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Ataxia onset prior to neurodegeneration in SCA6

Sriram Jayabal, Lovisa Ljungberg, Thomas Erwes, Alexander Cormier, Sabrina Quilez, Sara El Jaouhari and Alanna J. Watt

Department of Biology, McGill University, Montreal, Quebec, Canada.

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Correspondence should be addressed to: Alanna J. Watt, Department of Biology, McGill University, Bellini Life Sciences Complex, 3649 Sir William Osler, Montreal, QC, H3G 0B1, Canada. Tel: (514) 398-2806. alanna.watt@mcgill.ca

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3. List of Author Names and Affiliations:

Sriram Jayabal, Lovisa Ljungberg, Thomas Erwes, Alexander Cormier, Sabrina Quilez, Sara El Jaouhari, and Alanna J. Watt*

Department of Biology, McGill University, Montreal, Quebec, Canada.

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5. Correspondence should be addressed to:

Alanna J. Watt
Department of Biology, McGill University
Bellini Life Sciences Complex
3649 Sir William Osler, Montreal, QC, H3G 0B1, Canada
[1]alanna.watt@mcgill.ca (514) 398-2806

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Abstract

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar ataxia that has been associated with loss of cerebellar Purkinje cells. Disease onset is typically midlife, although it can vary widely from late teens to old age in SCA6 patients. Our study focused on an SCA6 knock-in mouse model with a hyper-expanded (84X) CAG repeat expansion that displays midlife-onset motor deficits at ~7 months old, reminiscent of mid-life onset symptoms in SCA6 patients, although a detailed phenotypic analysis of these mice has not yet been reported. Here, we characterize the onset of motor deficits in $SCA6^{84Q}$ mice using a battery of behavioral assays to test for impairments in motor coordination, balance, and gait. We found that these mice performed normally on these assays up to and including at 6 months, but motor impairment was detected at 7 months with all motor coordination assays used, suggesting that motor deficits emerge rapidly during a narrow age window in $SCA6^{84Q}$ mice. In contrast to what is seen in SCA6 patients, the decrease in motor coordination was observed without alterations in gait. No loss of cerebellar Purkinje cells or striatal neurons were observed at 7 months, the age at which motor deficits were first detected, but significant Purkinje cell loss was observed in 2-year-old $SCA6^{84Q}$ mice, arguing that Purkinje cell death does not significantly contribute to the early stages of SCA6.

Significance Statement

We confirm that disease onset in an 84Q-hyperexpanded polyglutamine mouse model of spinocerebellar ataxia type 6 (SCA6) occurs at 7 months of age, in agreement with a previous study by Watase and colleagues (2008). We characterize disease onset more precisely using a barrage of behavioral tests at multiple ages, and identify that motor coordination abnormalities emerge in a narrow time window between 6 and 7 months, in contrast to the variable age of onset observed in human

patients. We find that Purkinje cell degeneration occurs in this SCA6 mouse model at 2 years, nearly 1.5 years after the onset of motor deficits, demonstrating that Purkinje cell loss is not necessary for early SCA6 disease symptoms.

Introduction

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant neurodegenerative disease that leads to progressive ataxia of the limbs and gait abnormalities, and is one of the most common of the spinocerebellar ataxias (Ashizawa et al., 2013). SCA6 is caused by a CAG-repeat expansion in the gene *CACNA1A* encoding the α1A-subunit of voltage-dependent P/Q-type calcium channel, causing a polyglutamine (poly-Q) expansion (Zhuchenko et al., 1997). P/Q channels are widely expressed in the brain, including in cerebellar Purkinje cells (Westenbroek et al., 1995; Craig et al., 1998), which undergo degeneration in SCA6 (Yang et al., 2000). In patients, SCA6 symptoms typically present in midlife, with an average onset of ataxic symptoms around ~40-50 years (Matsumura et al., 1997; van de Warrenburg et al., 2002; Ashizawa et al., 2013), although disease onset has been observed across a wide range of ages, from late teens to old age (Yabe et al., 1998).

The size of the repeat expansion that gives rise to SCA6 is short compared to other triplet repeat diseases (Gatchel and Zoghbi, 2005): unaffected individuals have <20 repeats, while pathological repeat length is 20-33 (Yabe et al., 1998; van de Warrenburg et al., 2002; Gatchel and Zoghbi, 2005). Consistent with several other triplet-repeat diseases, there is an inverse relationship between CAG repeat expansion length and age of onset in SCA6: longer repeats are correlated with earlier onset of symptoms (Matsumura et al., 1997; van de Warrenburg et al., 2002; Ashizawa et al., 2013). However, the repeat length relationship with the age of disease onset is estimated to account for only 52% of the variance in the age of onset of SCA6 (van de Warrenburg et al., 2002), meaning that individuals who have the same repeat length can differ in the age at which they are first affected by SCA6 by decades.

We wondered whether similar variability is observed in animal models of SCA6, since this may give insight into the origin of variability of disease onset in human patients.

Several mouse models have been developed for SCA6 that show a broadly similar relationship between repeat length and gene dosage on disease onset and severity as observed for human patients, although typically shifted towards longer repeat lengths than those observed in human patients. Mice with human-length triplet repeats ($SCA6^{30Q}$) have not been observed to develop motor deficiencies (Watase et al., 2008), while a homozygous knock-in mouse model that harbors a hyper-expanded 84-CAG repeat in the encoding region of the P/Q channel subunit ($SCA6^{84Q}$) displays late-onset motor symptoms similar to human patients: homozygous mice show no motor abnormalities at 3 months but exhibit motor deficits at 7 months (Watase et al., 2008). Furthermore, a mouse with an even longer CAG repeat ($SCA6^{118Q}$) displays motor impairment as early as 6 weeks old (Unno et al., 2012). While Purkinje cell loss has been reported to rapidly follow motor deficits in the $SCA6^{118Q}$ transgenic mouse (detected at 10 weeks, Unno *et al.*, 2012), no Purkinje cell loss has been reported to date in the late onset $SCA6^{84Q}$ mouse (Watase *et al.*, 2008). More recently, mice overexpressing P/Q-type calcium channel C-terminal fragments containing human-length triplet-repeat insertions have been developed that display motor phenotype (Du et al., 2013; Mark et al., 2015).

Since the variable onset of disease symptoms in SCA6 patients is only partially explained by differences in repeat length (van de Warrenburg et al., 2002), we wondered whether variability existed in disease onset in a mouse model of SCA6 as well. Since the $SCA6^{84Q}$ mouse best recapitulates the midlife-onset observed in human SCA6 (Watase et al., 2008), we chose to study the onset of motor coordination symptoms in more detail in the $SCA6^{84Q}$ mouse in order to pinpoint the age of onset of disease symptoms more accurately. We assayed motor coordination of $SCA6^{84Q}$ mice at multiple postnatal ages using several motor coordination assays including Rotarod, elevated beam, and

swimming. We found that motor deficits were detected simultaneously with all motor coordination assays, suggesting that there is a narrow and rapid age of onset in this SCA6^{84Q} mouse model, which is strikingly different from the high variability in the age of onset observed in human patients. Motor coordination deficits occurred in 7-month-old mice without any observable difference in gait or changes in Purkinje cell number or morphology, and gait abnormalities were not found even in 2-year-old mice. Although Purkinje cell degeneration was not observed at 7 months in SCA6$^{84Q/84Q}$ mice, these mice have fewer Purkinje cells than WT mice at 2 years, arguing that although Purkinje cell death may contribute to disease progression in SCA6, it does not significantly contribute to early stages of SCA6.

Materials and Methods

Animals. Transgenic SCA6^{84Q} mice were purchased from Jackson Laboratories (strain B6.129S7-*Cacna1atm3Hzo*/J) and heterozygous mice were bred in order to produce litter-matched male and female transgenic SCA6^{84Q} (homozygous, SCA6$^{84Q/84Q}$, and heterozygous, SCA6$^{84Q/+}$) and wildtype (WT) mice. At each age, behavioral assays were performed on naive animals with no prior exposure to the assays during a period of 5 consecutive days (see Table 1 for animal numbers at each age), and all data was acquired blind to genotype. Animals were moved from the housing room to the experiment room and allowed 30 min to acclimatize before beginning experiments on each day of testing. Assays were performed in same order: (1) Rotarod, (2) elevated beam assays on days 1−4, (3) swimming; (4) gait was tested last on day (D)5 of testing for 3-7 month-old mice. For 1- and 2-year-old mice, gait was tested first on the first day of testing prior to Rotarod.

