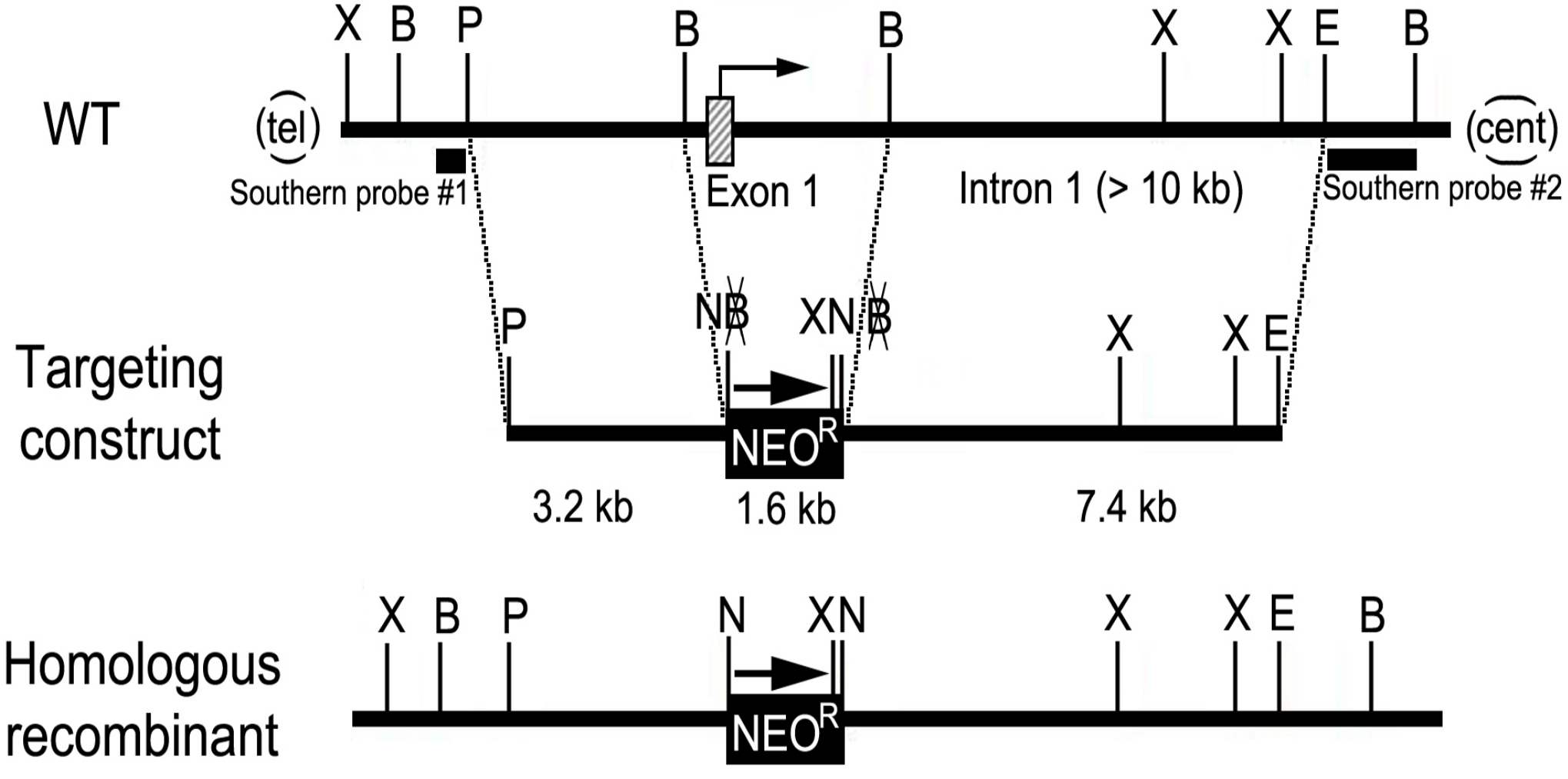
ABSTRACT

Spinocerebellar ataxia type 2 (SCA2) is a human neurodegenerative disorder caused by expansion of a polyglutamine repeat in the ataxin-2 gene. To elucidate the normal function of ataxin-2 and to determine whether loss-of-function contributes to SCA2 neurodegeneration, we generated a mouse model with a targeted deletion in the *SCA2* gene by homologous recombination in a mixed C57B6/129J background. Deficiency of ataxin-2 was confirmed by Northern and Western blot analyses. Despite embryonic lethality of ataxin-2 deficiency in *C. elegans* and the widespread expression of ataxin-2 during rodent development, $SCA2^{-/-}$ mice were viable and had no obvious defects or increased morbidity. Although $SCA2^{-/-}$ mice had normal body weight at birth, we observed subsequent excessive weight gain. At 6 months, the average body weight of $SCA2^{-/-}$ mice was 26% more than that of wildtype, but we observed no differences in $SCA2^{+/-}$ mice. Controlled feeding experiments demonstrated that obesity was the result of increased food intake. When maintained in isolation on a restricted diet, no differences in food intake or weight were observed for the three genotypes. However, when provided with an *ad libitum* diet, heterozygote and homozygote mice showed increased food intake accompanied by increased weight. Average weekly food intake was 4.45 g for wildtypes, 5.60 g for heterozygotes, and 6.0 g for knockout mice ($p<0.01$). Average weekly weight gain was 0.59 g for wildtypes, 0.65 g for heterozygotes, and 0.79 for knockout mice under *ad libitum* conditions ($p<0.01$). These results suggest that obesity observed in $SCA2^{-/-}$ mice is predominantly the result of hyperphagia and not of primary metabolic abnormalities Intermediate obesity observed in heterozygous animals strongly demonstrates that weight control is highly sensitive to ataxin-2 dosage.

