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Generation and characterization of Sca2 (ataxin-2) knockout mice

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Abstract

Ataxin-2, the gene product of the *Spinocerebellar Ataxia Type 2* (*SCA2*) gene, is a protein of unknown function with abundant expression in embryonic and adult tissues. Its interaction with A2BP1/Fox-1, a protein with an RNA recognition motif, suggests involvement of ataxin-2 in mRNA translation or transport. To study the effects of in vivo ataxin-2 function, we generated an ataxin-2 deficient mouse strain. Ataxin-2 deficient mice were viable. Genotypic analysis of litters from mating of heterozygous mice showed segregation distortion with a significant reduction in the birth of $Sca^{-/-}$ females. Detailed macroscopic and microscopic analysis of surviving nullizygous *Sca2* knockout mice showed no major histological abnormalities. On a fat-enriched diet, ataxin-2 deficient animals had increased weight gain. Our results demonstrate that ataxin-2, although widely expressed, is not essential in development or during adult survival in the mouse, but leads to adult-onset obesity.

Keywords: Spinocerebellar ataxia type 2; SCA2; Ataxin-2; A2BP1; Fox-1; Knockout; Obesity; Rotarod; Segregation distortion

Spinocerebellar Ataxia Type 2 is an autosomal dominant neurodegenerative disease that is caused by expansion of a CAG repeat in the coding part of exon 1 [1–3]. Ataxin-2, the gene product of the SCA2 gene, is a protein of 1312 amino acid (aa) residues with an apparent molecular mass of 145 kDa [4,5]. Most normal alleles contain 22 or 23 CAG repeats encoding glutamine, flanked by a region of proline and serine rich domains, whereas disease alleles have more than 31 repeats [6]. Ataxin-2 has a cytoplasmic localization in normal brain and is expressed in Purkinje cells and specific groups of brain stem and cortical neurons [4]. Mutant ataxin-2 aggregates in the cytoplasm of Purkinje cells of SCA2 patients [4]. Expression of mutant ataxin-2[Q58] in mice results in morphologic alterations and functional deficits [5]. Expression of mutant ataxin-2 in vitro causes abnormalities of the Golgi and increases cell death [7,8]. These observations are consistent with a gain-of-function model or gain-of-toxic function model for mutant ataxin-2, but cannot exclude a dominant-negative action of mutant ataxin-2.

The *SCA2* gene is highly conserved in evolution. The mouse homolog of ataxin-2 is >90% identical to the human protein at the amino acid level [9]. However, it only contains one glutamine at the site of the human polyQ tract, suggesting that the normal function of ataxin-2 does not depend on the polyQ tract. Analysis of the cDNA sequence indicates that ataxin-2 is highly conserved in vertebrates [10] and orthologs exist in *Drosophila melanogaster*,

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Caenorhabditis elegans, *Xenopus laevis*, and *Arabidopsis thaliana*. Conservation of amino acid residues is particularly high in the LSm (like Sm) domain, which contains Sm1 and Sm2 motifs implicated in RNA binding, and in the LsmAD (Lsm-associated) domain, which contains a clathrin-mediated trans-Golgi signal [7,10,11]. Ataxin-2 also has a highly conserved domain in its C-terminal third, which can bind poly(A)-binding protein (PABP) [12]. This domain has been designated PAM-2 (PABP-interacting motif 2) domain and is contained in a number of proteins including ataxin-2 related protein (A2RP) [10,13].

The search for interacting partners of ataxin-2 using yeast two-hybrid screening led to the discovery of ataxin-2-binding-protein 1 (A2BP1) [14,15], which binds to the C-terminus of ataxin-2. A2BP1 contains an RNA recognition motif (RRM), which led Shibata et al. [14] to suggest a function for A2BP1 and ataxin-2 in the regulation of translation [14]. The yeast homolog of ataxin-2, designated Pbp1 (Pab1-binding protein 1, also known as Mrs16), has also been implicated in RNA metabolism and contains Lsm and LsmAD domains like ataxin-2. Human ataxin-2 can functionally substitute for Pbp1 in yeast [16].

Ataxin-2 has widespread expression in adult and embryonic tissues [1,9]. Northern blot analysis detected a 4.4 kb *Sca2* transcript in various tissues of the adult mouse, predominantly in the brain, but also in heart, muscle, intestine, spleen, liver, kidney, and lung. In contrast, little or no SCA2 mRNA was found in the human kidney and lung [1]. Total RNA extracts of whole mouse embryos contain significant amounts of *Sca2* mRNA with increasing levels from gestational days 8 to 16 [9]. By immunocytochemistry, ataxin-2 is strongly expressed in neuronal cells of the adult mouse brain such as large pyramidal neurons and subpopulations of hippocampus, thalamus, and hypothalamus [9]. Very strong staining was seen in the cerebellar Purkinje cells, the primary site of SCA2 neurodegeneration. Non-neuronal tissues also showed high-level expression, in particular heart and skeletal muscle.

