were reports suggested that the trehalose had low toxicity and could help the cell to protect against the stress threatening the cell survival (Chen and Haddad, 2004). In addition, the trehalose had been reported to have potential in rescuing neuron pathology, molecular dysfunction and abnormal behavior in lots of neurodegenerative diseases, such as AD (Beranger et al., 2008), prion disease (Aguib et al., 2009), HD (Tanaka et al., 2004) and SCA14 (Seki et al., 2010). After applying the trehalose in slice culture at DIV1, we could observed the TBP aggregation was significantly reduced at DIV7, indicating that the trehalose might prevent the aggregation formation.

Previous study reported that trehalose might play a role as a chaperon to prevent the abnormal protein aggregation formation (Tanaka et al., 2004, Seki et al., 2010). To further understand whether trehalose coulddecrease TBP aggregation and rescue the SCA17 pathology in vivo, we applied the 2% and 4% trehalose, respectively, into mouse drinking

water. The trehalose was stable during our treatment condition; however, we could not observe significant rescuing effect of trehalose determined by rota-rod test. It could be that the first rota-rod condition was too strict to distinguish treatment group from vehicle group. After modifying the rota-rod condition from 4-30 rpm accelerated speed to 26 rpm fixed speed, we could observe the differences between these two groups. In addition, the footprint of trehalose treated mice had better performance than vehicle treated mice. These data reveal that although trehalose had some neuron protective effect on SCA17 mice, which could only be detected by a mild analysis protocol.

In our previous study, hyperactivity was reported to be one of behavior markers in our SCA17 transgenic mice (Chang et al., 2011). In the present study, we could also observe that after 4% trehalose treatment, the total distance analyzed by locomotor was slightly reduced in transgenic group, indicating that trehalose could ameliorate the hyperactivity of SCA17 mice.

From the *in vivo* study, we found the dendritic tree of transgenic mouse Purkinje cell had better performance after trehalose treatment, however, the TBP aggregation was not significantly reduced as the results found in slice culture. Trehalase is suspected to be the reason to cause the effect difference between mice and slice culture. Trehalase is the enzyme to digest trehalose into two glucoses. It was reported that the trehalase is present in the intestine of mammals including rabbit (Ruf et al., 1990), rat

(Oesterreicher et al., 1998), mouse (Oesterreicher et al., 2001) and human (Ishihara et al., 1997), which could digest the trehalose drunk by SCA17 transgenic mice. It might reduce the trehalose concentration and explain why there was no significant improvement in transgenic mice identified from both the rota-rod and locomotor test after trehalose treatment. Although the glucoses also showed some positive effect on HD mice (Tanaka et al., 2004), the trehalose was much better than it (Tanaka et al., 2004, Kruger et al., 2011). Therefore, finding a compound with similar potent as trehalose and also working as a trehalase inhibitor might be a potential strategy to solve the polyQ aggregation. For example, validamycin A is the trehalase inhibitor and was used to improve the trehalose biosynthesis (Xue et al., 2005) and accumulation (Lopez et al., 2009). It would be interesting to see whether the validamycin A could inhibit the abnormal TBP aggregation.

In this study, we also monitor the trehalose drinking level (data not show). We found that the mice drunk more water than vehicle treatment. However, the blood glucose did not change after treatment, indicated that the trehalose treatment did not affect the blood glucose and harm the mice. The interesting thing is that we found the blood glucose was significantly reduced in transgenic group at 5-week-old. Although there was no report pointed out the blood glucose was affected in SCA patients, the hypometabolism phenomena was observed by Positron emission tomography (PET) with 2-[fluorine18]-fluoro-2-deoxy-D-glucose in cerebellum of SCA patients (Wang et al., 2007), indicating that the

dysfunction of energy metabolism might be another pathology of SCAs. However, at this moment, we do not know whetherour SCA17 mice have any defect in energy metabolism and the trehalose treatment would have any effect on the hypometabolism phenomena of cerebellum or not.

Gliosis is observed as a neuron degenerative marker in SCA17 mice (Friedman et al., 2007, Chang et al., 2011). In this study, we could also detect the astrocytes was highly activated in the transgenic mice. After trehalose treatment, we found the activation of astrocytes was reduced, indicating that the trehalose could delay the neuron degeneration. However, we could also detect the microglia cells were activated after trehalose treatment, especially in SCA17 transgenic mice. The microglia contributed about 12% cells in whole brain and was known as biosensors in the central nervous system (Penfield, 1932). However, it had not been clarified yet that the activation of microglia would exert positive or negative effect on neurons (Li et al., 2007). For example, many studies reported that activating microglia cells would increase neuronal cell death through releasing glutamate, nitric oxide and toxic cytokines (Chao et al., 1992, Piani et al., 1992, Viviani et al., 1998). In contrast, some evidences indicated activating microglia cells could secrettrophic factor which is good for neurons, such as neurotrophins (Elkabes et al., 1996) and transforming growth factor-β (TGF-β) (Lehrmann et al., 1998). In a previous study, the astrocyte gliosis upregulated and neurodegeneration phenomena were observed when the hippocampal slice was cultured in microglia cell-depleted condition (Montero et al., 2009), indicating a

protective role of microglia cell in this system. In this study, we could also observe the astrocytes activation was reduced and microglia cells activation was upregulated.

Taking together, our data suggest that the trehalose treatment has positive effect in our neuronal pathology of SCA17 transgenic mice. This natural disaccharide might have potential to delay the polyQ diseases. However, adding the trehalase inhibitor with trehalose might be a more efficiency way than trehalose only for the treatment.

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Wang PS, Liu RS, Yang BH, Soong BW (2007) Regional patterns of cerebral glucose metabolism in spinocerebellar ataxia type 2, 3 and 6 : a voxel-based FDG-positron emission tomography analysis. Journal of neurology 254:838-845.

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Yamada K, Fukaya M, Shibata T, Kurihara H, Tanaka K, Inoue Y, Watanabe M (2000) Dynamic transformation of Bergmann glial fibers proceeds in correlation with dendritic outgrowth and synapse formation of cerebellar Purkinje cells. The Journal of comparative neurology 418:106-120.

Table 1. Primary antibody list in this study

Protein	Manufacturer	Titer	Source	MW (kDa)
Calbindin	Sigma	1:1000	mouse	28
Calbindin	Sigma	1:1000	rabbit	28
IP3R1	SantaCruz	1:1000	goat	313
1TBP18	QED	1:30000	mouse	
GFAP	Millipore	1:1000	mouse	51
S100	Millipore	1:1000	mouse	10
Iba1	Wako Pure Chemical	1:1000	rabbit	17
β-actin	Millipore	1:1000	mouse	42
MoSOD	Cell signaling	1:1000	mouse	24
pp38	Cell signaling	1:1000	rabbit	38
GAD67	Millipore	1:1000	rabbit	65
p-ERK (Thr202/Tyr204)	Cell signaling	1:1000	rabbit	42/44
ERK 1/2	Cell signaling	1:1000	rabbit	42/44
HSP70	Cell signaling	1:1000	rabbit	72
p-JNK (Thr183/Tyr185)	Cell signaling	1:1000	rabbit	46/54
JNK	Cell signaling	1:1000	rabbit	46/54

Table 2. Secondary antibody list in this study

Antibody	Manufacturer	Titer	Source
anti-mouse IgG, Alexa Fluor 488	Invitrogen	1:500	donkey
anti-mouse IgG, Alexa Fluor 555	Invitrogen	1:500	donkey
anti-rabbit IgG, Alexa Fluor 555	Invitrogen	1:500	donkey
anti-goat IgG, Alexa Fluor 488	Invitrogen	1:500	donkey
anti-goat IgG, Alexa Fluor 555	Invitrogen	1:500	donkey
Biotinylated Goat Anti-Mouse IgG	Vector	1:200	goat
Biotinylated Goat Anti-Rabbit IgG	Vector	1:200	goat
anti-rabbit IgG, HRP-linked	Cell Signaling	1:10000	goat
anti-mouse IgG, HRP-linked	Cell Signaling	1:10000	goat