Rotarod assay. Animals were placed on a Rotarod (Stoelting Europe, Ireland) using a standard 10-minute long accelerating assay where the rod accelerates from 4-40 RPM in the first 5 minutes and then continues to rotate at 40 RPM for the last 5 minutes (Carter et al., 2001; Watase et al., 2007) (Fig. 1*A*; Movie 1). The latency to fall was recorded for each mouse as a measure of cerebellar-related motor

92 coordination (Watase et al., 2007). Mice performed four trials (T1−4) per day, and had at least a 15 min
93 resting period between trials, over five consecutive days of testing (D1−5).

94 ***Elevated Beam assay.*** Animals walked along a custom-built apparatus consisting of raised round
95 wooden beams (100 cm long), towards a dark escape box as previously described (Carter et al., 2001)
96 (Fig. 2*A*; Movie 2). Bright light shining on the starting point was used as an aversive stimulus to
97 encourage mice to traverse the beam. D1−2 were training days, during which mice were trained to
98 cross a beam of 22 mm diameter. On days 3 and 4, correspond to testing day 1 and 2, each mouse
99 performed a trial on beams of the following diameters: 22, 18, 15 and 12 mm, totaling to 4 trials per
100 day. The time taken to traverse 80 cm was recorded, and the number of times the mouse's foot slipped
101 while crossing the beams was counted during post-hoc video analysis (see Movie 3 for an example of a
102 mouse whose feet slip 3 times during the assay).

103 ***Swimming assay.*** Animals were trained to swim across a custom-built Plexiglas swimming tank (100
104 cm long by 6 cm wide) towards a dry, boxed-in escape platform (Carter et al., 1999)(Fig. 3*A*; Movie 3).
105 Bright light at the starting location was used as an aversive stimulus to encourage swimming across the
106 tank. The mice were initially trained to swim across the swim tank towards the escape platform for two
107 trials per day. D1−2 were considered training days, while testing days correspond to D3−5 Mice were
108 videotaped and latency to traverse a 60 cm distance was recorded. The number of hind limb kicks to
109 cross the tank was counted during post-hoc video analysis. After the assay, mice were towel-dried and
110 monitored in their home cage for 20 minutes after the assay.

111 ***Gait Analysis.*** Gait was analyzed as previously described (Carter et al., 2001). The forelimbs and hind
112 limbs of each mouse were coated with distinct colors using non-toxic paint. Mice were prompted to
113 walk across a white sheet affixed to an elevated platform (10 cm high by 10 cm wide) towards a

custom-built dark escape box (Fig. 4*A*), leaving a trace of their paw prints on the sheet (Fig. 4*B*). Stride length (distance between subsequent left and right forelimb and hind limbs; Fig. 5*E-F*) and stance width (distance between forelimbs and hind limbs; Fig. 5*G, H*) were measured for 4-6 consecutive strides (measured between the centers of footprints). The co-efficient of variation (CV) between stride lengths as well as the degree of overlap between forelimb and hind limb footprints were recorded from 6 consecutive strides. This assay was performed in a single trial on either day 1 (at 1 and 2 years) or day 5 of testing (at 4, 6, and 7 months).

Immunocytochemistry. Mice were deeply anesthetized and perfused intracardially with 4% PFA (EMS, Hatfield, PA). The brain was extracted and stored in PFA at 4°C for 24 hours, then transferred to PBS with 0.5% sodium azide. The cerebellar vermis was sliced into 100 µm thick parasagittal slices, and the striatum was sliced into 100 µm thick coronal slices on a Leica Vibratome 3000 plus (Concord, ON, Canada). Staining was performed in a blocking solution consisting of 5% BSA, 0.05% sodium azide, and 0.4% Triton X in 0.01 M PBS. The primary antibodies used were rabbit anti-Calbindin D-28k (Swant, Switzerland) at a dilution of 1:1000, and mouse-anti NeuN (Millipore MAB377) tagged with Alexa 488 at a dilution of 1:500, and slices were incubated with this at room temperature on a rotary shaker at 70 RPM for 72 hours. Slices were then rinsed 3X in a solution of 0.4% Triton X in 0.01 M PBS, and for calbindin staining, a secondary antibody (Alexa Fluor 594 anti-rabbit (Jackson ImmunoResearch, West Grove, PA) was used at a dilution of 1:1000 in blocking solution and incubated for 90 minutes at room temperature while shaking. Sections were then rinsed and immediately mounted onto slides with Prolong gold anti-fade mounting solution (Life Technologies, Ontario, Canada) and stored in the dark at 4°C. Slices were imaged with a custom-built two-photon microscope with a Ti:Sapphire laser (MaiTai; SpectraPhysics, Santa Clara, CA) tuned to 775 nm. Image acquisition was done using ScanImage (Pologruto et al., 2003) running in Matlab (Mathworks,

Natick, MA). Purkinje cell numbers were counted in anterior (lobule 3) or posterior (lobule 9) vermis as a density per 100 µm Purkinje cell layer, and molecular layer thickness was measured from the distance between the Purkinje cell layer at multiple evenly-spaced locations in the lobule. Chemicals were purchased from Sigma unless otherwise indicated.

Data Analysis and statistics. All data was analyzed blind to genotype. For each behavioral assay, mouse performance was compared between the three genotypes using one-way ANOVA; when significance was found, this was followed by Tukey's HSD post-hoc test using JMP Software (SAS, Cary, NC). Purkinje cell density and molecular layer height were similarly compared with a one-way ANOVA followed by Tukey's HSD post-hoc test, and imaging data was acquired and analyzed blind to condition. Striatum cell counts were compared with Student's t-test. Data are reported as mean ± SEM.

Results

Rapid onset of motor coordination abnormalities at 7 months old

An accelerating Rotarod assay was used to test the motor coordination of mice at several postnatal ages ranging from 3 to 7 months (Fig. 1*A*). The performance of mice on Rotarod was age-dependant, since younger mice (3 and 4 months old) performed better than older ones (6 months old) on all three genotypes tested: $SCA6^{84Q/84Q}$, $SCA6^{84Q/+}$, and WT (Age: $F_{4,\ 105} = 10.67$; $P < 0.0001$; Fig. 1*B*). Similarly, we found that mice of all genotypes significantly increased their performance across days within an experimental age (comparing D5 to D1 X Age; $F_{16,\ 420} = 4.62$; $P < 0.0001$ for 3, 4, 5 and 6 months; Fig. 1*B*) except at 7 months, where there was no significant increase in performance across days in any genotype ($P = 0.57$).

In agreement with an earlier study by Watase and colleagues (2008), we found that $SCA6^{84Q/84Q}$ mice exhibited no motor abnormalities at 3 months, but displayed significant motor deficits compared to

159 SCA6$^{84Q/+}$ and WT mice at 7 months (Fig. 1*B*). We wondered if the onset of motor deficits occurred
160 between these ages, but found no differences in Rotarod performance across genotypes at 4, 5 or 6
161 months (consult Table 1 for sample size at each age; Fig. 1*B*), suggesting that the onset of motor
162 abnormalities in SCA6$^{84Q/84Q}$ mice is not earlier than 7 months, and that disease onset is relatively rapid
163 between 6 and 7 months old. Since some studies have found that other assays are more sensitive than
164 Rotarod to detect early motor abnormalities (Stroobants et al., 2013; Lariviere et al., 2015), we chose to
165 test motor coordination with additional assays as well.

