Olfactory Performance of SCA2 KO mice

10-12-2012

General Hypothesis

- The presence of Atxn2 in the olfactory system suggests that knocking out the protein, or introducing the mutant protein, will have detrimental effects on basic olfactory function.

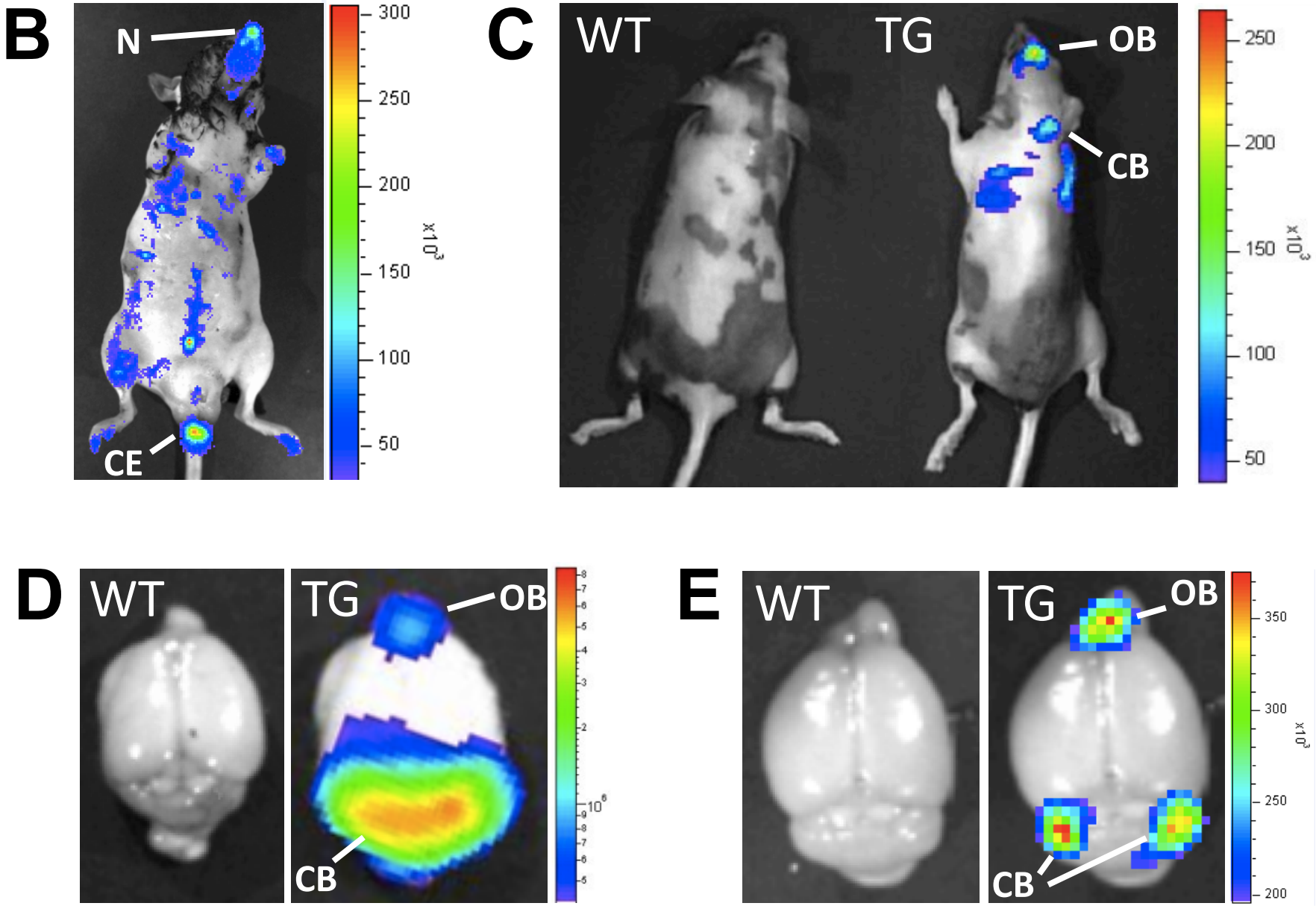
Rationale

- Dan and Steve have observed high expression of mATXN2 in the olfactory bulb (mitral cells – IHC staining by Steve) in the luciferase mice.