Spinocerebellar Ataxia Type 2 (SCA2)

- Autosomal dominant ataxia
- CAG expansion in the coding region of a novel gene on CHR 12q24 is causative.
- Polyglutamine disease
- cerebellar dysfunction (gait ataxia)
- ophthalmoparesis / saccadic eye movements
- pronounced dementia, frontal executive dysfunction
- Loss of neurons in brain stem and cerebellum
- Gene knock-down by RNA interference in C. elegans is an early embryonic lethal.

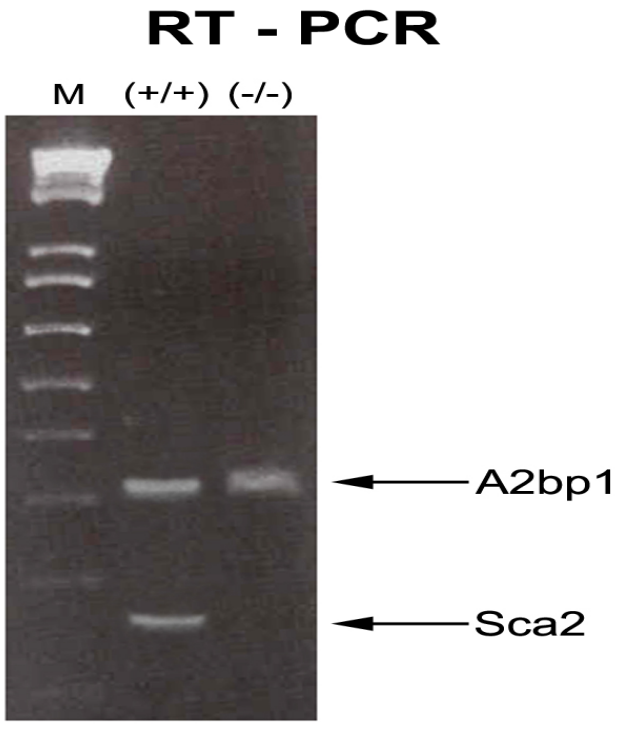
Targeting Strategy



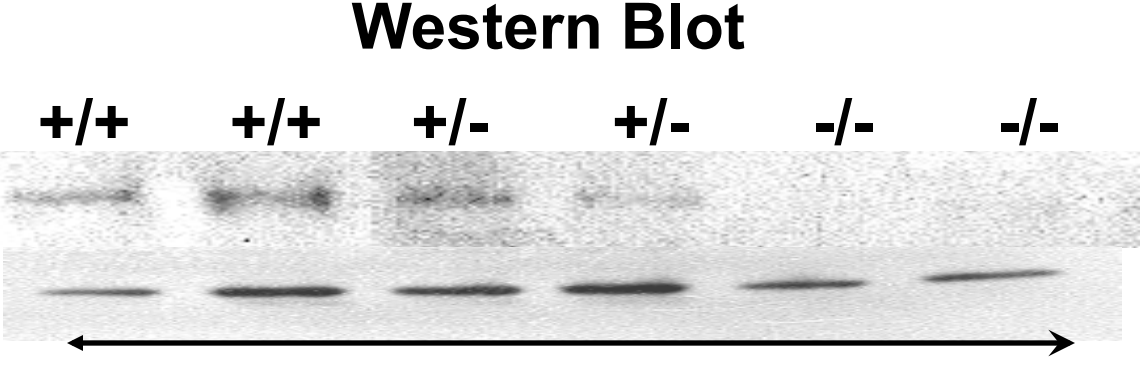
Confirmation of knockout

Absence of SCA2 mRNA :

- RT-PCR
- Microarray



RT-PCR was perfomed simulatneously in same reaction utilizing both Ataxin-2 binding protein and SCA2 primers.



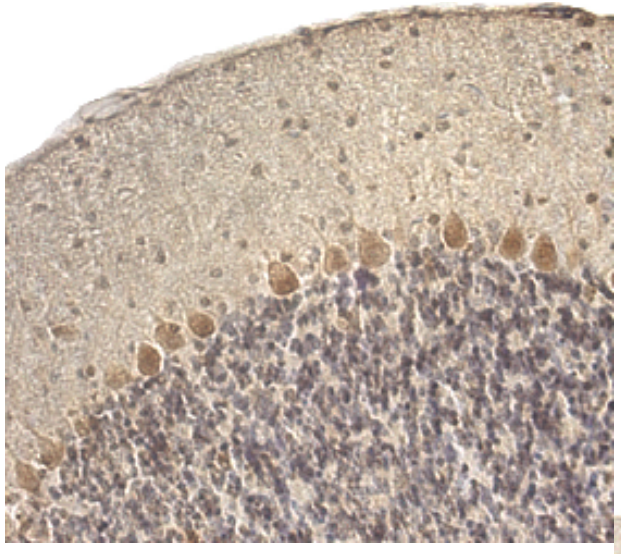
C-terminal ataxin-2 antibody “SCA2-B” was used for both Immunocytochemestry and Western blot.