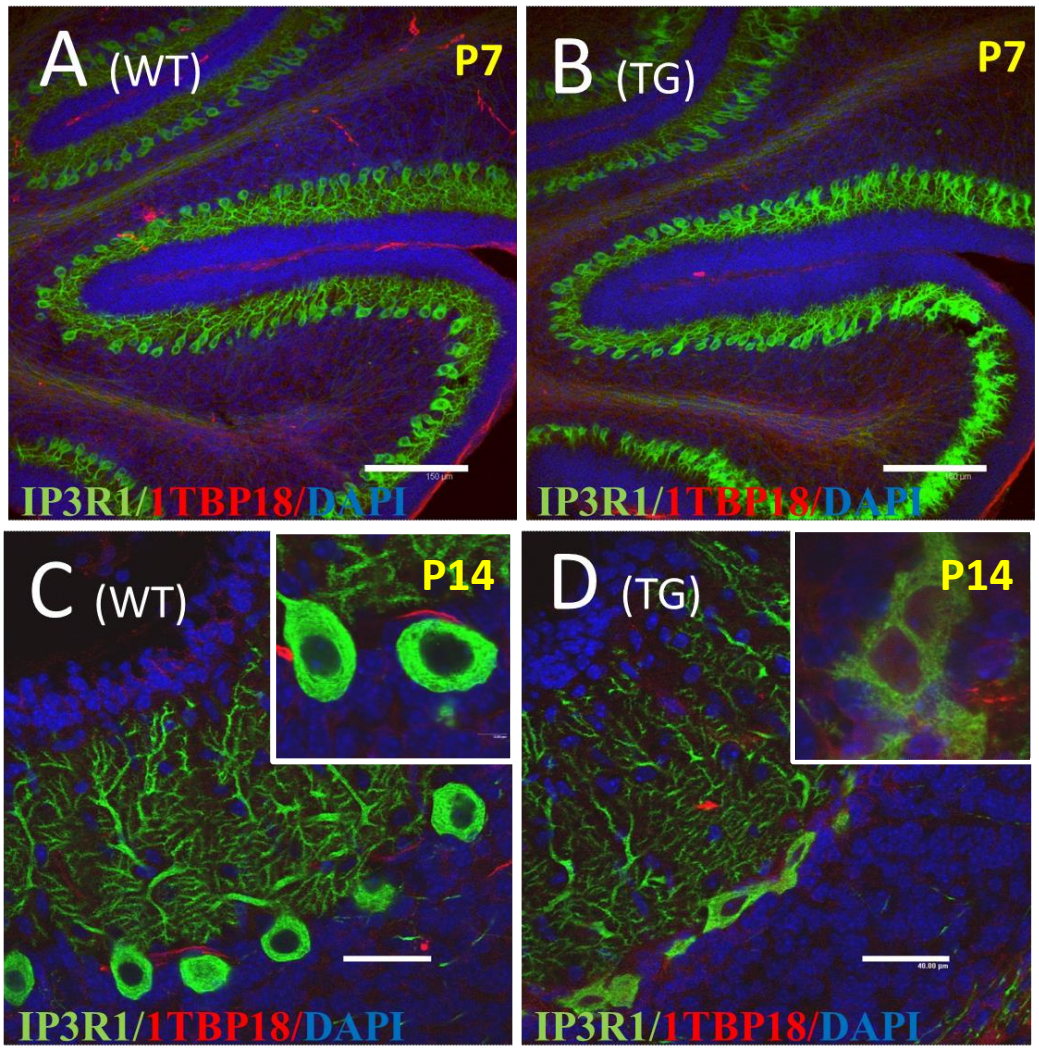


Figure 1.

No TBP aggregation was identified at postnatal day 7 transgenic mouse cerebellum.

The nuclear aggregation was analyzed by immunofluorescent staining with IP3R1 and 1TBP18 antibodies on postnatal day 7 (P7) **(A-B)**, and 14 (P14) **(C-D)** Wildtype and transgenic mice. We could not detect any 1TBP18 aggregaion in neither wildtype nor transgenic cerebella at these stages. However, there was high expression signal of 1TBP18 in transgenic cerebellum at p14. Scale bar = 150 μm (A-B), 40 μm (C-D)

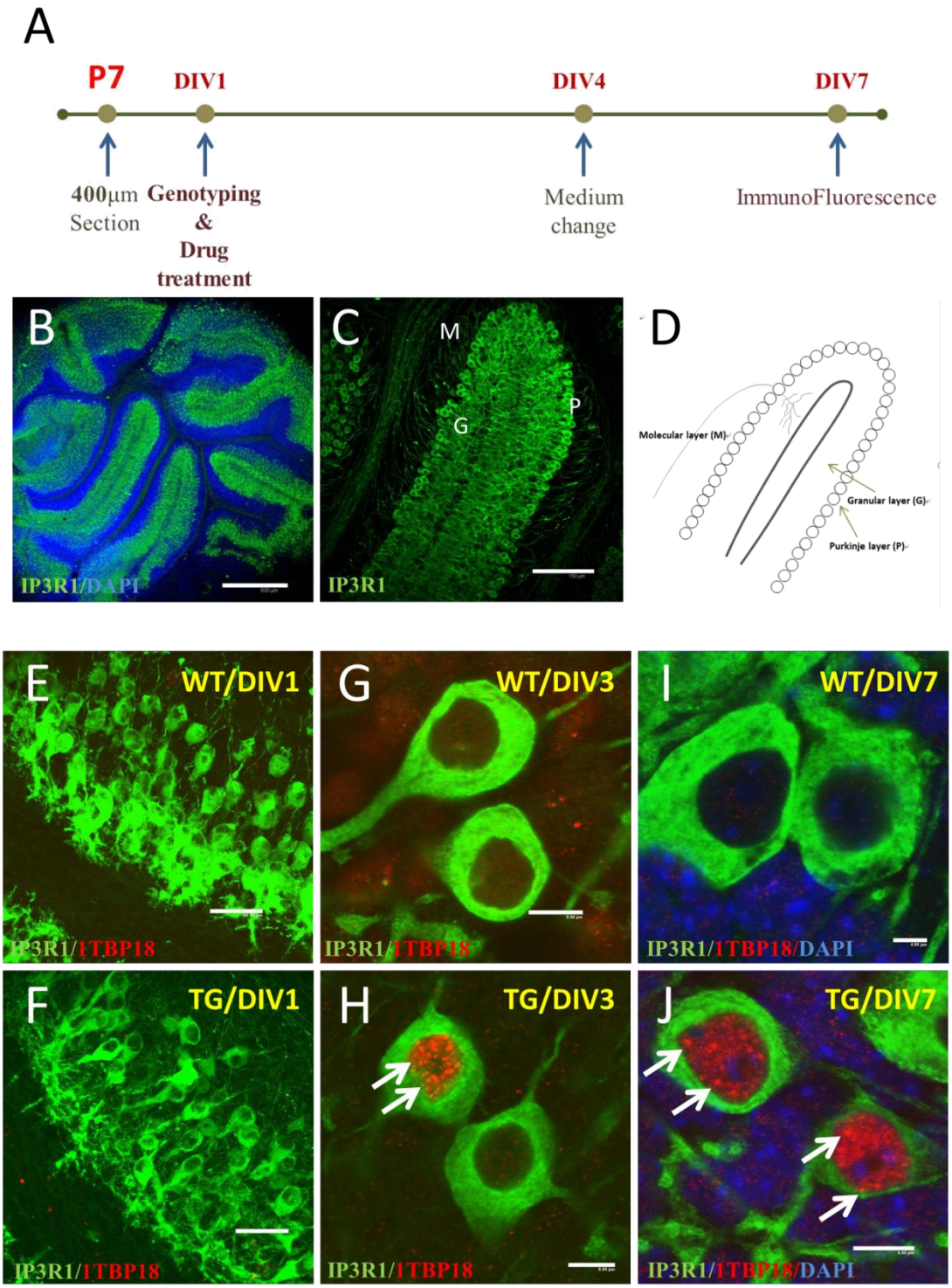
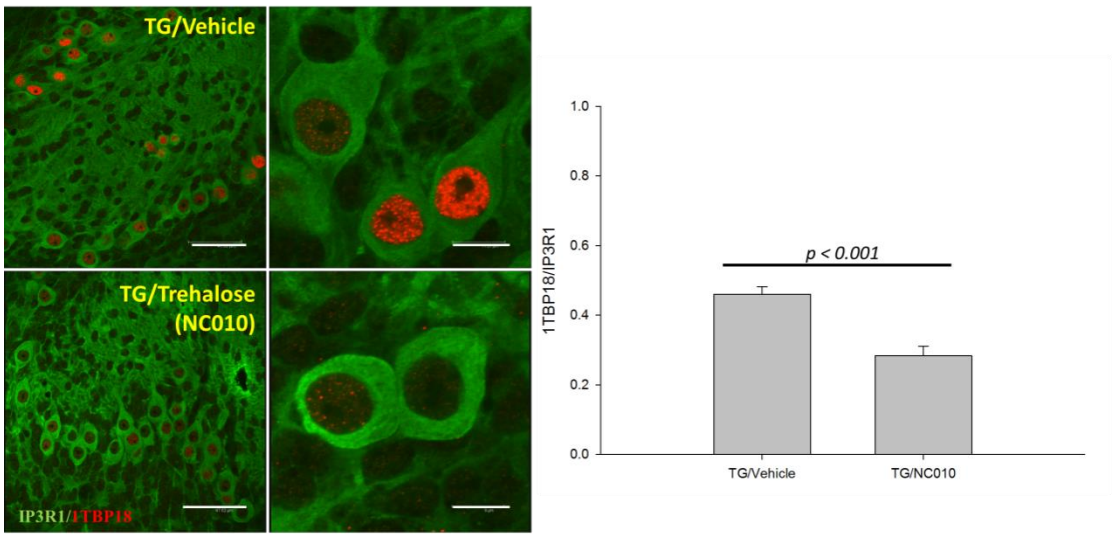