Using RNA interference, we previously demonstrated that the *C. elegans atx-2* gene had an essential role in early embryonic development [17]. The worm gene is expressed in the nervous system, the intestinal lining, the body wall muscle, and the germ line. The *Drosophila melanogaster SCA2* gene has been reported to be a dosage-sensitive regulator of cytoskeletal actin filament formation, although ataxin-2 did not directly interact with actin filaments [18]. Both reduction and overexpression of fly ATX-2 caused severe phenotypes such as female sterility, lethality, or degeneration of tissues due to defects in actin filament formation.

We are now reporting the generation of an ataxin-2 deficient mouse strain using homologous recombination. Despite widespread expression of ataxin-2 throughout development, homozygous $Sca^{-/-}$ pups were viable and displayed no obvious defects at birth. In late adult life, obesity and a subtle rotarod deficit were observed.

Materials and methods

Targeting construct, gene targeting, and generation of mutant mice. The mouse Sca2 sequence was isolated from a 129SV genomic library (Stratagene, La Jolla, CA), containing a 12.2 kb fragment that included exon 1 of the murine *Sca2* gene. Presence of exon 1 in this fragment was confirmed by restriction enzyme mapping and Southern blot analysis. A targeting construct was then generated in pBlueScript, in which a PGK-neomycin resistance cassette was inserted into a *Not*I site in exon 1, as illustrated in Fig. 1A. This construct was linearized and electroporated into 129/SvJ embryonic stem (ES) cells (Genome Systems, St. Louis, MO, now Incyte Genomics). Cells were grown under double selection in G418 and gancyclovir (Gibco-BRL, Rockville, MD) as described [19].

Animals and genotyping. Animals were housed under standard conditions in compliance with all relevant federal guidelines and institutional policies. Phenotypic analysis was performed on mice in a mixed C57BI/6x129SvJ background. Genotyping was performed by PCR with two sets of primers: The neo cassette was amplified with primers F3 (5′CCC GCA GGC CAT CCT CTA-3′) and R3 (5′-GGA ACA CGG CGG CAT CAG-3′). The forward primer is located 5′ of exon 1 and R3 is within the *Neo* cassette resulting in an amplicon of 830 bp. To amplify wildtype exon 1 two primers located within exon 1 were used with an amplicon of 106 bp. Primers were Ex-1 (5′-CGC CTC AGA CTG TTT TGG TAG-3′) and Ex-2 (5′-GAG CAG GAC AAC GAC GAA-3′). PCR conditions were as follows: 95 °C—15 min, then 5 cycles 95 °C 1 min, 65 °C 30 s, 72 °C 1 min, followed by 30 cycles of 95 °C 1 min, 60 °C 30 s, 72 °C 1 min, and final extension at 72 °C for 5 min.

Mice were housed 4–6 animals per cage under a 12:12 h light cycle with food and water provided ad libitum. The diet consisted of rodent chow (Purina mouse diet 5015: calories from protein 18.2%, fat 25.8%, and carbohydrates 55.9%). For rotarod experiments, mice were fed a low fat diet (Purina rodent diet: calories from protein 28.0%, from fat