Confirmation of knockout

Absence of ataxin-2 :

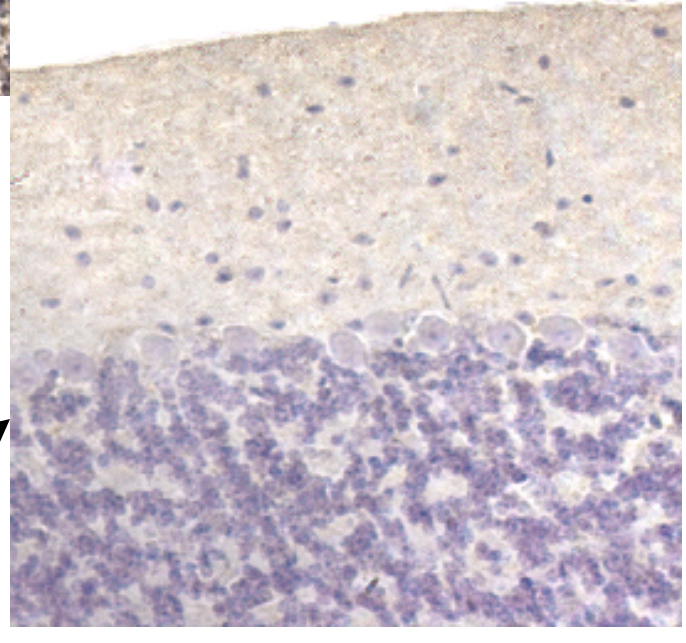
- Immunocytochemistry
- Western Blot

SCA2 (+/+)



Molecular layer

SCA2 (-/-)



Purkinje Cell

C-terminal ataxin-2 antibody "SCA2-B" was used for both Immunocytochemestry and Western blot.