A
P7
DIV1
DIV4
DIV7
400μm Section
Genotyping & Drug treatment
Medium change
ImmunoFluorescence
B
IP3R1/DAPI
C
M
G
P
IP3R1
D
Molecular layer (M)
Granular layer (G)
Purkinje layer (P)
E
WT/DIV1
IP3R1/1TBP18
G
WT/DIV3
IP3R1/1TBP18
I
WT/DIV7
IP3R1/1TBP18/DAPI
F
TG/DIV1
IP3R1/1TBP18
H
TG/DIV3
IP3R1/1TBP18
J
TG/DIV7
IP3R1/1TBP18/DAPI

Figure 2.

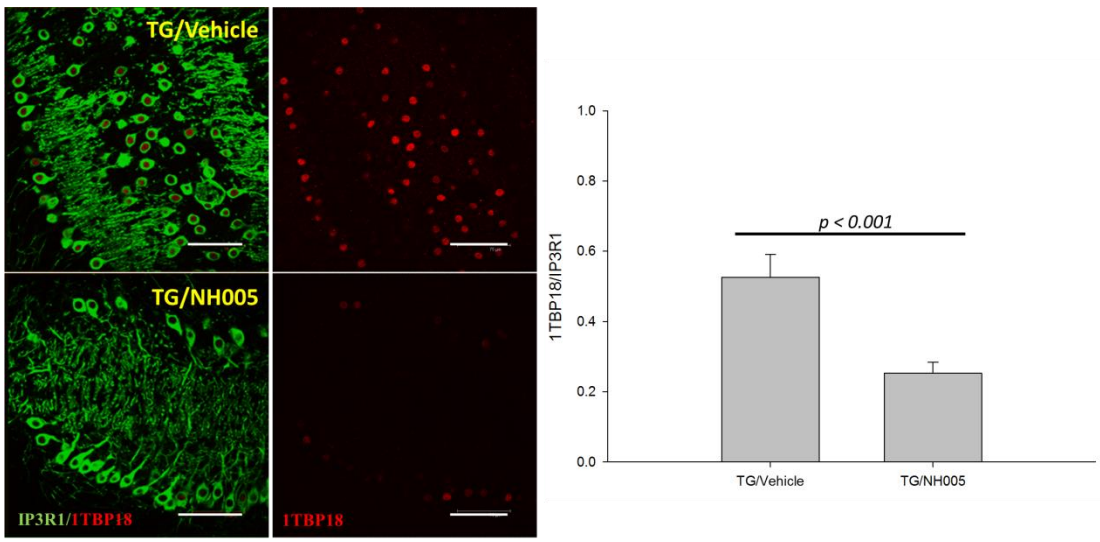
The TBP aggregation was identified in SCA17 transgenic cerebellar slice culture.

(A) The time line of cerebellar slice culture in this study. **(B-D)** The cerebellar slice kept normal morphology during the culture period. **(E-J)** The TBP aggregation formation was observed between DIV1 and DIV3 in transgenic slice culture, and no aggregation was identified in wildtype slice. Scale bar = 600 μm (B), 150 μm (C), 40 μm (E-F), 8 μm (G-H), 4 μm (I), 10 μm (J)

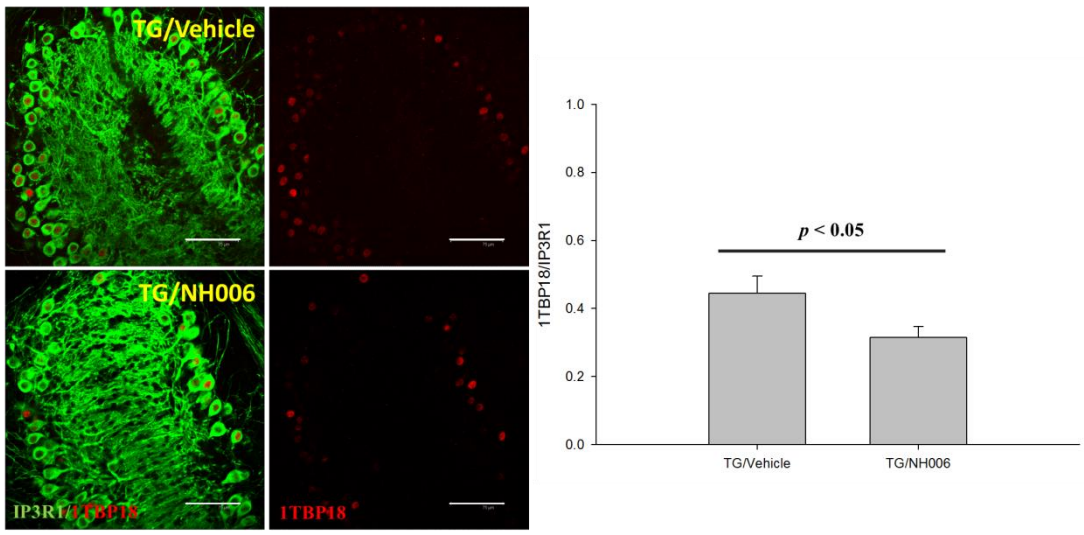
A


TG/Vehicle
TG/Trehalose
(NC010)
IP3R1/1TBP18
1TBP18/IP3R1
1.0
0.8
0.6
0.4
0.2
0.0
p < 0.001
TG/Vehicle
TG/NC010

B


TG/Vehicle
TG/NH005
IP3R1/1TBP18
1TBP18
1TBP18/IP3R1
1.0
0.8
0.6
0.4
0.2
0.0
p < 0.001
TG/Vehicle
TG/NH005

C


TG/Vehicle
TG/NH006
IP3R1/1TBP18
1TBP18
1TBP18/IP3R1
1.0
0.8
0.6
0.4
0.2
0.0
p < 0.05
TG/Vehicle
TG/NH006