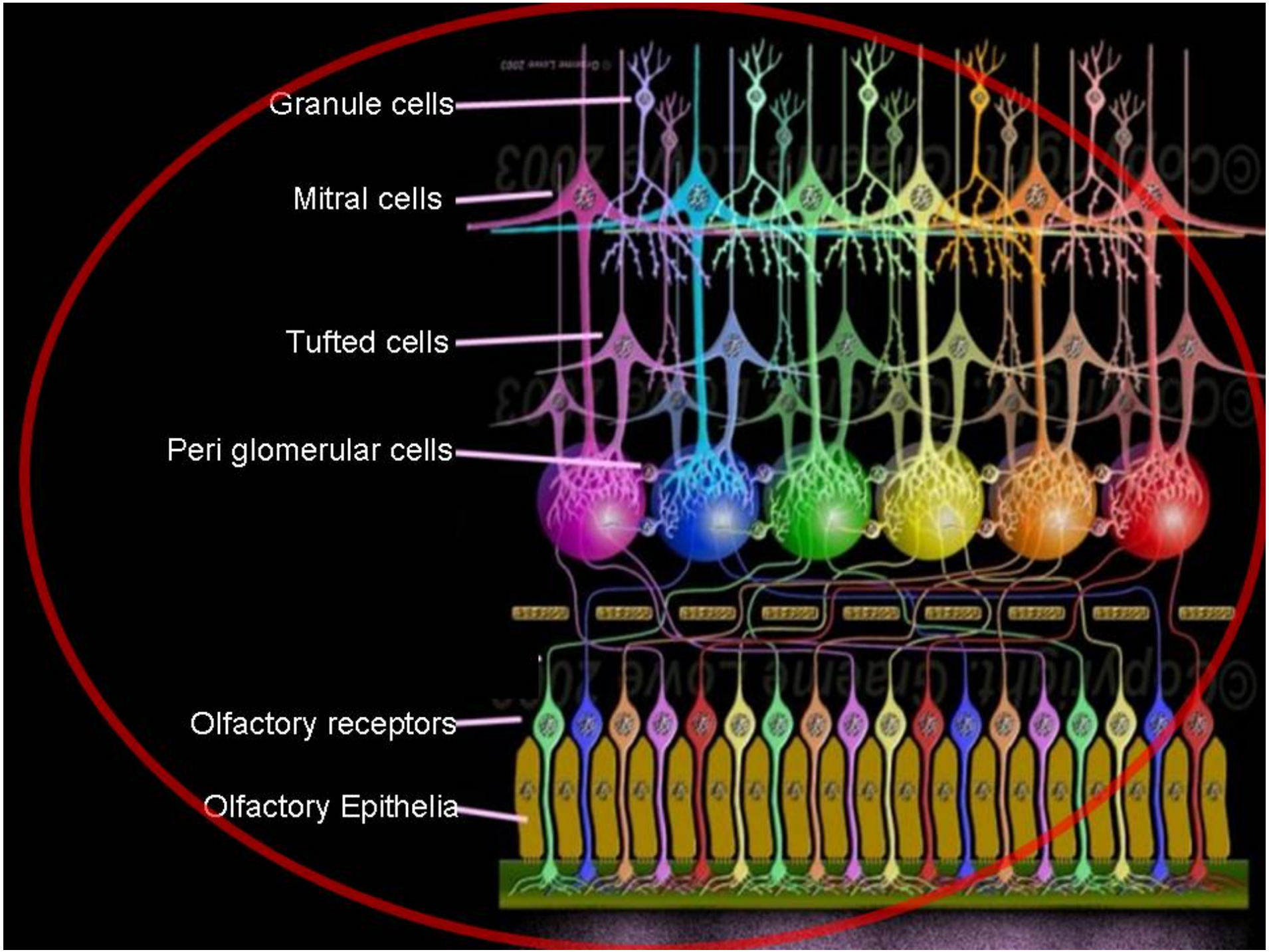
- Clinical trials have shown deficits in olfactory performance in SCA (2,3, and 7) patients (Velazquez-Perez et al., 2011; Connelly et al., 2003).