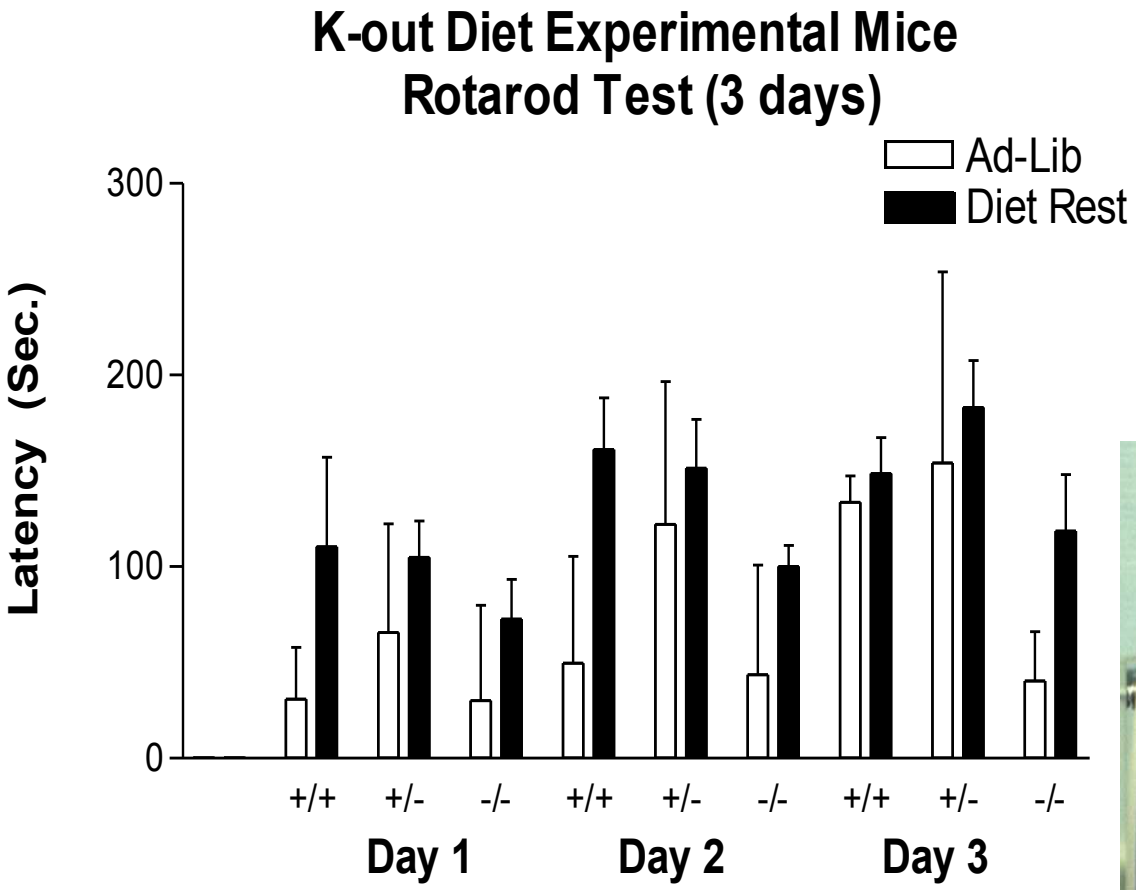
Granule layer

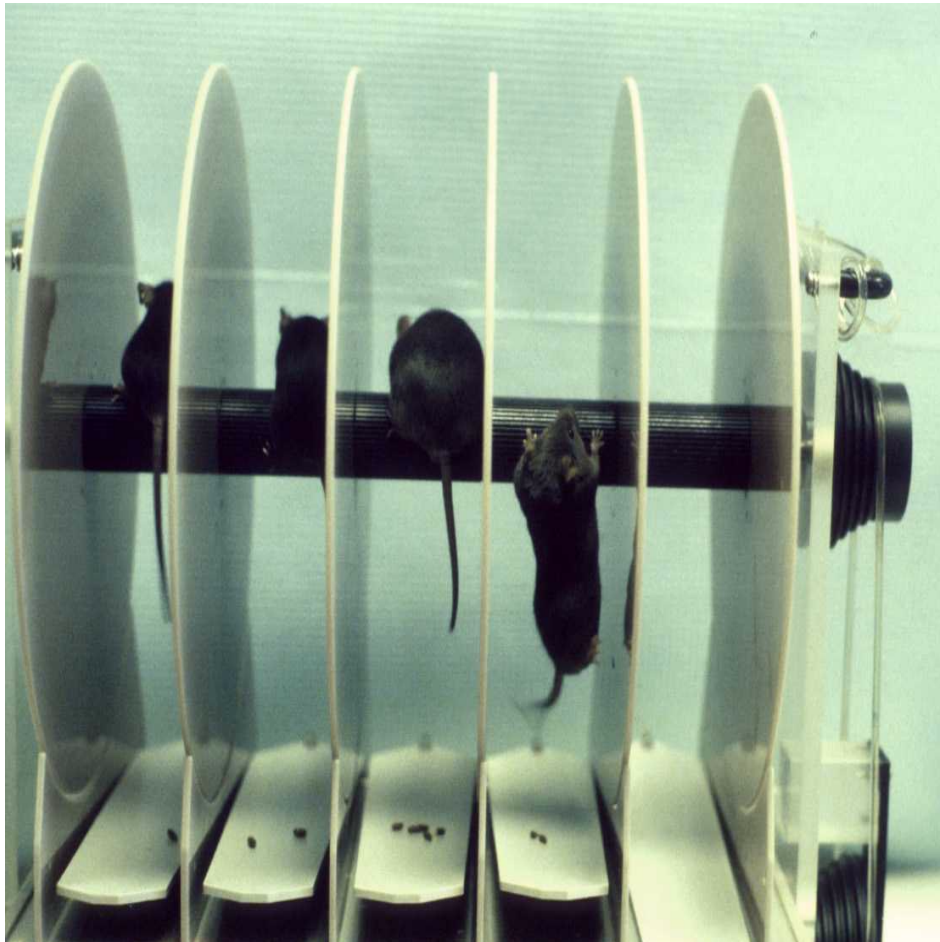
The ataxin-2 knockout mouse

SCA2$^{(-/-)}$ mice

- Phenotype
  - Mice are viable and fertile
  - Normal behavior
- Anatomy
  - No gross anatomic defects
  - structurally normal CNS
  - normal CNS histology