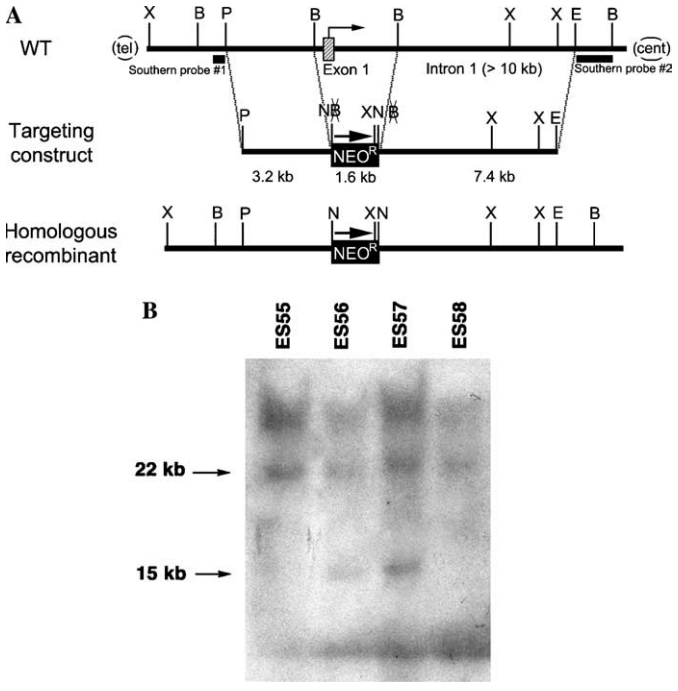

Fig. 1. (A) Graphic representation of *Sca2* gene and targeting strategy. Replacement of exon 1 and flanking sequences of the *Sca2* gene with the *Neo* cassette by homologous recombination. Restriction sites: B, *Bam*HI; E, *Eco*RI; N, *Not*I; P, *Pst*I; X, *Xho*I. (B) Identification of the targeted allele by Southern hybridization. The targeted allele is recognized by a 7 kb decrease in an *Xho*I restriction fragment when probed with the indicated 5′ external fragment.

12.1%, and from carbohydrates 59.8%). For determination of body weight, animals were weighed on a laboratory scale at weaning, 3, 6, 9, and 12 months. Animal protocols were approved by the Animal Care and Use Committees of Cedars-Sinai Medical Center and UCLA School of Medicine.

RT-PCR analysis. Total RNA was extracted from different regions of mouse brain and skeletal muscle using the RNeasy kit (Qiagen, Valencia, CA). RT-PCR was performed on cerebral cortex and skeletal muscle tissue with the Reverse Transcription System (Promega, Madison, Wl) according to the supplier's protocol. The primer pair used for detection of the Sca2 gene transcript was previously reported [5]. As a control, RNAs were amplified with a primer pair for mouse DSCAM [20] (M10For1: CTGGTATC CACCAAGGAGGT; M10Rev1:CCGCGAATGATCCCG TTTTG).

Protein extraction and Western blotting. Fresh brain tissue was obtained from two month old $Sca2^{+/-}$, $Sca2^{-/-}$ and wildtype control mice. Brain tissue was chopped into small pieces, and then resuspended in 10 ml of cold triple detergent buffer (100 mM Tris–HCl, pH 7.4, 1 mM EGTA, 1% NP40, 0.5% SDS, 0.5% deoxycholic acid, 1 mM Pefabloc SC, 1 μg/ml and pepstatin A, 2 μg/ml aprotinin, 50 μg/ml leupeptin) (all from Roche, Indianapolis, IN). The tissue was then homogenized with a VIRTIS polytron at maximal speed for 30 s. The lysates were first centrifuged at 1000*g* (3100 rpm in a JA17 rotor) for 5 min. The supernatants were centrifuged for 1 h at 4 °C at 105,000*g* (54,000 rpm in a TLN100 rotor). Each protein lysate was then aliquoted and stored at −80 °C. Protein concentrations were determined using the Bradford Protein Assay Kit (Bio-Rad, Hercules, CA). Prior to loading onto polyacrylamide gels, proteins were concentrated using a Microcon 10 column (Amicon, Bedford, MA). One hundred micrograms of total protein was loaded per lane in a precast 4–20 % gradient SDS–polyacrylamide mini-gel (BioRad) and electrophoresed at 100 V for 1–2 h. Proteins were transferred to nitrocellulose filter (Amersham, Piscataway, NJ). The filter was rinsed briefly with TBS (150 mM NaCl, 50 mM Tris–HCl, pH 8.0), and blocked for 1 h with 5 % nonfat dried milk (Bio-Rad). The filter was then incubated with 1 μg/ml rabbit SCA2B antibody [5], or mouse anti-β-actin antibody (Sigma). The primary antibody was detected with the ECL Western blotting detection system (Amersham) using anti-rabbit IgG or anti-mouse IgG antibody conjugated with horseradish peroxidase.

Antibodies and immunocytochemistry. Adult mouse tissues were obtained from wildtype and homozygous Sca2 knockout animals and fixed by perfusion with 4% paraformaldehyde. Sections were cut at 5 μm and mounted onto Superplus microscopic slides (Fisher Scientific, Pittsburgh, PA). The sections were rehydrated by rinsing twice at 5-min intervals in xylene, 100% ethanol, 95% ethanol, and 70% ethanol. Stains for hematoxylin–eosin (H&E) were performed on all slides. In addition, H&E/Luxol fast blue stains for myelin were performed on sections from the brain.

For immunocytochemistry, sections were treated with Auto/Zyme buffer (a protease mixture from Fisher/Biomedia), blocked with avidin/biotin and 3% normal goat serum. Sections were then incubated with 10–20 μg/ml of affinity purified ataxin-2 antibody or calbindin 28K antibody (Chemicon) overnight at 4 °C [4,5]. Primary antibody was detected using the Vector rabbit ABC elite Peroxidase kit (Vector, Burlingame, CA) with DAB enhancer, and visualized with diaminobenzidine (DAB, Biomeda, Hayward, CA). Sections were counterstained with aqueous hematoxylin (Zymed, S. San Francisco, CA). Controls consisted of antibody preabsorbed with 100 μM of the respective peptide and preimmune sera at comparable concentrations (1/500). All slides for direct comparison were processed in a single batch to minimize variability.