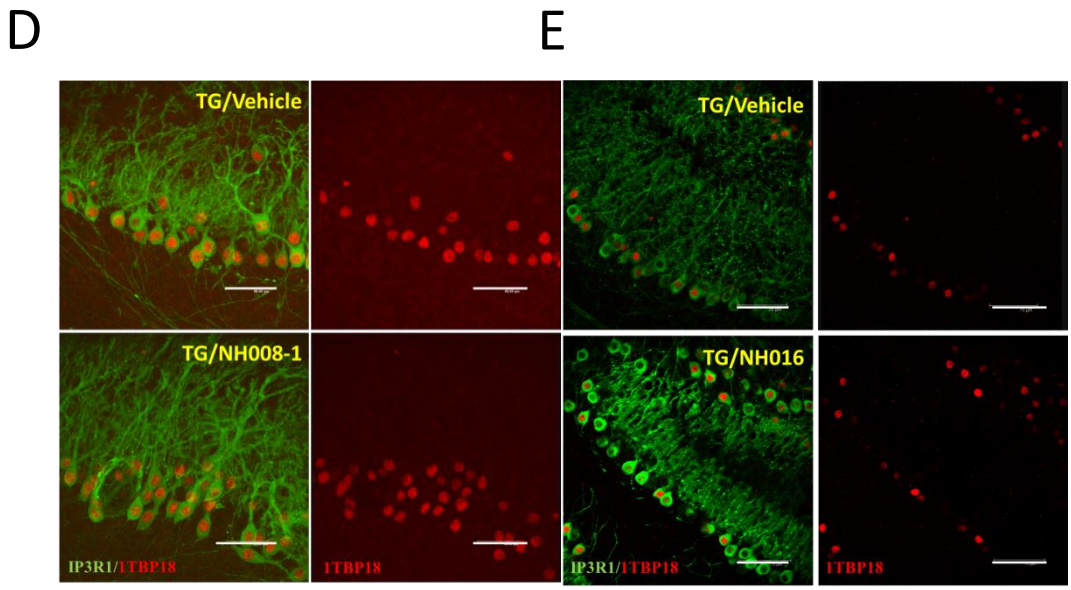
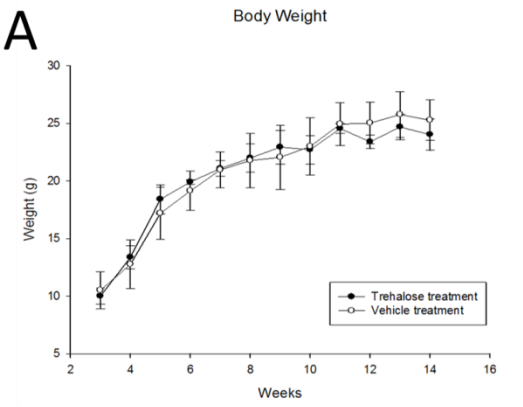
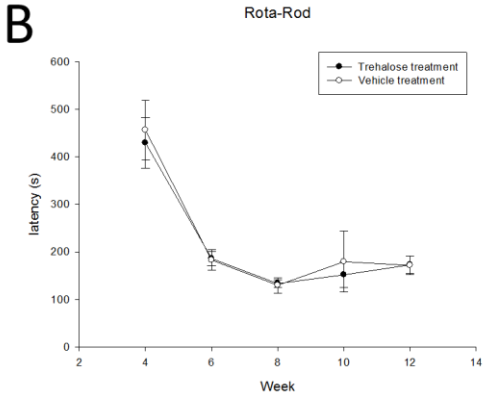
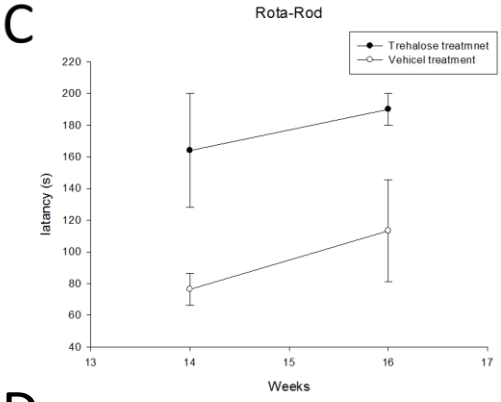


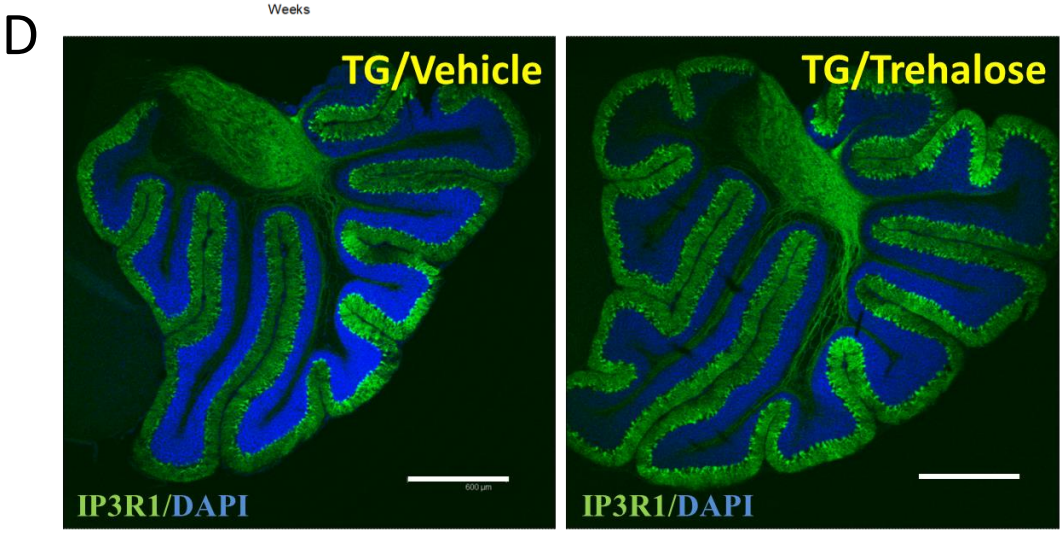
Figure 3.

Screen potential herbs/compounds for SCA17 using organotypic cerebellar slice culture system.1TBP18 aggregation was significatly decreased after 100 μM trehalose**(A),** 100 μg/ml NH005 **(B),** or NH006 **(C)** treatments.No significant difference in 1TBP18 aggregation was identified after 100 nM NH008-1**(D) ,**and 100 μg/ml NH016**(E)** treatments. Scale bar = 50 μm (A, left), 10 μm (A, right), 75 μm (B-E), 40 μm (D)

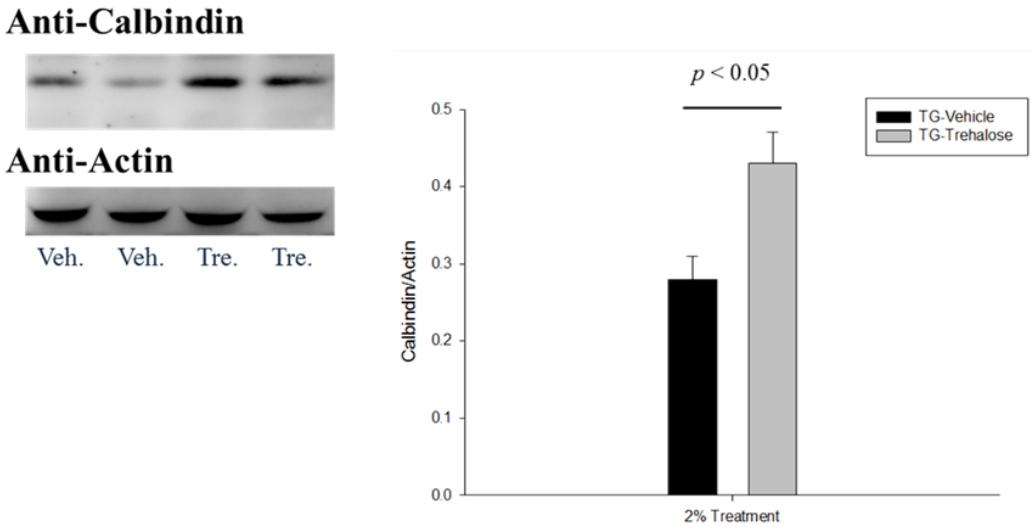

A
Body Weight
Weight (g)
Weeks
Trehalose treatment
Vehicle treatment


B
Rota-Rod
latency (s)
Week
Trehalose treatment
Vehicle treatment


C
Rota-Rod
latancy (s)
Weeks
Trehalose treatmnet
Vehicel treatment


D
TG/Vehicle
IP3R1/DAPI
TG/Trehalose
IP3R1/DAPI

E


Anti-Calbindin
Anti-Actin
Veh.
Veh.
Tre.
Tre.
p < 0.05
TG-Vehicle
TG-Trehalose
Calbindin/Actin
0.5
0.4
0.3
0.2
0.1
0.0
2% Treatment