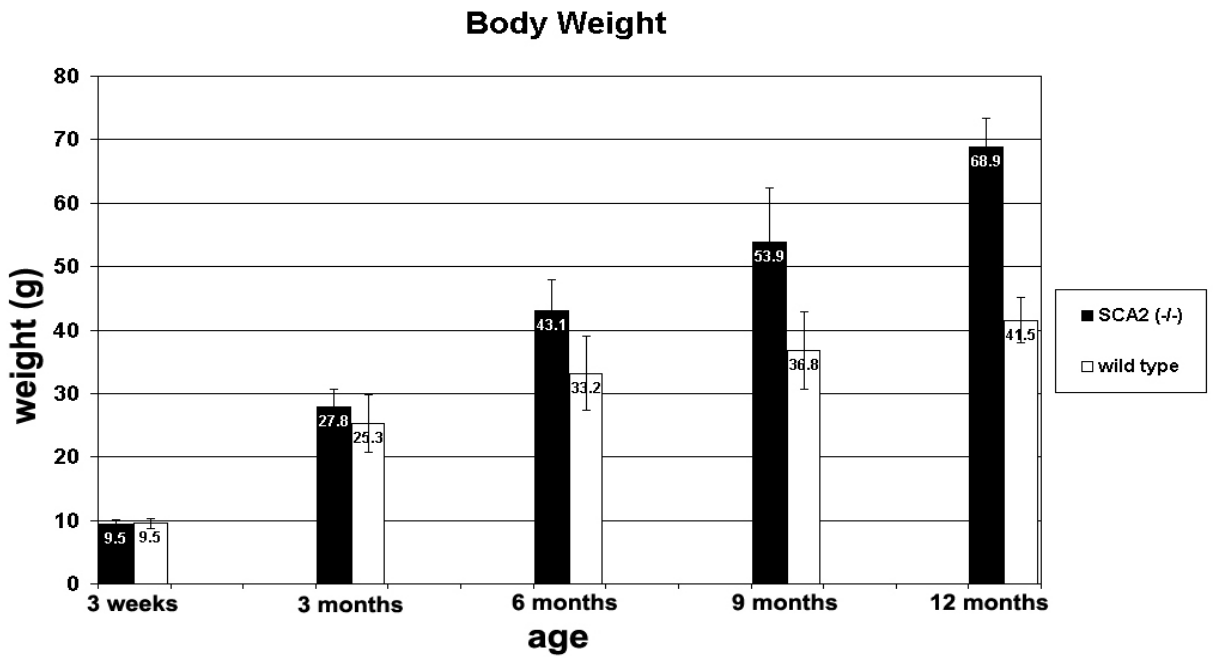
Functional Analysis





Diet accounts for approx. 10.37% of the total variance $P = 0.0003$

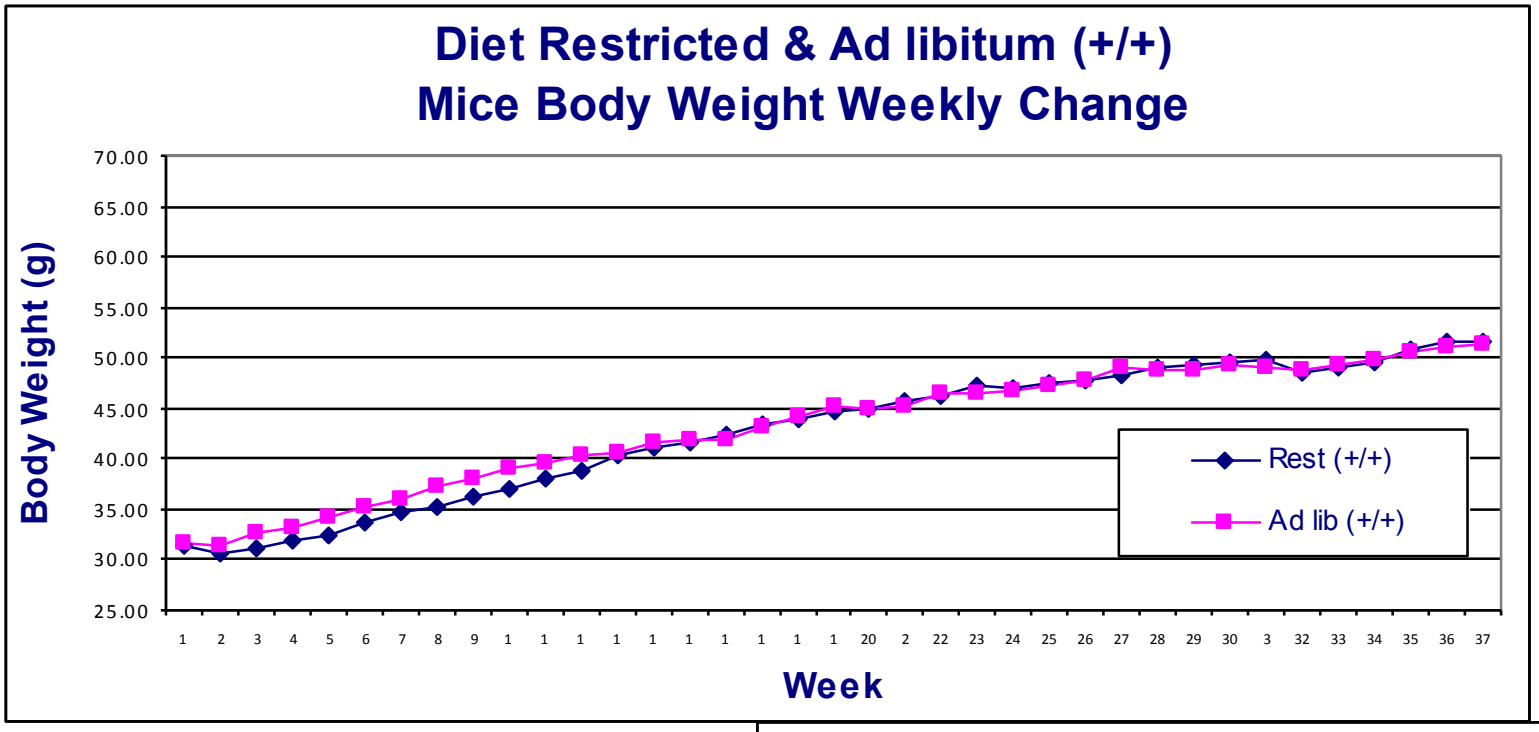
Genotype accounts for approx. 27.55% of the total variance $P < 0.0001$