166 We next conducted an elevated beam assay to gain insight into motor coordination and balance in
167 SCA6^{84Q} mice (Fig. 2*A*). Using beams of varying diameter, we measured the mice's latency to cross the
168 beam as an assay of motor coordination. Wide beams are typically easier for mice to walk across than
169 narrow beams, and we reasoned that a range of beam size might capture subtle motor deficits of fine
170 motor coordination that were not detectable with Rotarod. In contrast to what we found for Rotarod,
171 older mice (6 months) performed better by crossing the beams faster than younger mice (3 months) for
172 all genotypes tested, although this was only true for wide beams (Age: 22 mm beam: $F_{4, 105} = 4.72$; P =
173 0.003; 18 mm beam: $F_{4, 105} = 2.36$; P = 0.04; Figs. 2*B* and 2*C*), while no age-dependence was observed
174 for narrow diameter beams (15 mm: P = 0.38; and 12 mm: P = 0.14; Figs. 2*D* and 2*E*).

175 SCA6$^{84Q/84Q}$ mice took longer to traverse the elevated beam at 7 months in comparison to WT and
176 SCA6$^{84Q/+}$ mice for the majority of beams used, while their performance was indistinguishable at
177 earlier months (Fig. 2*B*-*E*). To look in more detail at the elevated beam phenotype, we measured the
178 number of times each mouse's hind leg feet slipped during beam crossings (Movies 2 and 3). For most
179 trials, the majority of mice crossed the beam without any footslips irrespective of genotype, age, or
180 beam width (P>0.05 for all beams and ages excluding 12 mm beam at 7 months; Fig. 3). However, at 7
181 months, the majority of SCA6$^{84Q/84Q}$ mice experienced footslips when crossing the narrowest beam, and

this was significantly different from WT or SCA6$^{84Q/+}$ mice (12 mm beam at 7 months: $F_{2, 37} = 4.19$; P = 0.02; Fig. 3*D*). Thus, the increased latency to cross most beams for 7-month-old SCA6$^{84Q/84Q}$ mice likely reflects motor coordination and/or balance abnormalities. SCA6$^{84Q/+}$ mice showed no significant differences compared to WT for any age or beam diameter (P>0.05; Fig. 2, 3). In summary, like Rotarod, the elevated beam assay found motor coordination and balance abnormalities in SCA6$^{84Q/84Q}$ mice at 7 months and no earlier, suggesting that the two assays are broadly similar in their ability to detect SCA6 motor abnormalities.

While Rotarod and elevated beam are standard assays for motor coordination and balance deficits, we wanted to explore whether less standard motor assays might be useful to detect a motor phenotype in SCA6 mice. Swimming assays have been shown to detect subtle motor deficits at an earlier age than both Rotarod and the elevated beam assay in Huntington's Disease (HD) mice (Carter et al., 1999), and we wondered if this might be similar in SCA6. We used a swimming assay to further characterize motor performance in SCA6^{84Q} mice (Fig. 4*A*). There were no obvious visual differences in the coordination of the limbs when SCA6^{84Q} mice swam. Unlike with Rotarod and the elevated beam assay, swimming performance showed no age-dependent differences across genotypes at all 5 ages tested, and the latency to cross the tank was not significantly different at any age across genotypes (3, 4, 5, 6, and 7 months; Age: $F_{4, 104} = 1.13$; P = 0.35; Fig. 4*B*). Mice appear to rely mainly on hind limbs for propulsion through the water when swimming. To determine if there were changes in swimming performance that was not captured by measuring latency, we also counted the number of hind limb swim kicks that were produced to traverse the tank. SCA6$^{84Q/+}$ and WT mice had a similar number of kicks across all ages (P>0.05; Fig. 4*C*). However, SCA6$^{84Q/84Q}$ mice produced a small but significant increase in hind limb kicks at 7 months on the third day of testing (Fig. 4*C, D*). These results strengthen our findings from Rotarod and elevated beam assays that SCA6$^{84Q/84Q}$ mice have normal motor ability and coordination

up until 6 months, and significant motor deficits are detected one month later at 7 months old, when more hind limb kicks are required to traverse the swim tank.

No gait abnormalities observed in SCA6$^{84Q/84Q}$ mice

Gait abnormalities have been recently reported for presymptomatic SCA6 patients (Rochester et al., 2014), and have also been observed in an SCA6 mouse model with an even longer 118Q expansion repeat (Unno et al., 2012). While no differences in gait were observed by eye, we examined gait in SCA6$^{84Q/84Q}$ mice using foot print analysis (Fig. 5*A, B*), and found no differences across genotypes for stride length (Fig. 5*C*−*F*) or stance width (Fig. 5*G, H*), and no differences across age 3−7 months for all genotypes (SCA6$^{84Q/84Q}$, SCA6$^{84Q/+}$, and WT, $P > 0.05$ for all measurements).

To further test for possible changes in gait, we looked at the variance of stride lengths and paw overlap since stride lengths of mice are known to be very precise with minimal variation (low CV) (Carter et al., 1999). We measured the CV of inter-stride distances from 6 consecutive strides to detect whether changes in this variance could be observed in SCA6^{84Q} mice. We found that the CV of hind limb and forelimb strides was low and not significantly different across genotypes (hind limb stride: Genotype: $F_{2, 63} = 0.27$; $P = 0.77$; forelimb stride: Genotype: $F_{2, 63} = 0.10$; $P = 0.91$; data not shown). We next compared the variance in paw overlap from forelimb and hind limb paws, but found that consistent with our other gait analyses, no significant differences in the CV of inter-paw overlap was observed across genotypes (Genotype: $F_{2, 63} = 0.44$; $P = 0.56$; data not shown). Furthermore, there were no significant age-dependent changes for any genotype ($P > 0.05$ for each measure; refer to table 2). These results suggest that SCA6$^{84Q/84Q}$ mice have normal gait at 7 months despite exhibiting motor coordination deficits that were detected using Rotarod (Fig. 1), elevated beam (Figs. 2 and 3), and swimming assays (Fig. 4).

We next tested gait in older SCA6$^{84Q/84Q}$ mice to determine if gait abnormalities emerged as the disease progressed, as has been observed in other mouse models (Unno et al., 2012). Using the same analyses as we performed at younger ages, we observed a difference comparing 1- and 2-year-old mice on several gait measurements across genotype (Age: $P < 0.005$ for left and right forelimb and hind limb stride, left and right stance, and CV of inter-paw overlap; ANOVA followed by post-hoc Tukey's test; Fig. 6) although not all measures showed significant changes (no significant difference in the CV of left or right forelimb or hind limb strides, $P > 0.05$; Fig. 6). These data suggest that there is a general age-related alteration in gait in aging mice. Surprisingly, there were no significant differences in any measure of gait in 1- or 2-year-old SCA6$^{84Q/84Q}$ mice compared to age-matched WT mice (Fig. 6). This result strongly argues that gait abnormalities are not observed in SCA6$^{84Q/84Q}$ mice throughout the majority of their lifespan, although they, like WT mice, experience aging-related gait alterations.

To assess disease progression over the same older mice, we examined Rotarod performance in 1- and 2-year-old mice. Motor coordination abnormalities were observed in 1 and 2-year-old SCA6$^{84Q/84Q}$ mice on Rotarod (Fig. 6), and these deficits progressively worsened compared to deficits observed at 7-months-old (Age X Genotype $F_{2, 40} = 3.67$; $P = 0.034$; Figs. 1, 6). Taken together, our data illustrate that motor coordination deficits have a rapid midlife onset in a narrow time window in SCA6$^{84Q/84Q}$ mice and these symptoms progressively worsen without any alterations in gait.