Rotarod testing. Rotarod testing was performed in 11 wildtype and 14 homozygous knockout animals at 12 months of age. Mice were handled for 7 consecutive days prior to performing the rotarod experiment. On the day of the experiment, mice were placed onto the rotating rod (TSE Model 3375) that rotates at an accelerating speed from an initial speed of 5 rpm to the final speed of 60 rpm in 3 min and then remains at that speed for an additional 2 min. The time to falling off the rod was recorded by an infrared sensor placed under the rotating rod. Each animal was tested in two consecutive trials, after which it was allowed to rest for more than 60 min. Then, the same procedure was repeated. A total of six trials were performed.

Results

Targeted disruption of the mouse Sca2 geneA portion of the Sca2 gene containing the translation start site was isolated as described above to create a targeting vector (Fig. 1A). This construct contained a Neomycin resistance element, which replaced exon 1 of the mouse Sca2 gene. Targeted ES cell clones were screened by Southern blot analysis (Fig. 1B). Almost 25% of ES cell clones carried the correct mutation. Positive clones were injected into C57Bl/6 blastocysts to generate chimeras and implanted into pseudopregnant females. Germline transmission was achieved by crossing chimeric males with C57BI/6 females.

To confirm changes in *Sca2* expression, RNAs and proteins were extracted from various tissues of wildtype, heterozygous, and $Sca2^{-/-}$ mice. RT-PCR analysis revealed the absence of *Sca2* transcripts in $Sca^{-/-}$ mice compared with amplification of the *DSCAM* gene used as a control (Fig. 2A). By immunoblot (Western) analysis, mice homozygous for the *Sca2* gene disruption expressed no ataxin-2 (Fig. 2B). Protein expression in heterozygous animals was intermediate. Absence of the protein was also confirmed by immunocytochemical analysis of various tissues. Expression in cerebellar Purkinje cells was strong in wildtype animals (Fig. 2C). Cerebellar sections of $Sca^{-/-}$ animals were devoid of staining (Fig. 2D). The combined results of these studies demonstrated that no ataxin-2 was produced in homozygous Sca2 knockout mice. The lack of C-terminal fragments, which potentially could occur from an alternative promoter was confirmed by use of an antibody to a C-terminal ataxin-2 epitope.

Segregation analysis

In order to determine whether Sca2 deficiency resulted in embryonic lethality or in increased fetal loss we examined the genotypes of 70 litters derived from mating heterozygous $Sca2^{+/-}$ mice in a mixed C57Bl/6 × 129/SvJ background. Of 372 offspring, 216 were male and 156 female, which represented a highly significant deviation from the expected sex ratio ($p < 0.002$). Detailed analysis of the genotypes showed that there was a remarkable distortion of the expected genotype ratios (Table 1). Whereas equal number of wildtype and homozygote animals would have been expected, heterozygous and homozygous mice were underrepresented. This was particularly apparent for female animals. While the number of male and female wildtype animals was nearly identical, there was a significant reduction in the number of heterozygote and homozygote female mice.

Phenotype of $Sca2^{-/-}$ mice

Homozygous $Sca2^{-/-}$ mice were viable and fertile. Neither $Sca2^{+/-}$ nor $Sca2^{-/-}$ mice displayed any visible abnormalities at birth. Mice were of normal length and weight at birth. Open cage behavior appeared normal. There was no