F

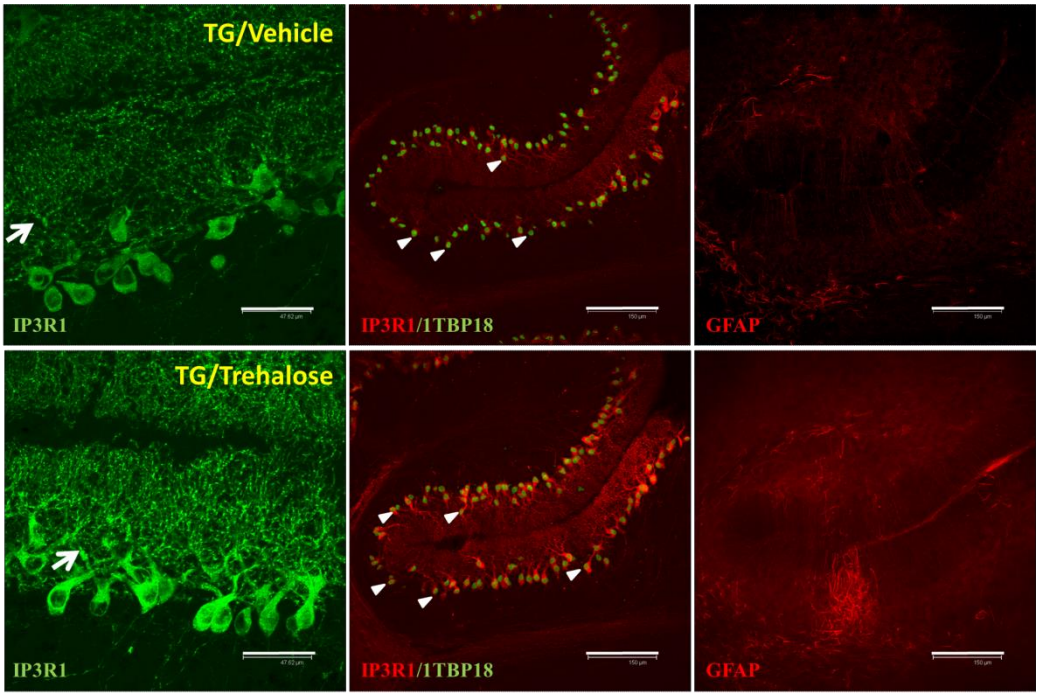

TG/Vehicle
IP3R1
IP3R1/1TBP18
GFAP
TG/Trehalose
IP3R1
IP3R1/1TBP18
GFAP

Figure 4.

The results of a pilot study in which 2% trehalose was applied to the SCA17 transgenic mice. **(A)** The bodyweight between vehicle and treatment groupsshowed no significant difference. **(B)** There was no significant difference identified between transgenic mice treated with trehalose or vehicle in a 4-30 rpm rota-rod task. **(C)** Under a 26 rpm fixed rota-rod condition, the different performance was identified between transgenic mice treated with vehicle and trehalose. **(D)** The cerebellar size of transgenic mice treated with trehalose was larger than that of vehicle treatment. **(E)** The calbindin expression level determined by western blot analysis showed significantly increased in transgenic mice with trehalose treatment than vehicle treatment. **(F)** The Purkinje cell showed higher dendritic density (arrow)and IP3R1 staining intensity after trehalose treatment than vehicle treatment; nevertheless, the TBP aggregation was not significantly altered after different treatments (arrow head).With GFAP staining, we observed the gliosis was slightly decreased after 2% trehalose treatment. Scale bar = 600 μm (D), 50 μm (F, left), 150 μm (F, middle and right)

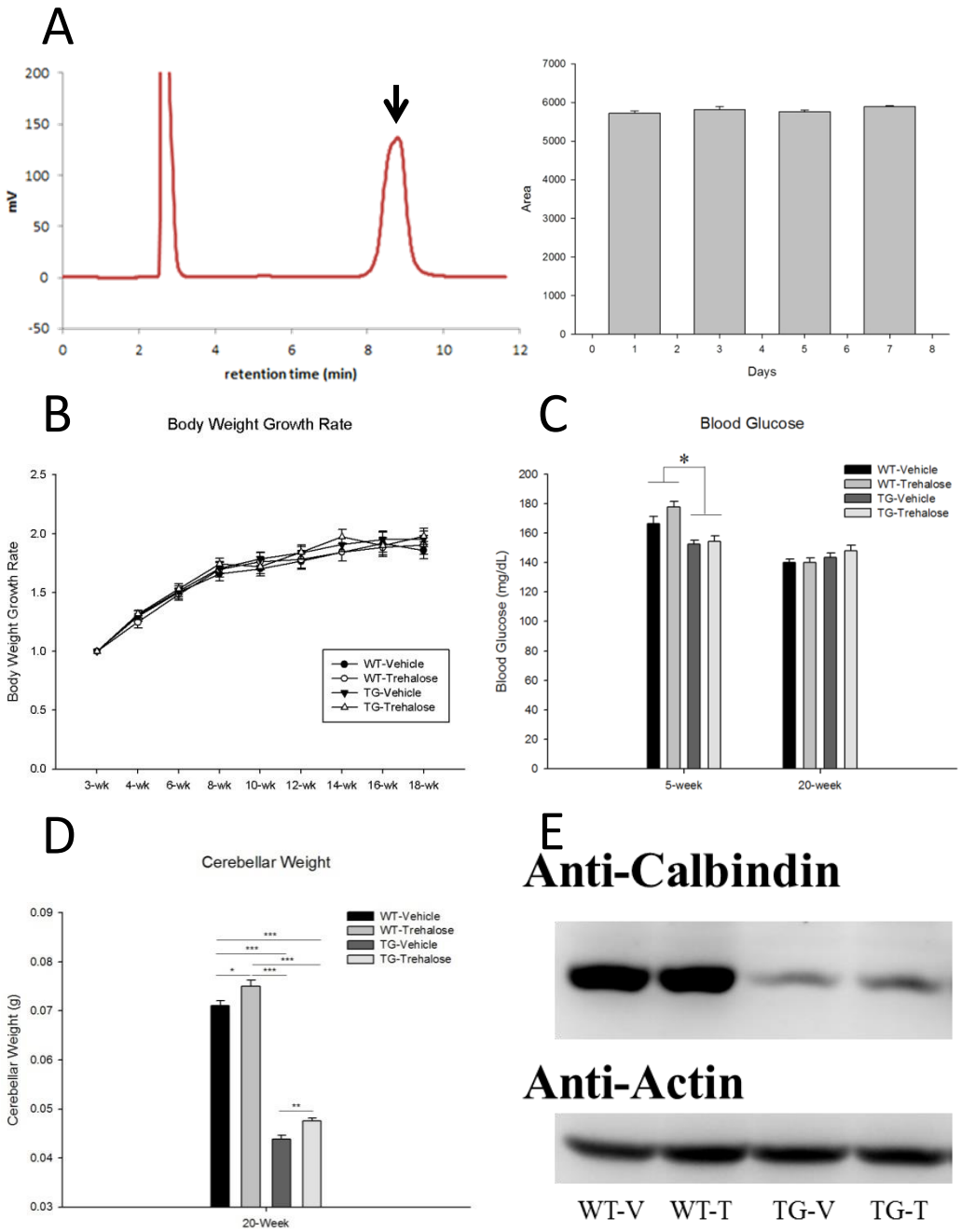
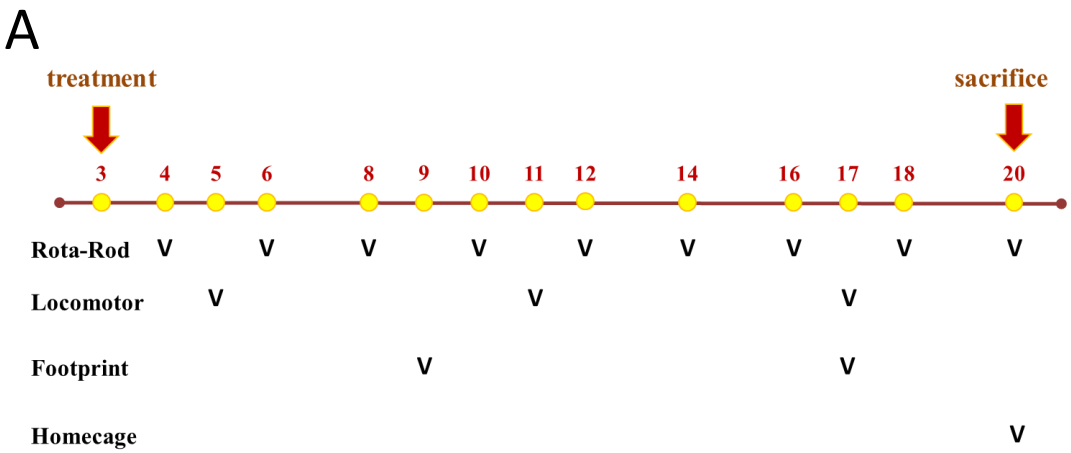
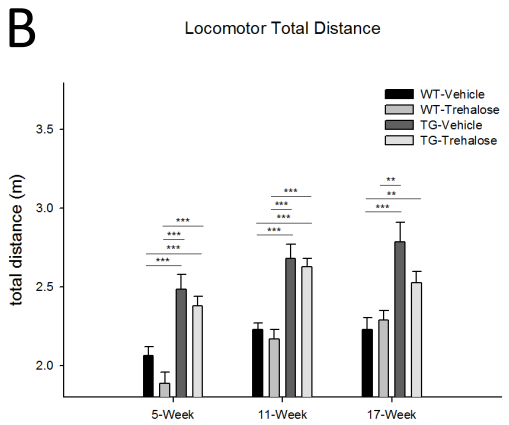
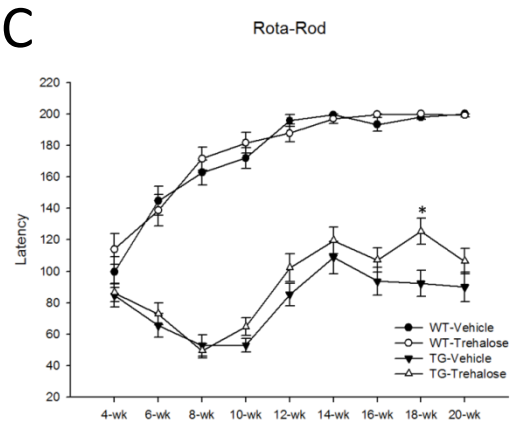