Late Purkinje cell degeneration in SCA6$^{84Q/84Q}$ mice long after the onset of motor coordination deficits

The transgenic SCA6$^{84Q/84Q}$ mice that we use in this study have previously been reported to exhibit no Purkinje cells degeneration at 20 months old (Watase et al., 2008), which is in contrast to degeneration observed early in 118Q hyper-expanded mice (Unno et al., 2012) and postmortem in human SCA6

patient data (Yang et al., 2000). We first examined Purkinje cell density in 7-month-old mice and found 5.2 ± 0.17 cells / 100 µm Purkinje cell layer in WT mice, with no significant differences in $SCA6^{84Q/84Q}$ mice (WT: 496 cells measured in 9.7 mm of Purkinje cell layer from N = 3 animals; $SCA6^{84Q/84Q}$: 490 cells in 9.7 mm of Purkinje cell layer from N = 3 animals; Fig. 7*A, B*), consistent with previous reports (Watase et al., 2008). We wondered whether subtle changes in Purkinje cell number or morphology might be restricted to only part of the cerebellum, since Purkinje cell degeneration has been reported to be more prevalent in anterior lobules of cerebellar vermis in some human patients (Gierga et al., 2009; Nanri et al., 2010). To address whether changes might be localised to subregions of cerebellar vermis, we measured Purkinje cell density in anterior and posterior lobules, but observed no significant differences in both WT and $SCA6^{84Q/84Q}$ mice (data not shown). To look in more detail at Purkinje cell morphology at disease onset, we measured the height of the molecular layer (Fig. 7*A*) as an estimate of the height of Purkinje cell dendritic trees and found no significant differences between WT and $SCA6^{84Q/84Q}$ mice at 7 months (WT: 291.8 ± 6.8 µm; $SCA6^{84Q/84Q}$: 301.2 ± 8.1 µm; Fig. 7*A, D*). Thus, the onset of disease symptoms in $SCA6^{84Q}$ mice is not associated with alterations in Purkinje cell number or gross dendritic morphology.

Since no changes in Purkinje cell number have been reported in 20-month-old $SCA6^{84Q/84Q}$ mice (Watase et al., 2008), we looked for degeneration in older mice at 2 years. We observed no significant reduction in Purkinje cell density in 2-year-old WT mice compared to 7 months, suggesting that little degeneration has occurred at this age in WT mice ($P = 0.99$; Fig. 7*B, D*). However, we observed a reduction in the density of Purkinje cells in $SCA6^{84Q/84Q}$ mice at 2 years compared to 7 months ($P < 0.0001$; Fig. 7*B, C*). Consistent with this, $SCA6^{84Q/84Q}$ mice had ~22% fewer Purkinje cells than their litter-matched WT siblings at 2 years (WT: 5.09 ± 0.18 cells / 100 µm, 538 cells measured in 10.3 mm of Purkinje cell layer from N = 3 mice; $SCA6^{84Q/84Q}$: 4.01 ± 0.16 cells / 100 µm, 385 cells in 9.8 mm of

Purkinje cell layer from N = 3 mice; Fig. 7*D*). To address whether these changes were localized across the vermis, we compared the cell density in anterior and posterior lobules in SCA6$^{84Q/84Q}$ mice, and found no significant differences at 2 years (anterior: 3.7 ± 0.19 cells / 100 µm; posterior: 4.2 ± 0.25 cells / 100 µm; P = 0.51). Rather, we observed a reduced Purkinje cell density in both anterior and posterior lobules of SCA6$^{84Q/84Q}$ mice at 2 years compared to age- and litter-matched WT mice (anterior: $F_{3, 92} = 10.16$; P < 0.0001; posterior: $F_{3, 106} = 3.37$; P = 0.02; data not shown), in contrast to the predominantly anterior Purkinje cell degeneration observed in some human SCA6 patients (Gierga et al., 2009; Nanri et al., 2010).

Since Purkinje cell degeneration has been associated with both a reduction in cell number as well as structural changes in Purkinje cell dendrites (Yang et al., 2000), we looked at the height of the molecular layer as a read-out of Purkinje cell dendritic alterations. We found the molecular layer height in 2-year-old WT mice was 299.5 ± 4.9 µm, which was not significantly different from younger (7 month) WT mice (P = 0.72; Fig. 7*A,C, D*). Together with the cell count data for 2-year-old WT mice (Fig. 7*B*), this suggests that very little Purkinje cell degeneration has occurred in the vermis in aged WT mice. However, the average molecular layer thickness of SCA6$^{84Q/84Q}$ mice at 2 years was 257.8 ± 5.3 µm, a ~15% reduction from the molecular layer height in 7-month-old SCA6$^{84Q/84Q}$ mice (P < 0.0001). We found that the molecular layer height in 2-year-old SCA6$^{84Q/84Q}$ mice was significantly reduced compared to age-matched WT mice (Fig. 7*C, D*). Both our cell count and molecular layer data suggest that there is significant Purkinje cell degeneration by 2 years in SCA6$^{84Q/84Q}$ mice.

Although SCA6 has been considered to be an example of a pure cerebellar ataxia (Solodkin and Gomez, 2012), non-cerebellar symptoms are present in some patients, with up to 25% of affected individuals having signs of basal ganglia-related symptoms (Solodkin and Gomez, 2012). Since several recent

studies have reported degeneration in the striatum of patients with other SCAs, including SCA2, SCA3 (Schols et al., 2015), and SCA17 (Brockmann et al., 2012), we looked at the number of striatal neurons in SCA6$^{84Q/84Q}$ mice at 7 months to determine if degeneration in the striatum was associated with the onset of motor abnormalities. We found that there were no significant differences between the density of cells in the striatum of SCA6$^{84Q/84Q}$ and WT mice at 7 months (SCA6$^{84Q/84Q}$: 1191 ± 36 cells/mm^2 striatum, total of 3332 cells counted from N = 4 mice; WT: 1212 ± 48 cells/mm^2, total of 2586 cells counted from N = 3 mice; not significantly different, P = 0.72; Fig. 8).

Discussion

We have performed an in-depth analysis of motor coordination and gait in a late-onset mouse model (84Q repeat length) of SCA6 in order to better understand the onset and progression of the SCA6 phenotype. We confirm that homozygous SCA6$^{84Q/84Q}$ mice display motor coordination deficits at 7 months, and that deficits were detectable simultaneously in all motor coordination assays tested, including Rotarod, elevated beam, and swimming. Prior to 7 months, the behavior of SCA6$^{84Q/84Q}$, SCA6$^{84Q/+}$, and WT mice were indistinguishable, arguing that motor coordination and performance in SCA6^{84Q} mice is normal prior to disease onset, and that motor deficits appear rapidly between 6 and 7 months in SCA6$^{84Q/84Q}$ mice. Although Saegusa and colleagues report that heterozygous mice expressing a human-length (28Q) repeat show enhanced motor coordination compared to WT (Saegusa et al., 2007), we observe no significant differences between heterozygous SCA6$^{84Q/+}$ and WT mice at any ages tested, in agreement with previous reports (Watase et al., 2008). In spite of observing motor coordination deficits in 7-month-old SCA6$^{84Q/84Q}$ mice, we observe no changes in any measures of gait, suggesting that this mouse model does not reproduce the progression of gait abnormalities typically observed for the human disease. Indeed, we were unable to detect changes in gait up to 2 years old, when motor coordination deficits had worsened, arguing that gait and motor coordination deficits can

present independently. Finally, the onset of disease symptoms in SCA6 is not accompanied by morphological changes or survival of cerebellar Purkinje cells, although Purkinje cell degeneration, reflecting both reductions in dendritic height and cell number, is observed nearly 1.5 years later in 2-year-old SCA6$^{84Q/84Q}$ mice. The long time delay between disease onset and Purkinje cell degeneration suggests that Purkinje cell loss does not significantly contribute to early stages of SCA6 pathophysiology, and suggests that the potential for therapeutic intervention before cell death occurs might be a promising avenue of future study.

Comparison of motor assays for detection of SCA6 and motor abnormalities

We studied a range of motor coordination assays in SCA6^{84Q} mice as it was unknown if one motor assay would be more sensitive than others to subtle changes in motor coordination in SCA6. With Rotarod, a mouse has the opportunity to slip only one time per trial, upon which it typically falls, which means that graded differences in motor performance may be underestimated. We reasoned that other motor coordination assays, like the elevated beam in which mice can slip multiple times during the completion of the task, might provide a more nuanced read-out of motor deficits, as has been observed in some animal models of ataxia (Lariviere et al., 2015), although there are animal models, like the SCA3 transgenic mouse, where deficits are detected with Rotarod before elevated beam (Switonski et al., 2015). We also used a swimming assay which has been shown to detect motor abnormalities in a HD transgenic mouse earlier than the elevated beam (Carter et al., 1999). Since we observe similar results with 3 different motor coordination assays, we argue that these assays have comparable power to detect motor coordination deficit onset in SCA6. However, in our hands Rotarod is simpler and easier to administer than the elevated beam or swimming assays, making it our preferred assay for SCA6 detection. It is possible, however, that even more sensitive assays exist to detect SCA6 motor abnormalities in rodents that we have not tested (e.g. (Vinueza Veloz et al., 2014; Jarrahi et al., 2015)).