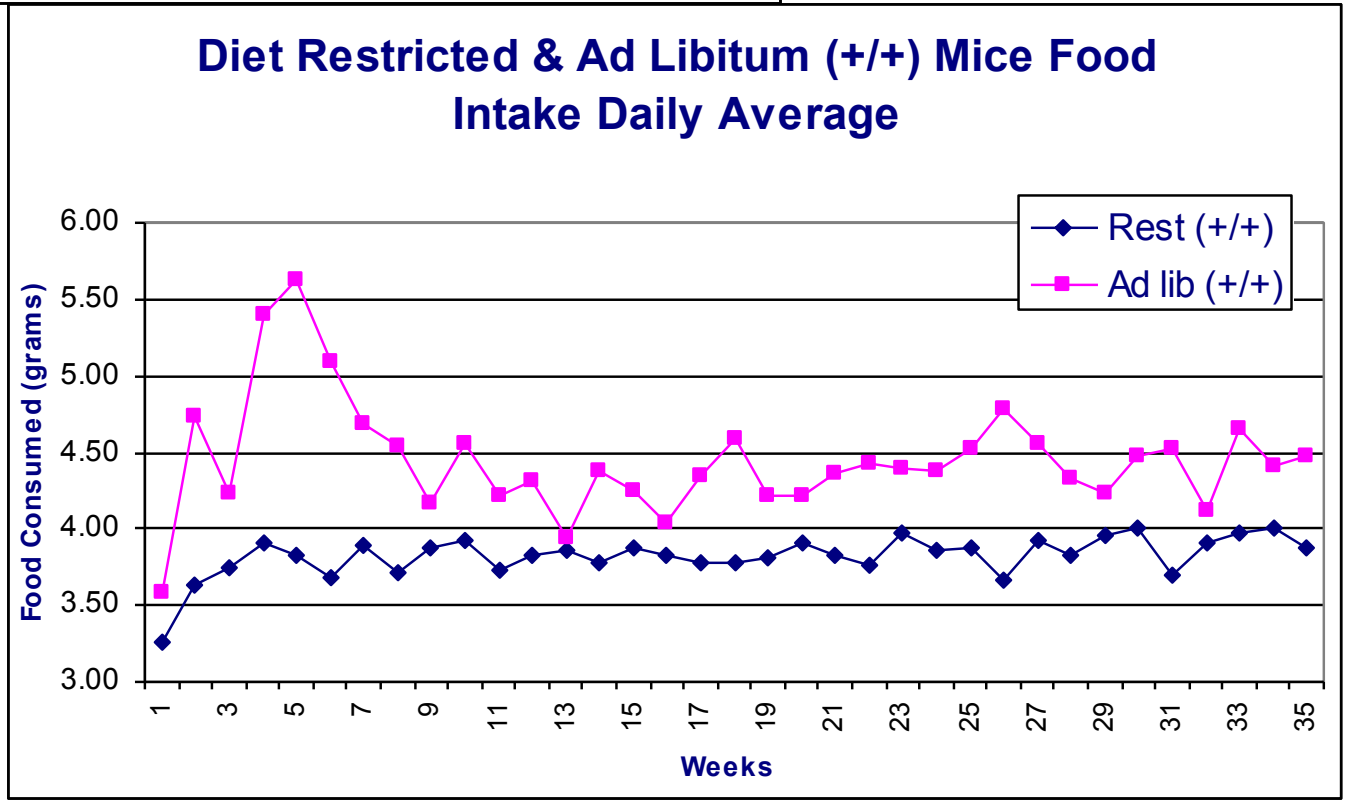


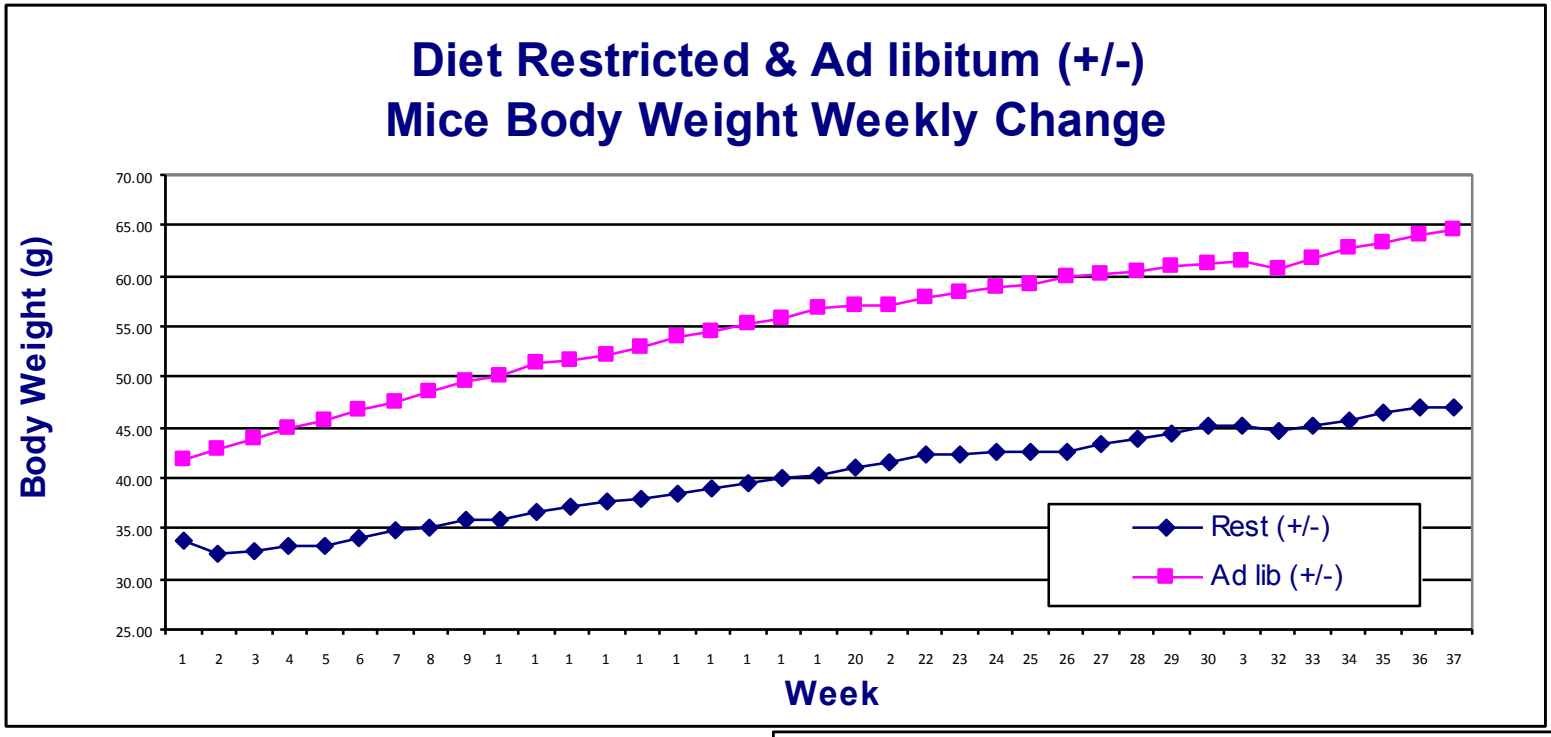
When maintained on a regular NIH rodent diet, homozygous SCA2 knockouts showed marked adult-onset obesity. While the body weight at birth and weaning age is identical to wild type mice, there is a progressive increase at 3 months (10% higher than WT), 6 months (29.8%) and 1 year of age (66%).