A
mV
retention time (min)
Area
Days
B
Body Weight Growth Rate
Body Weight Growth Rate
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
3-wk
4-wk
6-wk
8-wk
10-wk
12-wk
14-wk
16-wk
18-wk
C
Blood Glucose
Blood Glucose (mg/dL)
5-week
20-week
D
Cerebellar Weight
Cerebellar Weight (g)
20-Week
E
Anti-Calbindin
Anti-Actin
WT-V
WT-T
TG-V
TG-T

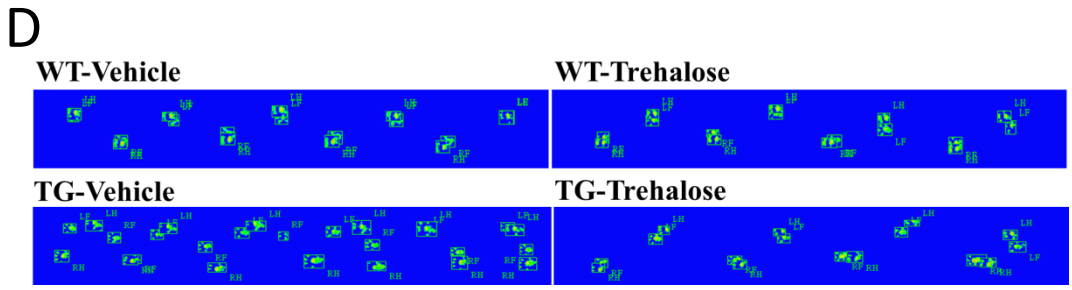
Figure 5.

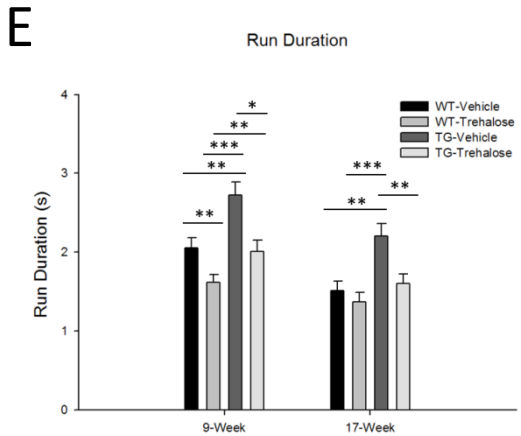
Evaluation of the effect of 4% trehalose treatment on SCA17 transgenic mice. **(A)**The 4% trehalose solution was stable for one week during the animal application identified by HPLC analysis and quantification. Arrow, the trehalose peak.**(B)** The mouse body weight showed no differences between these groups. **(C)** The mouse blood glucose were not altered after 4% trehalose treatment. **(D)** The cerebellar weight was partial recovered after 4% trehalose treatment. **(E)** The calbindin expression was slightly rescued after 4% trehalose treatment.(intepedent *t*-test; *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$)

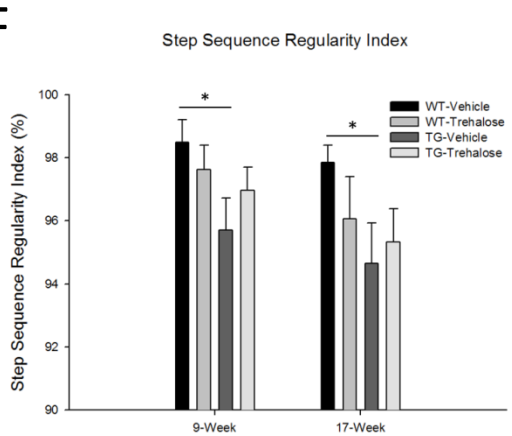

A
treatment
sacrifice
3
4
5
6
8
9
10
11
12
14
16
17
18
20
Rota-Rod
Locomotor
Footprint
Homecage


B
Locomotor Total Distance
total distance (m)
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
5-Week
11-Week
17-Week

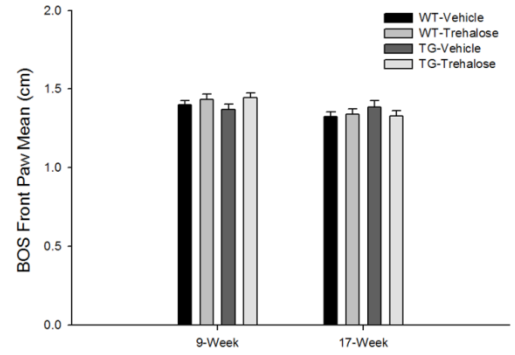

C
Rota-Rod
Latency
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
4-wk
6-wk
8-wk
10-wk
12-wk
14-wk
16-wk
18-wk
20-wk


D
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose

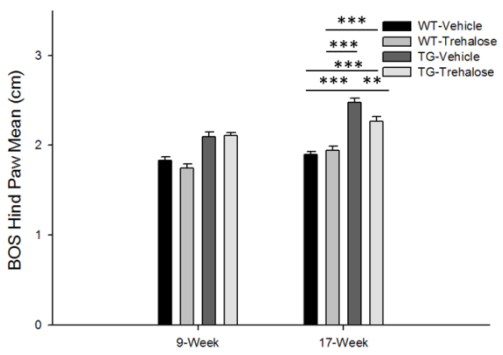

E
Run Duration
Run Duration (s)
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week


F
Step Sequence Regularity Index
Step Sequence Regularity Index (%)
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week

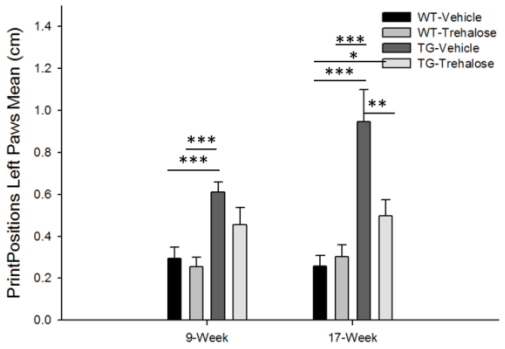
G


BOS Front Paw Mean
BOS Front Paw Mean (cm)
2.0
1.5
1.0
0.5
0.0
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week

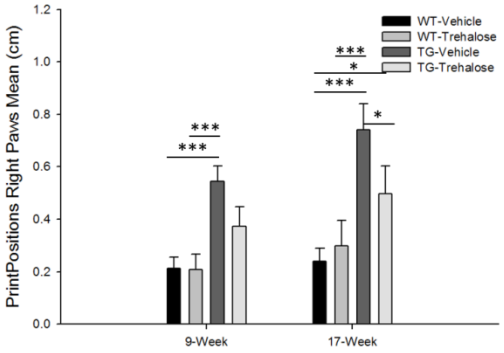
H


BOS Hind Paw Mean
BOS Hind Paw Mean (cm)
3
2
1
0
***
***
***
*** **
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week

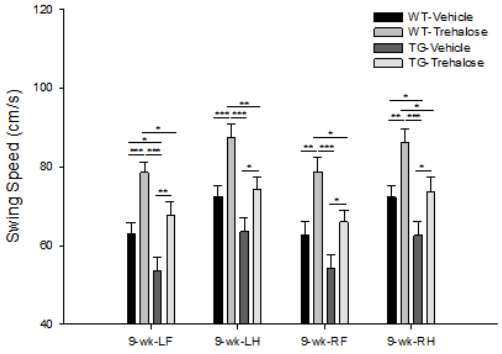
I


PrintPositions Left Paws Mean
PrintPositions Left Paws Mean (cm)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
*
***
**
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week