We found robust motor deficits at 7 months without any concomitant changes in gait, and indeed gait abnormalities were not observed even in 2-year-old SCA6$^{84Q/84Q}$ mice. This may at first appear surprising given that gait abnormalities are some of the first changes to be observed in SCA6 patients (Rochester et al., 2014), and gait abnormalities have been observed in an SCA6 mouse model with an even longer poly-Q expansion repeat (Unno et al., 2012). However, although motor coordination deficits and gait abnormalities often present together in mouse models (e.g. (Chen et al., 2015; Swarnkar et al., 2015)), this is not always the case: there are some ataxic models where gait abnormalities and motor coordination deficits are not temporally correlated (e.g. (Clark et al., 1997; Simon et al., 2004; Lariviere et al., 2015)), while in other transgenic mouse models, gait abnormalities have even been shown to accompany enhanced performance on motor coordination assays (Nakatani et al., 2009; Piochon et al., 2014). The absence of gait abnormalities with motor coordination changes in SCA6$^{84Q/84Q}$ mice highlights a limitation of this model to faithfully recapitulate human SCA6 symptoms (Rochester et al., 2014).

In addition to the reduced motor coordination observed with each motor assay for SCA6$^{84Q/84Q}$ mice at 7 months, we also observed age-dependent changes in performance across genotypes that were strikingly different for the different motor assays we used: Rotarod showed *decreased* motor performance with age, the elevated beam assay showed *increased* motor performance with age for wider beams, while swimming showed no apparent age-related differences in performance. While we and others have found decreased Rotarod performance in aged WT mice (>18 months for (Barreto et al., 2010); 2 years, Fig. 6*D*), which has been posited to arise because of neurodegeneration, we saw no significant reduction in WT Purkinje cell density at 2 years compared to 7 months (Fig. 7). In any case, degeneration cannot explain the age-dependent decreases in performance seen in 7-month-old mice of all genotypes with Rotarod (Fig. 1). All 3 genotypes showed reduced performance with Rotarod at 7

363 months compared to earlier performance; this suggests that there is a natural decline in performance
364 around 7 months that is exacerbated in SCA6$^{84Q/84Q}$ mice. Understanding the mechanism of aging-
365 dependent decline at 7 months in all genotypes may provide insight into disease onset in SCA6.

366 Another difference between Rotarod and the elevated beam assay is that for a given age, performance
367 tends to improve on successive trials per day (data not shown) as well as over experimental days with
368 Rotarod (Fig. 1), while on the elevated beam mice tend to perform worse on the second day of training
369 on most beam diameters (Fig. 2). In our experience, this day-on-day declining performance reflects at
370 least in part the mice's waning motivation to cross the beam, which could confound the evaluation of
371 motor coordination with this assay, while the day-on-day improvement observed with Rotarod may
372 involve cerebellar learning (Ly et al., 2013). For the swimming assay, there is little day-on-day or age-
373 dependent changes in performance, suggesting that this assay may not be best suited to measure some
374 aspects of motor performance. For these reasons we find that Rotarod is our best assay to detect motor
375 learning alterations in mouse models of SCA6.

376 ***Implications of SCA6^{84Q} Mouse model for human SCA6***

377 While motor coordination abnormalities have been observed in SCA6$^{84Q/84Q}$ mice at 7 months in a
378 previous study (Watase et al., 2008), since only a few time points were studied with a single motor
379 assay in this previous report, the onset of disease symptoms was not well understood. Here we have
380 characterized a rapid disease onset between 6 and 7 months that is detected with multiple behavioural
381 assays, suggesting that disease onset is relatively strong since it can be detected by assays of varying
382 sensitivity. This characterization of SCA6$^{84Q/84Q}$ mice helps to strengthen this transgenic mouse as a
383 model system for studying disease onset in SCA6. Our result of a narrow age of onset of disease
384 symptoms is in contrast to observations in human patients, where individuals with a given repeat length

can differ in age of onset by decades (van de Warrenburg et al., 2002). What might account for the discrepancy in disease onset variability between human patients and this transgenic mouse model? Transgenic SCA6^{84Q} mice are genetically homologous and live in a controlled environment, thus both genetic and environmental diversity that exists in SCA6 patients may be absent in our study. Future enquiry is required into understanding the contribution of epigenetic, environmental, and/or epistatic influences on SCA6 disease onset.

Since Purkinje cell loss is a common attribute of SCA6 (Yang et al., 2000), one of the limitations of the SCA6^{84Q} transgenic mouse model in the past has been the absence of reported Purkinje cell degeneration (Watase et al., 2008), unlike in other mouse models where Purkinje cell death is detected within weeks of disease onset (Unno et al., 2012). We observe Purkinje cell degeneration in 2-year-old SCA6$^{84Q/84Q}$ mice, nearly 1.5 years after the onset of motor deficits, which argues that Purkinje cell death does not contribute to early onset of motor abnormalities, and supports findings from other SCA6 mouse models suggesting that early symptoms of SCA6 arise from cellular alterations. Mechanisms that might contribute to the onset of motor dysfunction might be cellular inclusions in Purkinje cells (Watase et al., 2008; Mark et al., 2015), although Watase and colleagues show immunocytochemical evidence for these inclusions only at later ages (22 months), long after the onset of motor dysfunction in these mice (Watase et al., 2008). Other mechanisms contributing to early motor dysfunction might be due to a C-terminal fragment encoded by the *CACNA1A* gene that may act as a transcription factor (Du et al., 2013), and/or may have deleterious action in the cytoplasm of Purkinje cells (Mark et al., 2015). Changes in synaptic input to Purkinje cells may also be involved (Mark et al., 2015). Our demonstration of Purkinje cell degeneration, a key feature of human SCA6 (Yang et al., 2000), in aged SCA6$^{84Q/84Q}$ mice supports these mice as a good model system of SCA6. From a therapeutic perspective, these results are promising, as they suggest a window for therapeutic intervention might

exist where motor function could be ameliorated by the rescue of cellular abnormalities before cell death occurs. Rescue of motor function after cell death at later stages of the disease may be more challenging, and may require different therapeutic approaches.

Several recent studies on SCAs have found that neural degeneration is not limited to cerebellum. For instance, degeneration of neurons in the striatum has been observed in SCA2, SCA3 (Schols et al., 2015), and SCA17 (Brockmann et al., 2012). Interestingly, significant degeneration has been observed in the striatum without motor symptoms in both HD (Cowan and Raymond, 2006), and in several SCAs (Brockmann et al., 2012; Schols et al., 2015), which made us wonder whether changes in the striatum might be involved in the onset of motor symptoms in SCA6. However, we found no significant cell loss in the stratum of $SCA6^{84Q/84Q}$ mice at 7 months, suggesting that striatal degeneration is unlikely to contribute to early stages of disease onset in SCA6.

In summary, homozygous $SCA6^{84Q/84Q}$ mice display motor coordination deficits that arise rapidly between 6 and 7 months without gait abnormalities or Purkinje cell degeneration. Motor coordination deficits progress, and Purkinje cell degeneration is observed in 2-year-old $SCA6^{84Q/84Q}$ mice, confirming that these mice display this hallmark feature of human SCA6. The temporal lag between disease onset and neuronal degeneration argues that degeneration plays a role only in later stages of SCA6. These results are important as they suggest that a wide therapeutic window may exist after SCA6 disease onset before cell death occurs.

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Legends

Figure 1. Rotarod deficits at 7 months in SCA6$^{84Q/84Q}$ mice.