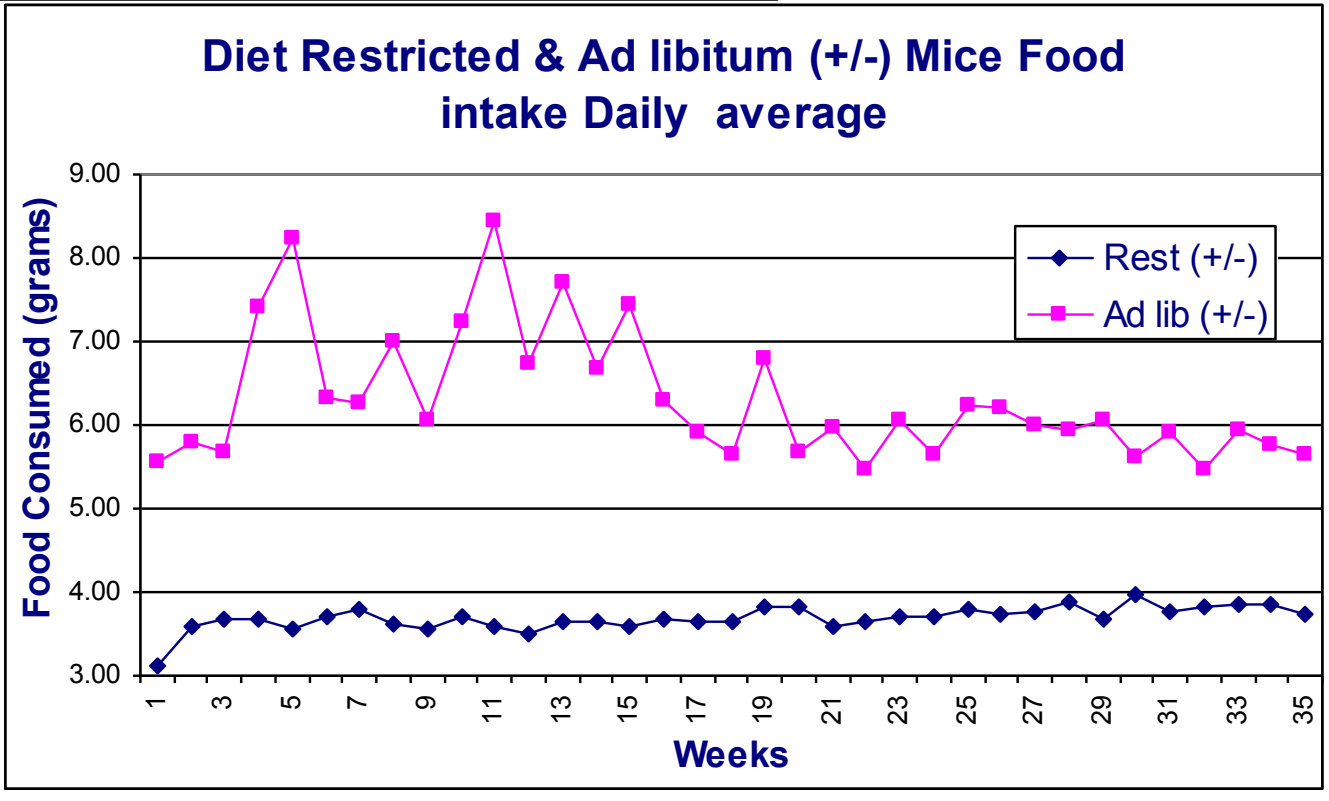
Methods

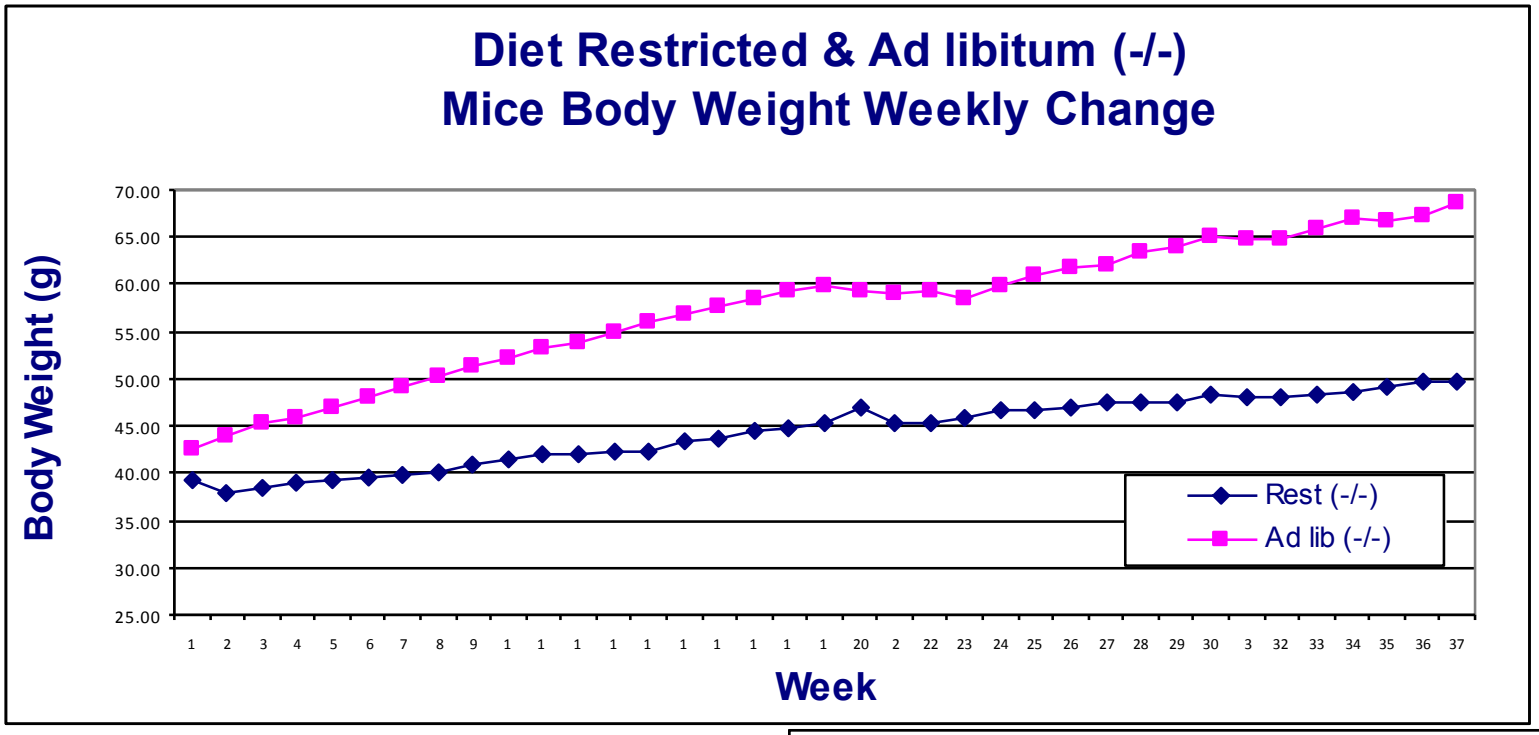
- For this experiment 36 three month old mice were housed in individual cages (12 homozygous Wt, 12 heterozygotes and 12 homozygous mice) and subsequently subdivided into 2 diet groups, a restricted diet group and an ad-libitum group.
- The restricted diet group was given a fixed amount of regular food (4grams) and water (capacity of container) per day. The ad-libitum group was allowed to consume as much food and water as desired. The amount of food consumed by each mouse was weighed daily.
- All mice were weighed once a week to monitor weight change.