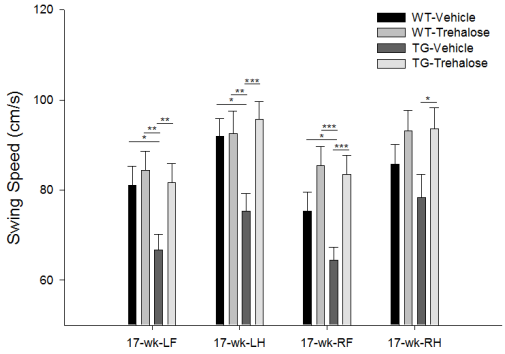
J


PrintPositions Right Paws Mean
PrintPositions Right Paws Mean (cm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
*
***
*
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-Week
17-Week

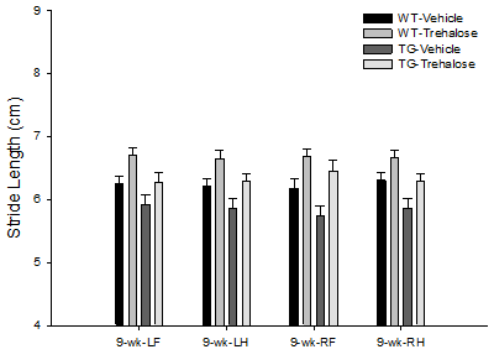
K


9-week-old Swing Speed (cm/s)
Swing Speed (cm/s)
120
100
80
60
40
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-wk-LF
9-wk-LH
9-wk-RF
9-wk-RH

L


17-week-old Swing Speed (cm/s)
Swing Speed (cm/s)
120
100
80
60
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
17-wk-LF
17-wk-LH
17-wk-RF
17-wk-RH

M


9-week-old Stride Length (cm)
Stride Length (cm)
9
8
7
6
5
4
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
9-wk-LF
9-wk-LH
9-wk-RF
9-wk-RH

N

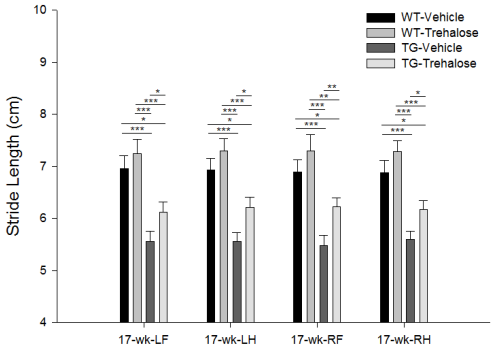
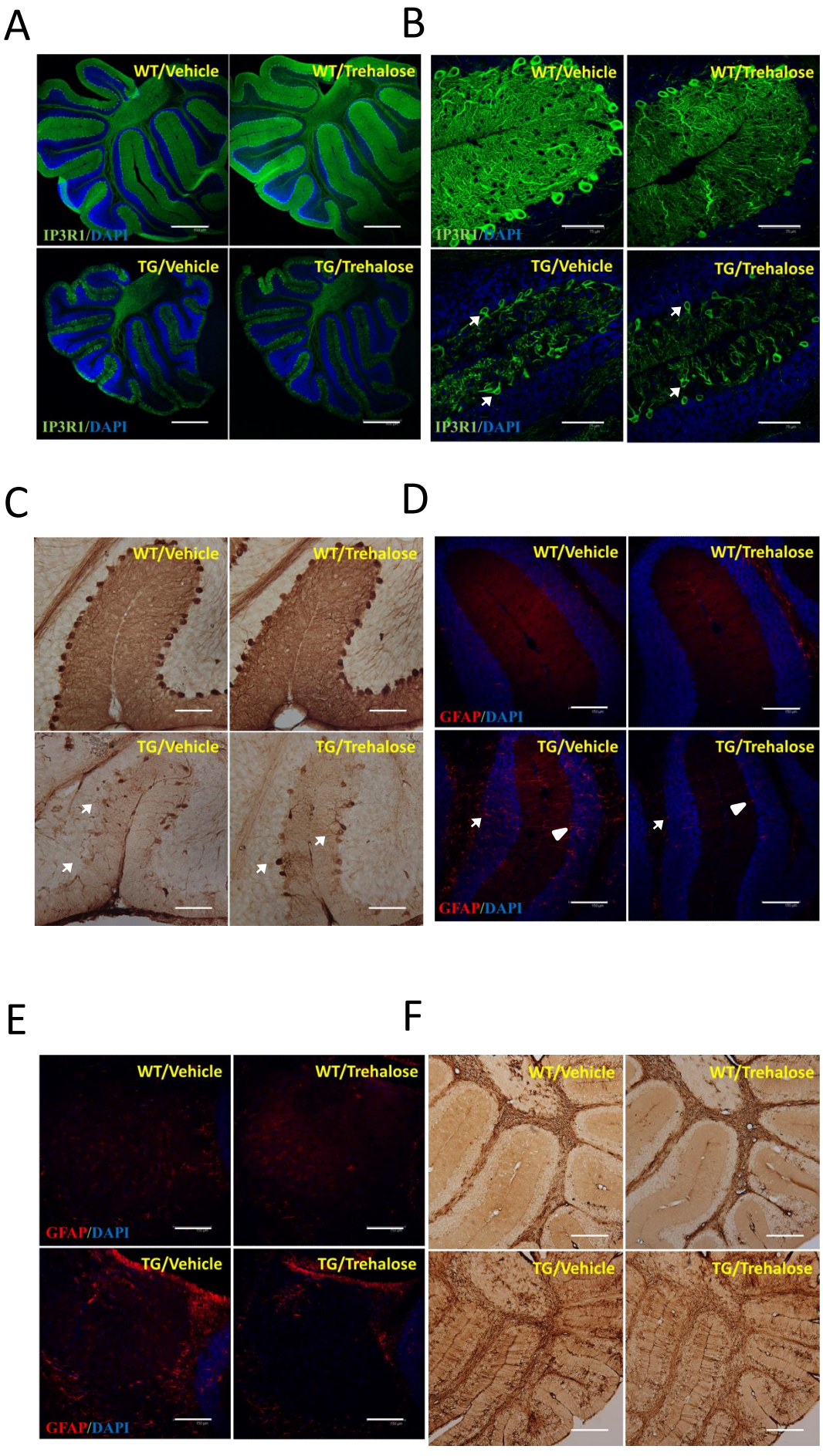
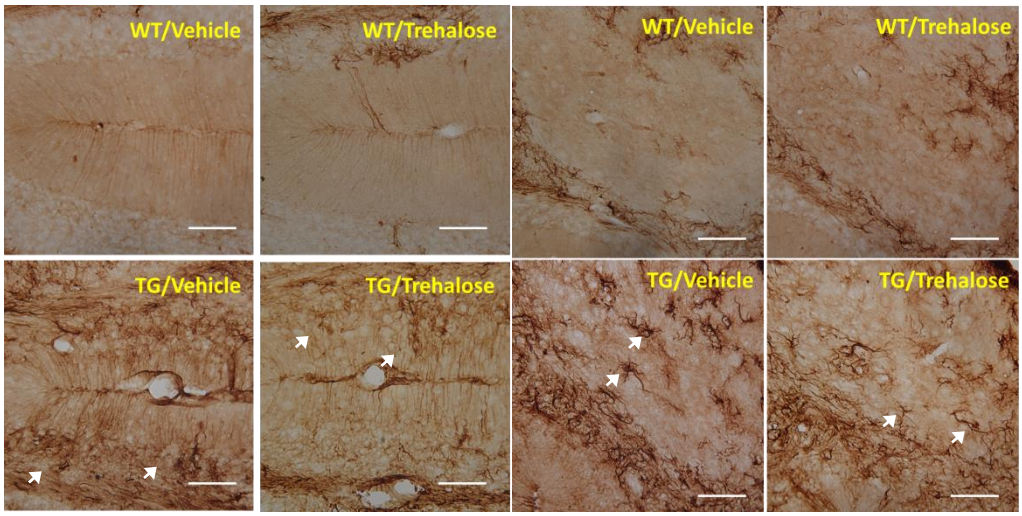

17-week-old Stride Length (cm)
Stride Length (cm)
10
9
8
7
6
5
4
WT-Vehicle
WT-Trehalose
TG-Vehicle
TG-Trehalose
17-wk-LF
17-wk-LH
17-wk-RF
17-wk-RH

Figure 6.