A, schematic of experimental paradigm: accelerating Rotarod experiments were conducted for 4 trials/day for 5 days of testing at each age. *B*, no significant differences on D4−5 were observed between SCA6$^{84Q/84Q}$, SCA6$^{84Q/+}$, and WT genotypes at 3, 4, 5 or 6 months old; however, SCA6$^{84Q/84Q}$ mice display poorer performance on Rotarod on D4−5 at 7 months compared to WT mice (Genotype: $F_{2, 37} = 12.19$; P = 0.0004, one-way ANOVA with post-hoc Tukey's test; ***P < 0.0005, P>0.05 where not indicated; N = 8−10 SCA6$^{84Q/84Q}$ mice depending on age, 5−9 SCA6$^{84Q/+}$ mice, and 6−9 WT mice, consult Table 1 for sample size at each age).

Figure 2. Increased latency on elevated beam at 7 months in SCA6$^{84Q/84Q}$ mice.

A, schematic of experimental design for elevated beam assay. Two days of training were followed by two days of testing (D1 and D2 in panels *B*−*E*). *B*−*E*, latency to cross beam was measured for each genotype at each age (3, 4, 5, 6, and 7 months) over D1 and D2. SCA6$^{84Q/84Q}$ mice were significantly slower at traversing the beam at 7 months on D2 for the following diameters: *B*, 22 mm ($F_{2, 17} = 7.36$; P = 0.005) *C*, 18 mm ($F_{2, 17} = 7.46$; P = 0.005), *D*, 15 mm ($F_{2, 17} = 4.34$; P = 0.03), and *E*, 12 mm ($F_{2, 17} = 5.27$; P = 0.017), while SCA6$^{84Q/+}$ mice were indistinguishable from WT. *P < 0.05, **P < 0.01, ***P < 0.005, P>0.05 where not indicated, one-way ANOVA followed by post-hoc Tukey's test; N = 8−10 SCA6$^{84Q/84Q}$ mice depending on age, 5−9 SCA6$^{84Q/+}$ mice, and 6−9 WT mice, consult Table 1 for sample size at each age.

Figure 3. Increased footslips on narrow elevated beam at 7 months in SCA6$^{84Q/84Q}$ mice.

A–*D,* the number of mice that display footslips (0 footslips = lightest colour, > 2 footslips = darkest colour, and 1 and 2 footslips graded in between) when crossing beams for 3 genotypes: WT (greyscale) SCA6$^{84Q/+}$ (orange-scale) and SCA6$^{84Q/84Q}$ mice (red-scale); see legend on the right. No differences were seen across genotypes and age for: *A,* 22 mm diameter beam ($F_{2, 37} = 0.17$; P = 0.85); *B,*18 mm beam ($F_{2, 37} = 1.91$; P = 0.16); *C,* 15 mm beam ($F_{2, 37} = 0.65$; P = 0.53); *D,* a significant increase in the number of footslips was observed for the 12 mm beam at 7 months for SCA6$^{84Q/84Q}$ mice ($F_{2, 37} = 4.19$; P = 0.02). *P < 0.05, P > 0.05 where not indicated, one-way ANOVA followed by post-hoc Tukey's test; N = 8–10 SCA6$^{84Q/84Q}$ mice depending on age, 5–9 SCA6$^{84Q/+}$ mice, and 6–9 WT mice, consult Table 1 for sample size at each age.

Figure 4. Reduced swimming deficits at 7 months in SCA6$^{84Q/84Q}$ mice.

A, schematic showing the experimental design for the swimming assay. Mice were trained for 2 days and subsequently tested over 3 days (D1-D3 in panels *B* and *C*). *B,* no significant differences in swim latency were observed for mice across ages and genotypes (Age X Genotype: $F_{8, 105} = 1.85$; p = 0.07; see Table 1 for N's). *C*, in contrast to latency, there was an increase in the number of hind-limb kicks performed to cross the tank at 7 months in SCA6$^{84Q/84Q}$ but not SCA6$^{84Q/+}$ compared to WT mice (Age X Genotype X Days: $F_{16, 210} = 1.81$; P = 0.03). N = 8–10 SCA6$^{84Q/84Q}$ mice depending on age, 5–9 SCA6$^{84Q/+}$ mice, and 6–9 WT mice, consult Table 1 for sample size at each age. *D,* summary data showing the number of kicks on Day 3 at 7 months old for the different genotypes. * P < 0.05 one-way ANOVA followed by Tukey's post-hoc test; N = 6 WT, 5 SCA6$^{84Q/+}$, 9 SCA6$^{84Q/84Q}$.

Figure 5. No abnormalities observed in gait in SCA6^{84Q} mice before or at the onset of motor coordination deficits.

A, schematic of painted footprint experiment used to study gait: forelimbs were painted blue and hind limbs were painted red. *B,* representative footprints from mice in each genotype reveal no significant differences in gait. (*C*−*H*) The distance between subsequent limb placements (stride length) at 4, 6, and 7 months were not significantly different across phenotypes for: *C,* left hind limb ($F_{2, 63} = 0.14$; $P = 0.87$); *D,* left forelimb ($F_{2, 63} = 0.25$; $P = 0.78$); *E,* right hind limb ($F_{2, 63} = 0.46$; $P = 0.64$); and, *F,* right forelimb stride lengths ($F_{2, 63} = 0.08$; $P = 0.91$). Likewise, no significant differences were observed for stance (distance between left and right limb placements) of: *G,* hind limbs ($F_{2, 63} = 1.23$; $P = 0.30$); and *H,* forelimbs ($F_{2, 63} = 0.53$; $P = 0.60$) at 4, 6, or 7 months. One-way ANOVA; N = 8−10 SCA6$^{84Q/84Q}$ mice depending on age, 5−9 SCA6$^{84Q/+}$ mice, and 6−9 WT mice, consult Table 1 for sample size at each age.

Figure 6. Disease progression marked by no gait abnormalities, but worsening motor coordination.

A−*F*, gait was examined in aging animals that were 1- and 2-years old to determine if differences in gait emerged as SCA6 progressed. The stride length at 1 and 2 years were not significantly different across SCA6$^{84Q/84Q}$ and WT mice for: *A,* left hind limb ($F_{1, 27} = 0.08$; $P = 0.77$); *B,* left forelimb ($F_{1,27} = 0.01$; $P = 0.90$); *C,* right hind limb ($F_{1, 27} = 0.002$; $P = 0.97$); and, *D,* right forelimb stride lengths ($F_{1, 27} = 0.10$; $P = 0.76$). Nor were significant differences observed for stance (distance between left and right limb placements) of: *E,* hind limbs ($F_{1, 27} = 0.0001$; $P = 0.99$); and *F,* forelimbs ($F_{1, 27} = 0.17$; $P = 0.68$). *G,* motor coordination abnormalities worsened with age for 1- and 2-year-old mice on Rotarod (1 year,

605 Genotype: $F_{1,30} = 56.01$; $P < 0.0001$; 2 year, Genotype: $F_{1,28} = 33.62$; $P < 0.0001$). *** $P < 0.001$ one-
606 way ANOVA followed by Tukey's post-hoc test; N = 8 WT and 8 $SCA6^{84Q/84Q}$ 1-year-old mice; n = 7
607 WT and 8 $SCA6^{84Q/84Q}$ 2-year-old mice.

608

609 **Figure 7. Purkinje cell degeneration is observed long after the onset of motor phenotype at 2**
610 **years in $SCA6^{84Q/84Q}$ mice.**

611 *A,* representative images of calbindin-stained Purkinje cells from 7-month-old WT (left) and
612 $SCA6^{84Q/84Q}$ (right) mouse cerebellar slices. The height of the molecular layer is indicated. Scale bar for
613 both images, 20 µm. *B,* density of Purkinje cells in 7-month-old cerebellum is not significantly
614 different in $SCA6^{84Q/84Q}$ compared to WT mice (Genotype: $F_{1, 109} = 0.002$, $P = 0.96$). However, reduced
615 Purkinje cell density is observed at 2 years in $SCA6^{84Q/84Q}$ mice (genotype: $F_{1, 97} = 18.76$, $P = <0.0001$;
616 right). *C,* representative images of 2-year-old WT (left) and $SCA6^{84Q/84Q}$ (right) Purkinje cells. Scale
617 bar for both images, 20 µm. *D,* no significant different in Purkinje cell molecular layer is observed at 7
618 months in $SCA6^{84Q/84Q}$ and WT mice ($F_{1, 203} = 0.79$, $P = 0.37$; left), while molecular layer thickness is
619 reduced at 2 years in $SCA6^{84Q/84Q}$ mice compared to WT ($F_{1, 189} = 33.12$, $P < 0.0001$; right). N = 3-4
620 animals for each genotype at each age; at least 12 mm of Purkinje cell layer was measured for each
621 comparison; one-way ANOVA with post-hoc Tukey's test. *** $P < 0.0001$, ** < 0.01, *$P < 0.05$,
622 $P>0.05$ where not indicated.