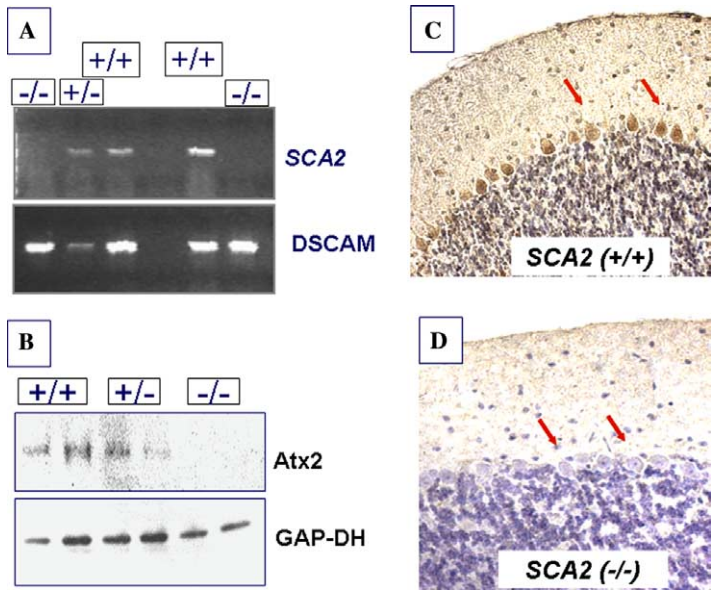

Fig. 2. SCA2 deficiency at the transcript and protein level. (A) RT-PCR analysis of mRNAs purified from wildtype, $Sca^{+/-}$, and $Sca2^{-/-}$ brains. Primers are located in different exons, and therefore do not amplify genomic DNA. As a control, the transcript of the DSCAM gene is well amplified in WT and $SCA^{-/-}$ RNA. (B) Western blot analysis of protein extracts prepared from WT and $Sca2^{-/-}$ brains. Blots are stained with an antibody to an epitope in the C-terminus of ataxin-2 (antibody SCA2B, [4]); staining of the blot with GAP-DH shows near equal loading. (C) Immunocytochemical staining of a section from a wildtype cerebellum with antibody SCA2B shows strong labeling of Purkinje cell bodies. (D) Section of the cerebellum from an $Sca^{-/-}$ mouse. Purkinje cells do not stain (red arrows). Note the normal width of the molecular layer. Sections are counterstained with hematoxylin.