Evaluation of the neurobehavior effect of 4% trehalose treatment on SCA17 transgenic mice. **(A)** The time line of behavior test in this study. **(B)** The total distance analysis by locomotor behavior test. The hyperactivity performance of SCA17 transgenic mice was not rescued after treatment. **(C)** The rota-rod performance was slightly rescued after 4% trehalose treatment. **(D-N)** The footprint behavior test showed that the 4% trehalose partial rescued the footprint phenotype of SCA17 transgenic mice. (independent *t*-test; *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$)

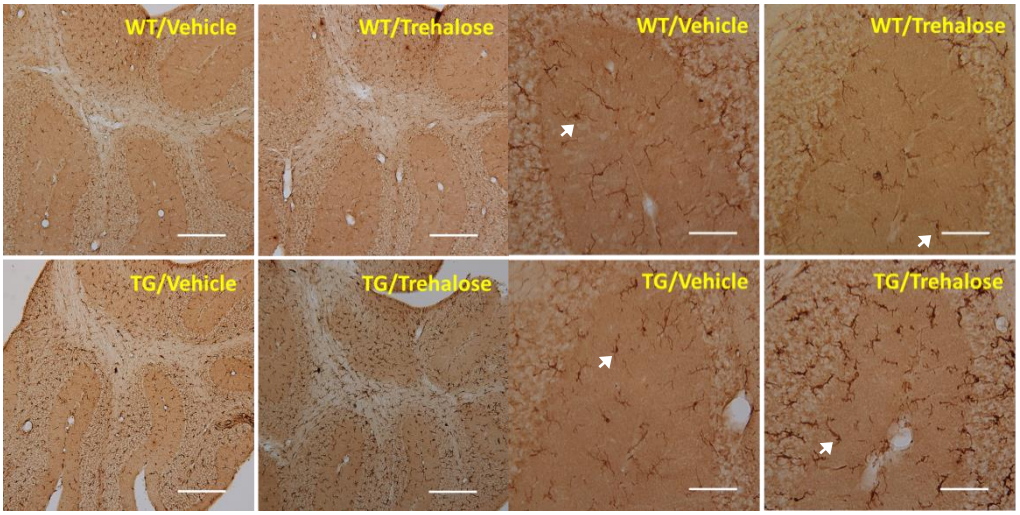

A
B
WT/Vehicle
WT/Trehalose
TG/Vehicle
TG/Trehalose
IP3R1/DAPI
C
D
GFAP/DAPI
E
F

G


WT/Vehicle
WT/Trehalose
WT/Vehicle
WT/Trehalose
TG/Vehicle
TG/Trehalose
TG/Vehicle
TG/Trehalose

H

I


WT/Vehicle
WT/Trehalose
WT/Vehicle
WT/Trehalose
TG/Vehicle
TG/Trehalose
TG/Vehicle
TG/Trehalose

J

K

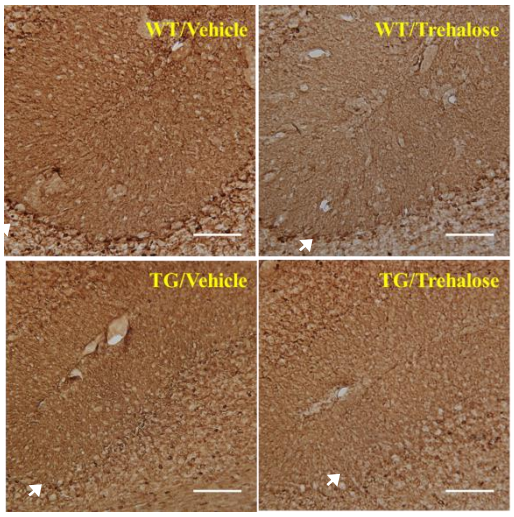

WT/Vehicle
WT/Trehalose
TG/Vehicle
TG/Trehalose

Figure 7

Evaluation of the neuropathological effect of 4%trehalose treatment on SCA17 transgenic mice. **(A)** The cerebellar atrophy was slightly rescued after 4% trehalose treatment. **(B)** The Purkinje cell dendritic tree showed more complex (arrow) in trehalose treatment group than vehicle treatment group. **(C)** The immunohistochemical staining of calbindin showed that the dendritic tree performance of Purkinje cell (arrow) was rescued after 4% trehalose treatment. **(D)** The gliosis was determined by GFAP staining (arrow) to show the Bergmann gliosis (arrow head) occurred in Purkinje cell layer. **(E)** The 4% trehalose treatment also reduced the gliosis in deep cerebellum nuclei (DCN).**(F-H)** The effect of trehalose in inhibiting gliosis was also identified by immunohistochemical staining, showed the astrocyte (arrow in H) and Bergmann glia (arrow in **G**) were significantly reduced. **(I-J)** Microglia cells (arrow) were activated after 4% trehalose treatment. **(K)** The Bergman glia in cerebellum stained by S100 showed no differences between vehicle-or trehalose-treated groups. Scale bar = 600 μm (A), 75μm (B-E), 150 μm (G-H and J-K), 600 μm (F and I)

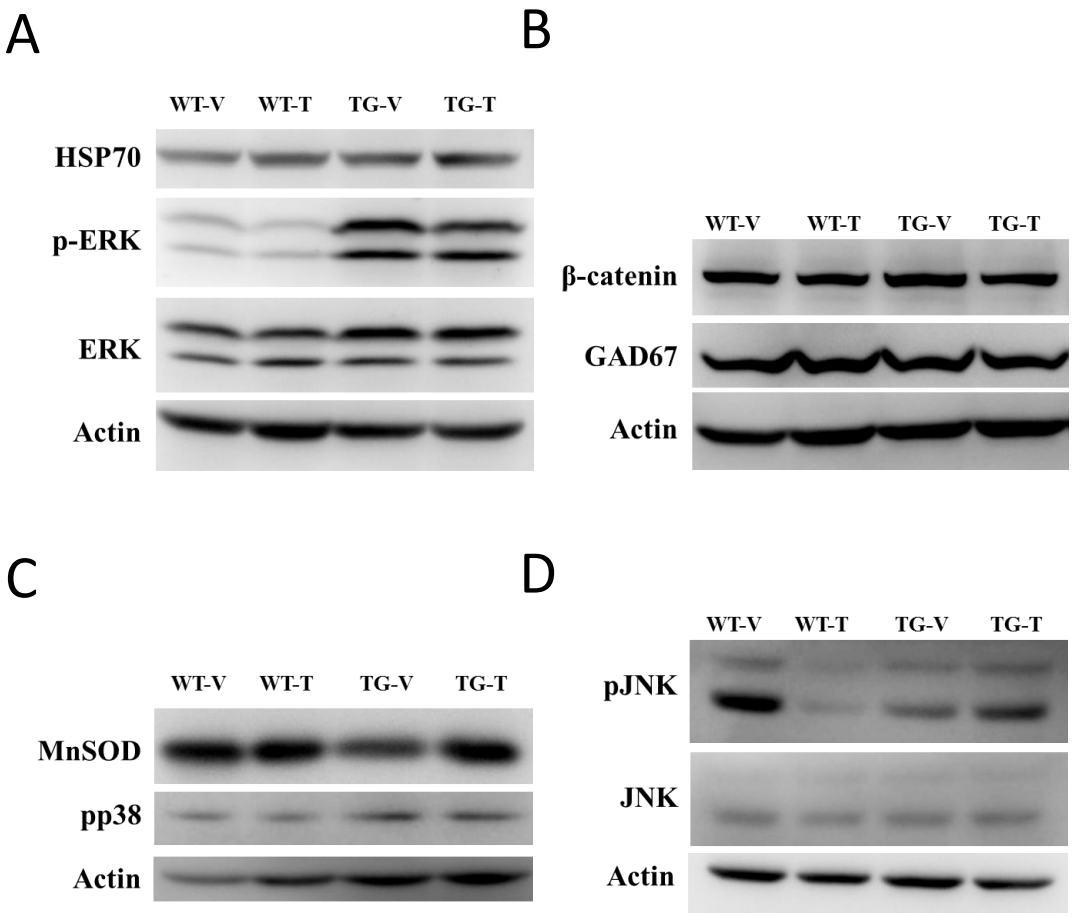


Figure 8.

The molecular effect of 4% trehalose treatment on SCA17 transgenic mice. **(A-B)** The western blot analysis at 20-week-old mice, the higher expression of p-ERK in SCA17 transgenic mice was observed, however, trehalose treatment did not reduced the expression level. No difference was identified in the HSP70, GAD67 and β-catenin expression level sin different groups. **(C)** The MoSOD level was upregulated after trehalose treatment in transgenic mice. **(D)** The pJNK was downregulated in trehalose treated wildtype mice, however it was upregulated in treated transgenic mice.