623 **Figure 8. No loss of striatal neurons in $SCA6^{84Q/84Q}$ mice accompanies the onset of motor**
624 **coordination deficits at 7 months.**

625 *A*, representative images of NeuN-stained cells from 7-month-old WT (left) and SCA6$^{84Q/84Q}$ (right)
626 mouse striatum. Scale bar for both images, 20 µm. *B*, density of striatal cells is not significantly
627 different in SCA6$^{84Q/84Q}$ compared to WT mice at 7 months (Student's t-test, P = 0.72).

628

Table 1. Sample size for each genotype at each experimental age

Genotype	N for each experimental age						
	3 months	4 months	5 months	6 months	7 months	1 year	2 years
WT	7	9	8	7	6	8	7
$SCA6^{84Q/+}$	6	9	7	8	5	-	-
$SCA6^{84Q/84Q}$	9	9	8	10	9	8	8

Summary of the number of animals (N) for each of three genotypes used at each experimental age (mice were naive at each age without any prior behavioral training).

Table 2. Statistical Table

Figure & Panel		Description	Test	Degrees of freedom	F-Value	P-Value	95% Confidence interval
1	B	Rotarod (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	10.6731	<0.0001	-
1	B	Rotarod (3 to 7 months) - Effect of days X age	ANOVA - Fit model	16, 420	4.6269	<0.0001	-
1	B	Rotarod (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	2.2818	0.0271	-
1	B	Rotarod (3 months) - Effect of genotype	One-WAY ANOVA	2,39	2.5509	0.091	-
1	B	Rotarod (4 months) - Effect of genotype	One-WAY ANOVA	2,51	0.2849	0.7533	-
1	B	Rotarod (5 months) - Effect of genotype	One-WAY ANOVA	2,45	1.5495	0.2235	-
1	B	Rotarod (6 months) - Effect of genotype	One-WAY ANOVA	2,53	0.6127	0.5457	-
1	B	Rotarod (7 months) - Effect of genotype	One-WAY ANOVA	2,37	12.1937	<0.0001	-
1	B	Rotarod (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0004	[14.17,52.89]
1	B	Rotarod (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.0009	[12.74,53.71]

1	B	Rotarod (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.9994	[-21.94, 22.54]
2	B	Balance beam latency, 22 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	4.7234	0.0026	-
2	B	Balance beam latency, 22 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	2.0755	0.0446	-
2	B	Balance beam latency, 22 mm (3 months) - Effect of genotype	One-WAY ANOVA	2,18	1.8397	0.1875	-
2	B	Balance beam latency, 22 mm (4 months) - Effect of genotype	One-WAY ANOVA	2,24	1.3117	0.288	-
2	B	Balance beam latency, 22 mm (5 months) - Effect of genotype	One-WAY ANOVA	2,21	1.8648	0.1797	-
2	B	Balance beam latency, 22 mm (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.1779	0.838	-
2	B	Balance beam latency, 22 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,17	7.3589	0.005	-
2	B	Balance beam latency, 22 mm (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0101	[0.78,5.88]
2	B	Balance beam latency, 22 mm (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.021	[0.46,5.86]
2	B	Balance beam latency, 22 mm (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.987	[-2.76,3.11]
2	C	Balance beam latency, 18 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	2.3582	0.0398	-
2	C	Balance beam latency, 18 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	3.0995	0.0035	-
2	C	Balance beam latency, 18 mm (3 months) - Effect of genotype	One-WAY ANOVA	2,18	1.8068	0.1927	-
2	C	Balance beam latency, 18 mm (4 months) - Effect of genotype	One-WAY ANOVA	2,24	0.0071	0.9929	-
2	C	Balance beam latency, 18 mm (5 months) - Effect of genotype	One-WAY ANOVA	2,21	2.0014	0.1601	-
2	C	Balance beam latency, 18 mm (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.7112	0.5007	-
2	C	Balance beam latency, 18 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,17	7.4618	0.0047	-
2	C	Balance beam latency, 18 mm (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0083	[1.67,11.43]
2	C	Balance beam latency, 18 mm (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.0243	[0.73,11.06]
2	C	Balance beam latency, 18 mm (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.9519	[-4.95,6.26]
2	D	Balance beam latency, 15 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	1.0691	0.3756	-
2	D	Balance beam latency, 15 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	2.0276	0.05	-
2	D	Balance beam latency, 15 mm (3 months) - Effect of genotype	One-WAY ANOVA	2,18	3.1614	0.0666	-

2	D	Balance beam latency, 15 mm (4 months) - Effect of genotype	One-WAY ANOVA	2,24	0.5249	0.5982	-
2	D	Balance beam latency, 15 mm (5 months) - Effect of genotype	One-WAY ANOVA	2,21	0.9172	0.4151	-
2	D	Balance beam latency, 15 mm (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.4909	0.6178	-
2	D	Balance beam latency, 15 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,17	4.3447	0.0299	-
2	D	Balance beam latency, 15 mm (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0382	[0.22,8.51]
2	D	Balance beam latency, 15 mm (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.1167	[-0.77,8.00]
2	D	Balance beam latency, 15 mm (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.9143	[-4.01,5.51]
2	E	Balance beam latency, 12 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	1.7763	0.1391	-
2	E	Balance beam latency, 12 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	2.1864	0.0342	-
2	E	Balance beam latency, 12 mm (3 months) - Effect of genotype	One-WAY ANOVA	2,18	0.4304	0.6568	-
2	E	Balance beam latency, 12 mm (4 months) - Effect of genotype	One-WAY ANOVA	2,24	1.5008	0.2431	-
2	E	Balance beam latency, 12 mm (5 months) - Effect of genotype	One-WAY ANOVA	2,21	0.332	0.7212	-
2	E	Balance beam latency, 12 mm (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.4932	0.6165	-
2	E	Balance beam latency, 12 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,17	5.2734	0.0165	-
2	E	Balance beam latency, 12 mm (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0427	[0.16,10.23]
2	E	Balance beam latency, 12 mm (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.0351	[0.33,9.85]
2	E	Balance beam latency, 12 mm (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.9987	[-5.37,5.57]
3	A	Balance beam footslips, 22 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	2.1833	0.0759	-
3	A	Balance beam footslips, 22 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	0.5829	0.79	-
3	A	Balance beam footslips, 22 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,37	0.1683	0.8458	-
3	B	Balance beam footslips, 18 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	1.589	0.1827	-
3	B	Balance beam footslips, 18 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	0.6673	0.7191	-
3	B	Balance beam footslips, 18 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,37	1.9098	0.1624	-