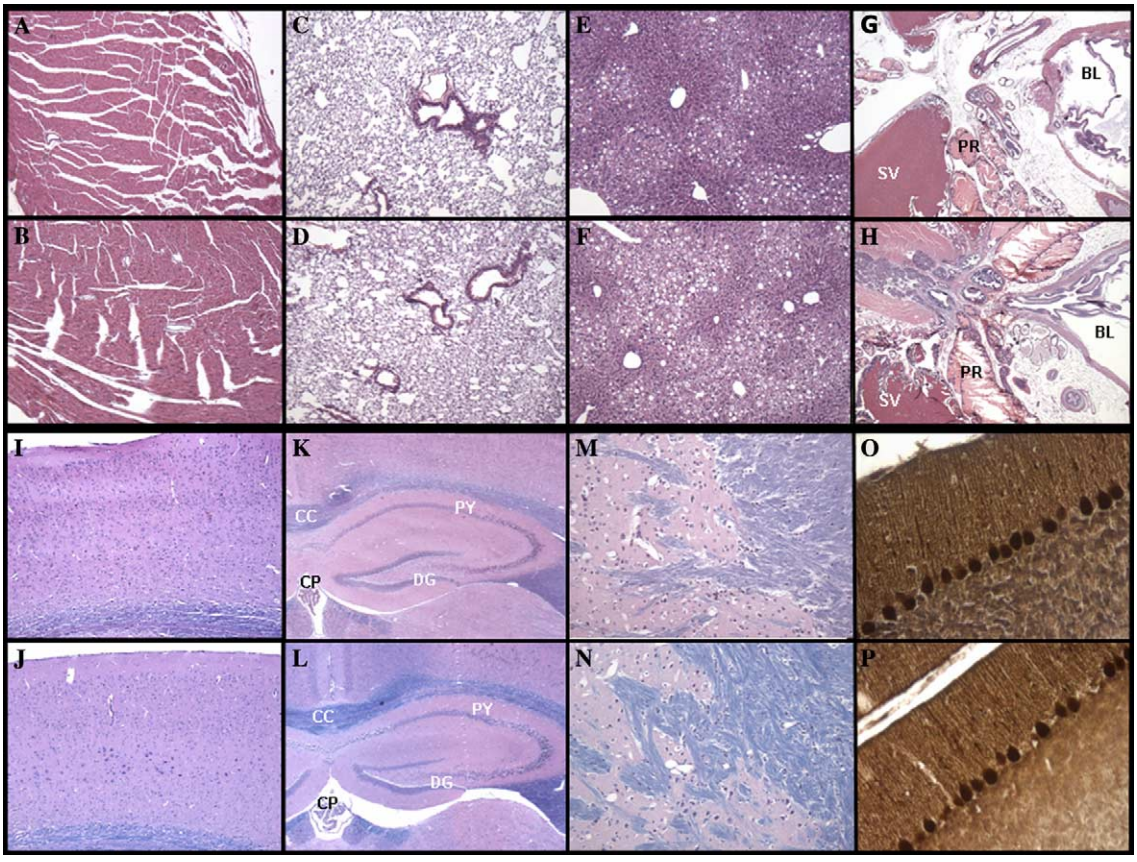

Fig. 3. Histomorphology of organs in wildtype (A, C, E, and G) and $Sca2^{-/-}$ mice (B, D, F, and H). (A,B) Heart, (C,D) lung, (E,F) liver, (G,H) lower genito-urinary tract: seminal vesicle (sv), prostate (pr), and bladder (bl). Sections from the central nervous system are shown in (I–P) (wt: I, K, and M; $Sca2^{-/-}$: J, L, and N). (I,J) Cerebral cortex; (K,L) hippocampus, dentate gyrus (dg); pyramidal cell layer (py); corpus callosum (cc); choroid plexus (cp); (M,N) putamen; (O,P) cerebellum. (I–N) Stained with luxol fast blue/H&E. (O,P) Immunohistochemistry for Calbindin D28K, counterstained with hematoxylin.

Table 1
Gender and genotypes of litters from heterozygote matings

Genotype	+/+	+/−	−/−	Total	P
Total	144	165	63	372	<0.00001*
Male	76	101	39	216	<0.002*
Female	68	64	24	156	<0.00001*

* χ^2 analysis comparing observed vs. expected genotypes.

evidence of ataxia, abnormal postures or seizure-like events from birth to 1 year of age. No propensity for the development of malignant tumors was detected. At one year of age, most knockout animals had a significantly increased abdominal fat pad. Some animals showed varying degrees of bilateral enlargement of the seminal vesicles. The size ranged from barely detectable to the very occasional animal that had to be sacrificed due to an enlarged abdomen.

Histopathological examination of the central nervous system and other organs demonstrated no morphologic abnormalities in Sca2$^{-/-}$ animals except for the liver (Fig. 3). At one year of age, the liver of wildtype and knockout animals showed fatty changes, i.e., micro- and macrovesicular steatosis (Figs. 3E and F). Changes were significantly more severe in the livers of knockout animals and included marked cholestasis. At that time, the animals had a significant increase in body weight. The histology of the seminal vesicle remained benign. No obstruction of seminal vesicle outflow or abnormalities of the prostate were identified (Figs. 3G and H). Kidney and skeletal muscle were microscopically unremarkable (not shown).

As ataxin-2 is widely expressed in neurons and glial cells, we examined various brain regions in wildtype and Sca2$^{-/-}$ mice (Figs. 3I–P). The cerebral cortex of the Sca2$^{-/-}$ mouse showed a regular six-layered anatomy (J). Hippocampal sections revealed normal cell populations of the dentate gyrus and pyramidal layer. The size and myelination of white matter structures such as corpus callosum and internal capsule were normal. The deep grey structures such as basal ganglia (N) were unremarkable. The cerebellar cortex showed no significant differences in cellularity or thickness of the molecular, Purkinje cell or granule cell layers (Figs. 2C and D; 3O and P). As ataxin-2 is strongly expressed in Purkinje cells [4] and expansion of the polyQ domain in ataxin-2 causes cerebellar degeneration and loss of dendritic arborization in humans and in transgenic mice [5], we examined the Purkinje cell arbor using immunohistochemistry for calbindin D28k. No differences in calbindin labeling of Purkinje cell bodies or dendrites were detected (Figs. 3O and P).

Obesity

On a moderately fat-enriched diet (calories from fat: 25.8%), knockout mice began to develop increased body weight that first became apparent at 3 months of age. The increase in bodyweight for male wildtype and knockout mice is shown in Fig. 4. While there were no significant weight differences at weaning, the differences in body weight between knockout and wildtype animals increased

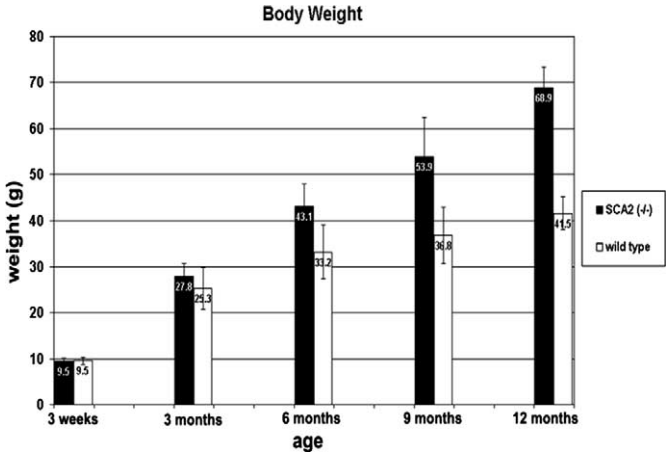

Fig. 4. Markedly obese phenotype in male Sca2$^{-/-}$ animals (black bars, $n = 5$) compared to male wildtype animals (white bars, $n = 5$). Body weight was determined at weaning (3 weeks), and at 3, 6, 9, and 12 months. Mean and standard deviation are shown. The differences in body weight between KO and WT animals increase with age. Differences in means are significant at 6, 9, and 12 months (two-tailed t test; $p < 0.008$).

with age. At 6 months weight was increased by 29.8% and at 1 year of age by 66%. No abnormal weight gain was observed on a low-fat diet (see below).