- *Drosophila* require Atx2 for long-term habituation of the olfactory system (McCann et al., 2011).

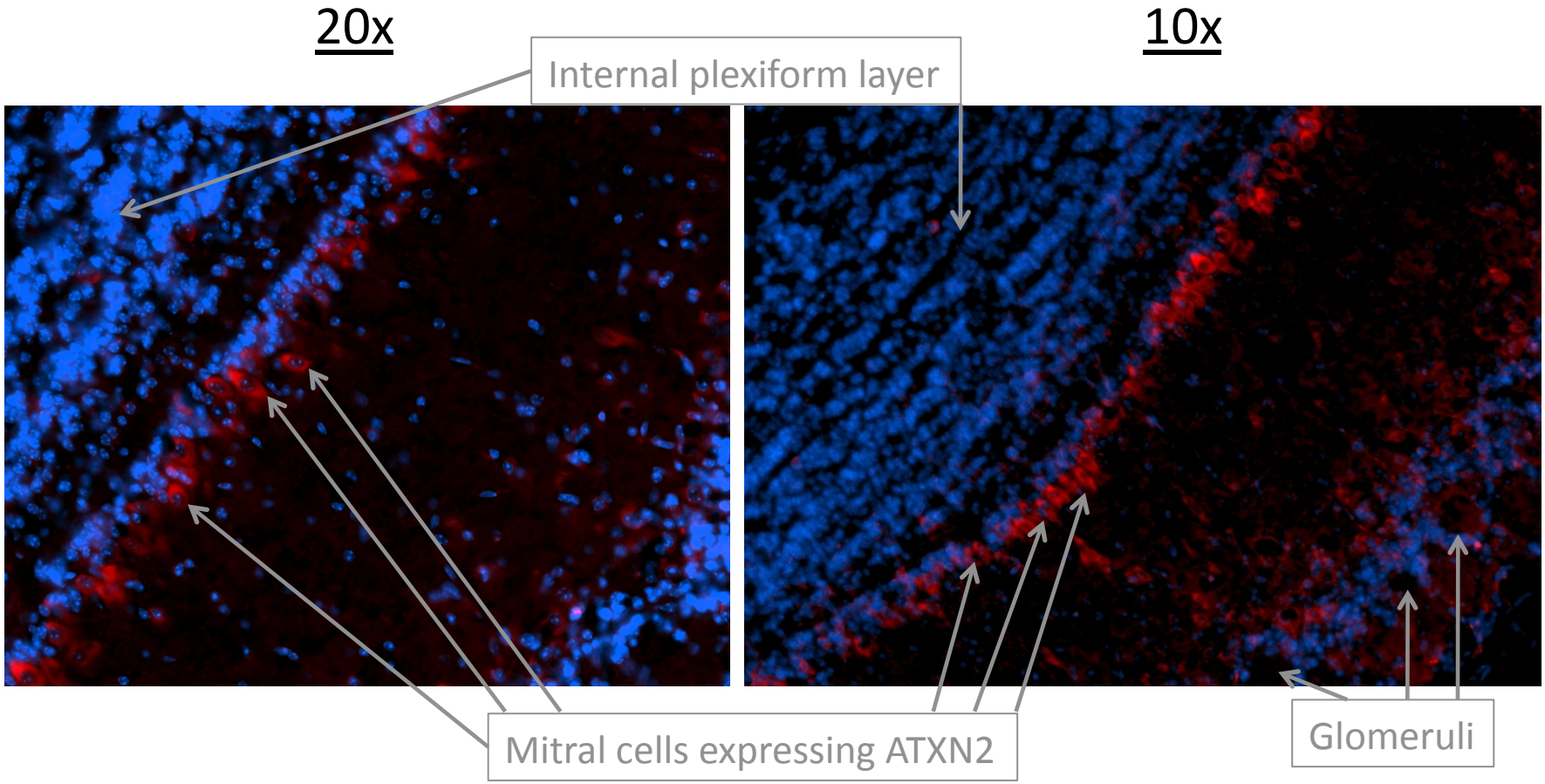
- SCA2 KO mice show a propensity for weight gain. Maybe related to olfaction?