3	C	Balance beam footslips, 15 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	2.1859	0.0756	-
3	C	Balance beam footslips, 15 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	0.5983	0.7779	-
3	C	Balance beam footslips, 15 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,37	0.6498	0.528	-
3	D	Balance beam footslips, 12 mm (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	2.1259	0.0827	-
3	D	Balance beam footslips, 12 mm (3 to 7 months) - Effect of genotype X age	ANOVA - Fit model	8, 105	2.1089	0.0412	-
3	D	Balance beam footslips, 12 mm (3 months) - Effect of genotype	One-WAY ANOVA	2,39	0.1375	0.8719	-
3	D	Balance beam footslips, 12 mm (4 months) - Effect of genotype	One-WAY ANOVA	2,51	0.2186	0.8044	-
3	D	Balance beam footslips, 12 mm (5 months) - Effect of genotype	One-WAY ANOVA	2,45	1.4268	0.424	-
3	D	Balance beam footslips, 12 mm (6 months) - Effect of genotype	One-WAY ANOVA	2,53	0.7188	0.492	-
3	D	Balance beam footslips, 12 mm (7 months) - Effect of genotype	One-WAY ANOVA	2,37	4.1923	0.0229	-
3	D	Balance beam footslips, 12 mm (7 months) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	0.0386	[0.05,2.12]
3	D	Balance beam footslips, 12 mm (7 months) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.0796	[-0.10,2.10]
3	D	Balance beam footslips, 12 mm (7 months) - WT X SCA6 84Q/-	Tukey-HSD	-	-	0.984	[-1.11,1.27]
4	B	Swimming latency (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	1.1257	0.3484	-
4	B	Swimming latency (3 to 7 months) - Effect of genotype	ANOVA - Fit model	2, 105	0.0301	0.9704	-
4	B	Swimming latency (3 to 7 months) - Effect of age X genotype	ANOVA - Fit model	8, 105	1.8585	0.0744	-
4	C	Swimming kicks (3 to 7 months) - Effect of age	ANOVA - Fit model	4, 105	1.8924	0.1421	-
4	C	Swimming kicks (3 to 7 months) - Effect of genotype	ANOVA - Fit model	2, 105	1.4178	0.2468	-
4	C	Swimming kicks (3 to 7 months) - Effect of age X genotype	ANOVA - Fit model	8, 105	1.6343	0.1238	-
4	C	Swimming kicks (3 to 7 months) - Effect of age X genotype X days	ANOVA - Fit model	16, 210	1.812	0.0312	-
4	D	Swimming kicks 7 months (Day 3 of testing) - SCA6 84Q/84Q X WT	Tukey-HSD	-	-	0.0375	[0.07, 6.71]
4	D	Swimming kicks 7 months (Day 3 of testing) - SCA6 84Q/84Q X SCA6 84Q/-	Tukey-HSD	-	-	0.0439	[0.09, 6.78]

4	D	Swimming kicks 7 months (Day 3 of testing) - SCA6 84Q/- X WT	Tukey-HSD	-	-	>1	[-3.68, 3.94]
5	C	Stride left hindlimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	0.1432	0.8741	-
5	C	Stride left hindlimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.1633	0.8504	-
5	C	Stride left hindlimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.0946	0.91	-
5	C	Stride left hindlimb (7 months) - Effect of genotype	One-WAY ANOVA	2,17	0.3729	0.6942	-
5	D	Stride right hindlimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	0.4552	0.6381	-
5	D	Stride right hindlimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.1701	0.8447	-
5	D	Stride right hindlimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.1729	0.8422	-
5	D	Stride right hindlimb (7 months) - Effect of genotype	One-WAY ANOVA	2,17	0.0981	0.9071	-
5	E	Stance hindlimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	1.2341	0.2981	-
5	E	Stance hindlimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.4725	0.6299	-
5	E	Stance hindlimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.0238	0.9765	-
5	E		One-WAY ANOVA	2,17	0.5018	0.6141	-
5	F	Stride left forelimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	0.2511	0.7804	
5	F	Stride left forelimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.3767	0.6907	-
5	F	Stride left forelimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.5051	0.6094	-
5	F	Stride left forelimb (7 months) - Effect of genotype	One-WAY ANOVA	2,17	0.4583	0.6399	-
5	G	Stride right forelimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	0.0758	0.9124	-
5	G	Stride right forelimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.2384	0.79	-
5	G	Stride right forelimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	0.2074	0.7653	-
5	G	Stride right forelimb (7 months) - Effect of genotype	One-WAY ANOVA	2,17	0.2588	0.775	-
5	H	Stance forelimb (4 to 7 months) - Effect of genotype	ANOVA- Fit model	2,63	0.5312	0.6042	-
5	H	Stance forelimb (4 months) - Effect of genotype	One-WAY ANOVA	2,21	0.3596	0.7022	-
5	H	Stance forelimb (6 months) - Effect of genotype	One-WAY ANOVA	2,25	1.2299	0.3094	-
5	H	Stance forelimb (7 months) - Effect of genotype	One-WAY ANOVA	2,17	0.5272	0.5996	-
6	A	Stride left hindlimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.08	0.7654	-
6	A	Stride left hindlimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0.4616	0.5079	-

6	A	Stride left hindlimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.1893	0.6707	-
6	B	Stride right hindlimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.002	0.9704	-
6	B	Stride right hindlimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0.2275	0.6407	-
6	B	Stride right hindlimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.3613	0.5581	-
6	C	Stance hindlimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.0001	0.9871	-
6	C	Stance hindlimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0.5178	0.4836	-
6	C	Stance hindlimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.5202	0.4835	-
6	D	Stride left forelimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.0117	0.9045	-
6	D	Stride left forelimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0	1	-
6	D	Stride left forelimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.0453	0.8348	-
6	E	Stride right forelimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.1049	0.7559	-
6	E	Stride right forelimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0.0692	0.7963	-
6	E	Stride right forelimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.0308	0.8635	-
6	F	Stance forelimb (1-2 year) - Effect of genotype	ANOVA- Fit model	1,27	0.1742	0.6841	-
6	F	Stance forelimb (1 year) - Effect of genotype	One-WAY ANOVA	1,14	0.5657	0.4644	-
6	F	Stance forelimb (2 year) - Effect of genotype	One-WAY ANOVA	1,13	0.0059	0.94	-
6	E	Rotarod (1 year) - Effect of genotype	One-WAY ANOVA	1,30	56.012	<0.0001	-
6	E	Rotarod (1 year) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	<0.0001	[44.07, 77.15]
6	E	Rotarod (2 year) - Effect of genotype	One-WAY ANOVA	1,28	33.6153	<0.0001	-
6	E	Rotarod (2 year) - WT X SCA6 84Q/84Q	Tukey-HSD	-	-	<0.0001	[19.35, 40.48]
7	B	Purkinje cell count/100 um (7 months) - Effect of genotype	One-WAY ANOVA	1, 109	0.0023	0.9616	-
7	B	Purkinje cell count/100 um (2 year) - Effect of genotype	One-WAY ANOVA	1, 97	18.7953	<0.0001	-
7	B	Purkinje cell count/100 um (2 year) - WT X SCA6 84Q/84Q	One-WAY ANOVA	-	-	<0.0001	[0.58, 1.57]
7	D	Molecular layer length (7 months) - Effect of genotype	One-WAY ANOVA	1, 203	0.7918	0.3746	-
7	D	Molecular layer length (2 year) - Effect of genotype	One-WAY ANOVA	1, 189	33.1151	<0.0001	-
7	D	Molecular layer length (2 year) - WT X SCA6 84Q/84Q	One-WAY ANOVA	-	-	<0.0001	[27.35, 55.87]

635

636

637 **Multimedia**

638 *Movie 1* Rotarod assay

639 $SCA6^{84Q/84Q}$ mouse (right chamber) spends less time on an accelerating rotating rod compared to the
640 litter-matched WT control mouse (left chamber) at 7 months.

641

642 *Movie 2* Sample Rotarod assay (entire trial at high speed).

643 $SCA6^{84Q/84Q}$ mouse (right chamber) spends less time on an accelerating rotating rod compared to the
644 litter-matched WT control mouse (left chamber) at 7 months. Mice are the same as in Movie 1, but the
645 entire trial is shown, at 4X speed.

646

647 *Movie 3* Elevated beam assay

648 A 7-month-old WT mouse crosses an elevated beam.

649

650 *Movie 4* Elevated beam assay illustrating footslips (in slow motion)

651 An $SCA6^{84Q/84Q}$ mouse slipping 3 times on the elevated beam assay, shown in slow motion.

652

653 *Movie 5* Swimming assay (top view)

654 A 7-month-old SCA6$^{84Q/84Q}$ mouse swims across the tank.

655

656 *Movie 6* Swimming assay – SCA6$^{84Q/84Q}$ mouse (side view).

657 A 7-month-old SCA6$^{84Q/84Q}$ mouse swims across the tank. Asterisks indicate right hind limb kicks; 19
658 kicks were counted.

659

660 *Movie 7* Swimming assay – WT mouse (side view).

661 A 7-month-old WT mouse swims across the tank. Asterisks indicate right hind limb kicks; 15 kicks
662 were counted.

663