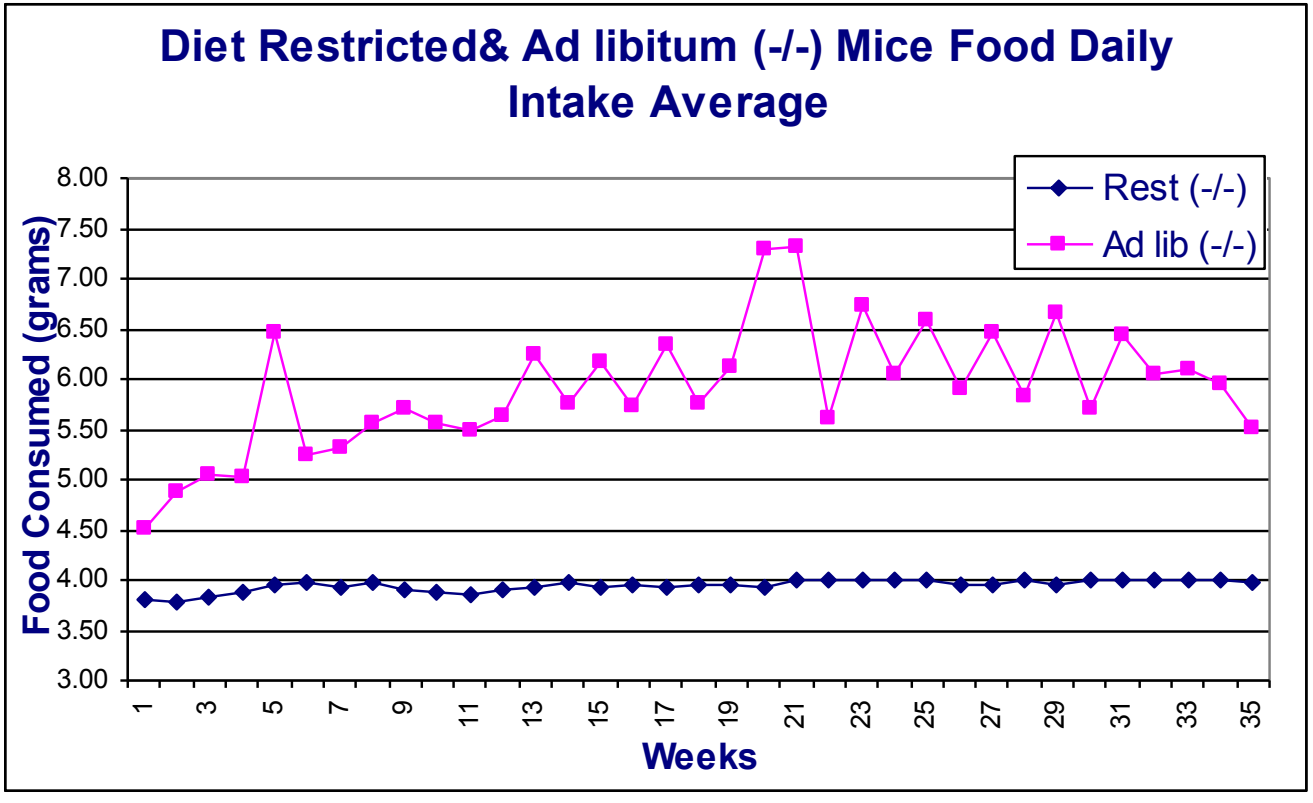

Diet Restricted & Ad libitum (+/+)
Mice Body Weight Weekly Change
Body Weight (g)
70.00
65.00
60.00
55.00
50.00
45.00
40.00
35.00
30.00
25.00
Week
Rest (+/+)
Ad lib (+/+)


Diet Restricted & Ad Libitum (+/+) Mice Food
Intake Daily Average
Food Consumed (grams)
6.00
5.50
5.00
4.50
4.00
3.50
3.00
Weeks
Rest (+/+)
Ad lib (+/+)


Diet Restricted & Ad libitum (+/-)
Mice Body Weight Weekly Change
Body Weight (g)
70.00
65.00
60.00
55.00
50.00
45.00
40.00
35.00
30.00
25.00
Week
Rest (+/-)
Ad lib (+/-)


Diet Restricted & Ad libitum (+/-) Mice Food intake Daily average
Food Consumed (grams)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
Weeks
Rest (+/-)
Ad lib (+/-)


Diet Restricted & Ad libitum (-/-)
Mice Body Weight Weekly Change
Body Weight (g)
70.00
65.00
60.00
55.00
50.00
45.00
40.00
35.00
30.00
25.00
Rest (-/-)
Ad lib (-/-)
Week


Diet Restricted& Ad libitum (-/-) Mice Food Daily Intake Average
Food Consumed (grams)
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
Rest (-/-)
Ad lib (-/-)
Weeks

Conclusions

- Ataxin-2 deficient mice are viable
- K-out mice are near normal and do not develop Purkinje

Cell neurodegeneration

- Ataxin-2 deficiency causes obesity by hyperphagia