Motor function

Although no abnormalities of motor function in open cage behavior were observed, we wanted to test motor ability and motor learning under more stringent conditions using the rotating rod apparatus. As body weight may influence performance on the rotating rod, we maintained mice for these experiments on a low fat diet (calories from fat 12.1%). At 12 months, weights for 11 wildtype mice were 41.0 ± 4.8 g (means $\pm$ SD), and for 14 Sca2$^{-/-}$ mice it was 43.6 ± 2.2 g, which was not significantly different (Fig. 5). There was a subtle but statistically highly significant difference in motor performance on the accelerating rotarod. Although knockout mice showed improved performance in trials 2–6, their performance was worse in each trial compared to wildtype mice (two-way ANOVA $p < 0.001$).

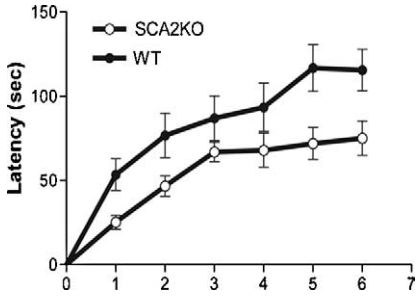

Fig. 5. Rotarod performance of wildtype ($n = 11$) and ataxin-2 ($n = 14$) deficient animals. Mice were tested in six individual trials during an 8 h period. Latency to fall off the accelerating rotating rod is shown for each trial (means $\pm$ SD). Differences were significant (two-way ANOVA, $p < 0.001$).

Discussion

This study is the first description of a targeted disruption of the mouse *Sca2* gene. No loss-of-function mutations or deletions in the *Sca2* gene in humans have been described that could have elucidated the in vivo consequences of ataxin-2 deficiency. On the other hand, mutations of *A2BP1* [14], one of the proteins interacting with ataxin-2, have been described. Heterozygous loss of function mutations in the *A2BP1* gene caused by chromosomal translocations are associated with varying degrees of mental retardation, autistic features, and epileptic seizures [21].

In light of previous research of ataxin-2 deficiency in invertebrates [18,22,23] and in human tumors [24], the results of this study were surprising in several respects. Ataxin-2 has widespread expression in embryonic and adult tissues including very strong expression in brain, skeletal and cardiac muscles [1,9]. *Sca2* mRNA can be detected from day 8 of gestation, the earliest time point examined [9]. Starting at day E11, there is a rapid increase of ataxin-2 in spinal chord and brain. The expression pattern of mouse A2bp1 and ataxin-2 are almost identical [15].

In this context it was not surprising that ataxin-2 deficiency or knockdown in the worm or fly were associated with embryonic lethality [17,18]. Ciosk et al. [22] followed up on these initial observations and determined that in the absence of ataxin-2, the germline was abnormally masculinized. In the worm, ataxin-2 physically interacts with PAB-1, one of the two *C. elegans* poly(A) binding proteins. The developmental defects appear to result from inappropriate translational regulation which is normally mediated by the conserved KH-domain protein GLD-1 and a second KH-domain protein MEX-3.

In contrast to deficiency in invertebrates, *Sca2* knockout in mice produced viable offspring. The only indication for abnormalities in germline development or embryogenesis came from our genotypic analysis of a large number of offspring from mating heterozygous animals. The number of heterozygous and in particular homozygous offspring was reduced compared to expected ratios (Table 1). This was especially pronounced for female knockout animals, where the number of $Sca^{-/-}$ females was only about a third of expected. More studies will be needed to dissect the pathogenesis of the segregation distortion.

Based on studies in human neuroblastomas [24], which demonstrated a strong anti-apoptotic effect for ataxin-2, one might have expected the development of malignant tumors in ataxin-2 deficient animals. However, no malignant tumors were detected within the observation period of one year. Furthermore, no decreased survival was seen within this timeframe. Obviously, we cannot exclude the development of tumors later in life or the presence of microscopic lesions in a small subset of animals or in organs that were not examined (Fig. 3).

The only exception to these observations was the development of enlarged seminal vesicles in older animals. Some degree of enlargement was seen in all male $Sca2^{-/-}$ animals. The seminal vesicles themselves had normal histology (Figs. 3G and H). No obvious morphologic cause for a distal obstruction could be identified making, a functional abnormality more likely.

The surprisingly non-essential role of ataxin-2 in rodents despite its widespread expression in development could relate to the presence of orthologs and redundant mechanisms that may rescue the function. For instance, ataxin-2-related protein (A2RP) on chromosome 16 is similar in amino acid sequence to ataxin-2, especially in regard to presence of domains [10,25]. Although there is considerable divergence from a putative ancestral gene, A2RP may still rescue some ataxin-2 function in knockout animals. The motifs shared between the two proteins include the Lsm, LsmAd, and PAM2 domains, and A2RP is expressed in tissues that express ataxin-2 [10]. These observations are consistent with at least partially overlapping functions of both proteins.

The initial impetus for undertaking an in vivo knockout approach was to examine whether loss of ataxin-2 function would result in neurodegeneration. Expansion of the polyglutamine domain in ataxin-2 causes human spinocerebellar ataxia type 2. Expression of a mutant ataxin-2[Q58] in mice results in Purkinje cell dysfunction and morphologic changes [5]. Although these animal studies were consistent with a gain-of-function or toxic gain-of-function of mutant ataxin-2, they could not exclude a dominant-negative action of mutant ataxin-2. A dominant-negative action has recently been suggested for aspects of SCA7 pathogenesis, another polyQ disorder [26].

In contrast to transgenic animals expressing mutant ataxin-2 [Q58] [5], mice deficient in ataxin-2 did not show Purkinje cell loss or marked changes in the Purkinje cell dendritic tree (Figs. 2 and 3). Both of these features are typical for human patients with SCA2 or mice expressing mutant ataxin-2[Q58] [4,5]. Similarly, mice lacking ataxin-1 are viable, fertile, and do not show any evidence of ataxia or neurodegeneration [27]. This further supports the notion that polyglutamine expansion in ataxin-2 does not cause a loss of function or has dominant negative properties.

The subtle deficits in rotarod performance are interesting and deserve more detailed future studies (Fig. 5). The improved performance with each trial indicates that $SCA2^{-/-}$ mice were able to learn the task, but were not able to perform it as well as wildtype animals. Abnormalities in the rotarod paradigm are not restricted to cerebellar lesions, but are also found in abnormalities in motor learning and neuromuscular deficits [28]. In this context it is interesting to note that ataxin-2 has strong expression in skeletal and cardiac muscle. Although no morphologic changes were detected in cardiac muscle (Fig. 3) or skeletal muscle (data not shown), it is conceivable that a compromise in skeletal muscle or cardiac function contributed to reduced rotarod performance.

An unexpected aspect of the *Sca2* deficiency phenotype was the development of adult-onset obesity (Fig. 4). The gain in total body weight reached statistically significant

levels at 6 months of age, although more subtle increases could be observed as early as 3 months of age. Larger numbers of animals are needed to examine whether weight gains at earlier ages are significant. Of note, the development of obesity was dependent on food composition. When mice were offered an *ad libitum* low-fat diet, body weights were not significantly different between wildtype and knockout animals, whereas on a fat-enriched diet, body weight was significantly increased. In this context it is important to emphasize that rotarod studies were performed in animals that were on a low-fat diet. The obesity associated with Sca2 deficiency requires further analysis in individually housed animals. Initial studies suggest that increased body weight is caused by hyperphagia. Owing to the widespread expression of ataxin-2 within and outside the CNS, it is currently not known whether obesity is the result of a central or peripheral effect.

The SCA2 region was recently identified as one of the regions in the human genome that had undergone strong selection in Europeans [29]. Using the long range haplotype (LRH) test, which compares the length of each haplotype to that of others at the locus, the SCA2 region had a similar outlier status as the LCT gene, which is involved in digestion of dairy products and is known to be subject to strong selection. More detailed examination of this region revealed that selection likely acted upon the CAG_8CAA-$CAG_4CAACAG_8$ allele and possibly on close flanking single nucleotide polymorphisms [30]. As the selected allele differs from the other common allele in Europeans only by the number of CAA interruptions, the selection may relate to mRNA stability, transport or interaction with RNA binding proteins. Based on our results with Sca2 knockout mice we can speculate that the selected allele may influence energy balance and resistance to famine or to fertility, although effects on resistance to pathogens such as RNA viruses should also be considered given the presence of an Lsm domain in ataxin-2.

In summary, despite embryonic lethality in ataxin-2 deficient invertebrates, Sca2 knockout animals were viable. Although a subtle rotarod deficit was identified, there was no evidence for neuronal loss or morphologic alterations in the cerebellum consistent with the notion that SCA2 mutations in humans do not result in loss-of-function. Segregation analysis indicated reduced frequency of homozygotes among offspring especially of female pups. Adult obesity was an unexpected feature and warrants further evaluation.

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