- Scoles et al., 2012


Granule cells
Mitral cells
Tufted cells
Peri glomerular cells
Olfactory receptors
Olfactory Epithelia

IHC - mouse olfactory bulb



- Data provided by Dr. Steve (commercial Ab)

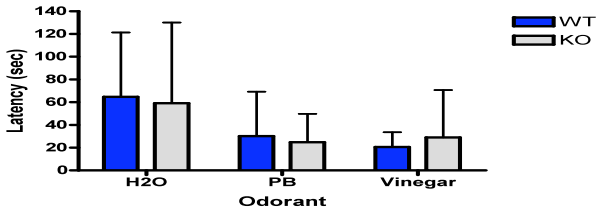
Methods – Experimental Design

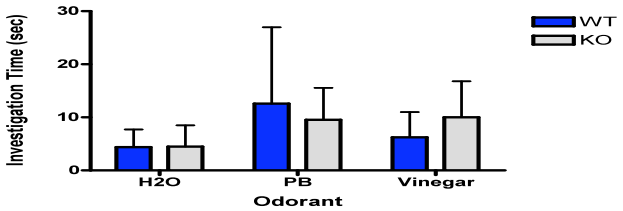
- Test for Smell Recognition
  - Present each mouse with 3 odorants (peanut butter, vinegar, water control) for three minutes.
  - Record latency to locate the smell origin
  - Record total time investigating the odorant

Methods – Experimental Design

- Test for Habituation/Dishabituation
  - Habituation - present each mouse with an odorant (lime concentrate) for six exposures of 30 seconds each.
  - Dishabituation - on 7th trial, introduce a similar, but novel, odorant (orange concentrate).
  - Record total time investigating the odorant for each trial.

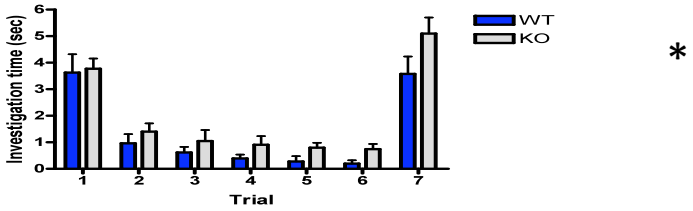
Results – Olfactory Recognition





N = 21 for both groups. By two-way ANOVA (Bonferroni post-hoc), there are no differences between groups. Means ± s.d.

Results – Habituation/Dishabituation



Habituation (trials 1-6):

N = 21 for both groups. By two-way ANOVA (Bonferroni post-hoc), there is a difference between groups ($p = 0.0242$) but not within a given trial. Mean ± s.e.m.

Dishabituation (trial 7):

N = 21 for both groups. By two-tailed, paired t-test, there is a difference between groups ($*p = 0.0304$) in trial 7. Mean ± s.e.m.

Conclusions – Olfactory Assays

- SCA2 KO mice (compared to WT littermates) do not show behavioral differences in response to novel odorants.

- SCA2 KO mice (compared to WT littermates) habituate more slowly to a repeated exposure of a common odorant. In semi-agreement (involved in habituation) with role of Atxn2 in Drosophila that showed Atxn2 was required for long-term habituation but not short-term habituation (McCann et al, 2011). Here, we show evidence of short-term habituation in the KO mice.

- After habituation, SCA2 KO mice spend more time investigating a novel, but similar, odorant.

Could the observed differences in olfactory performance be an artifact that results from hyperactive behavior in the KOs?

Metabolic Characterization of SCA2 KO, BAC-Q22, and BAC-Q72 mice
Pilot Study

10-12-2012

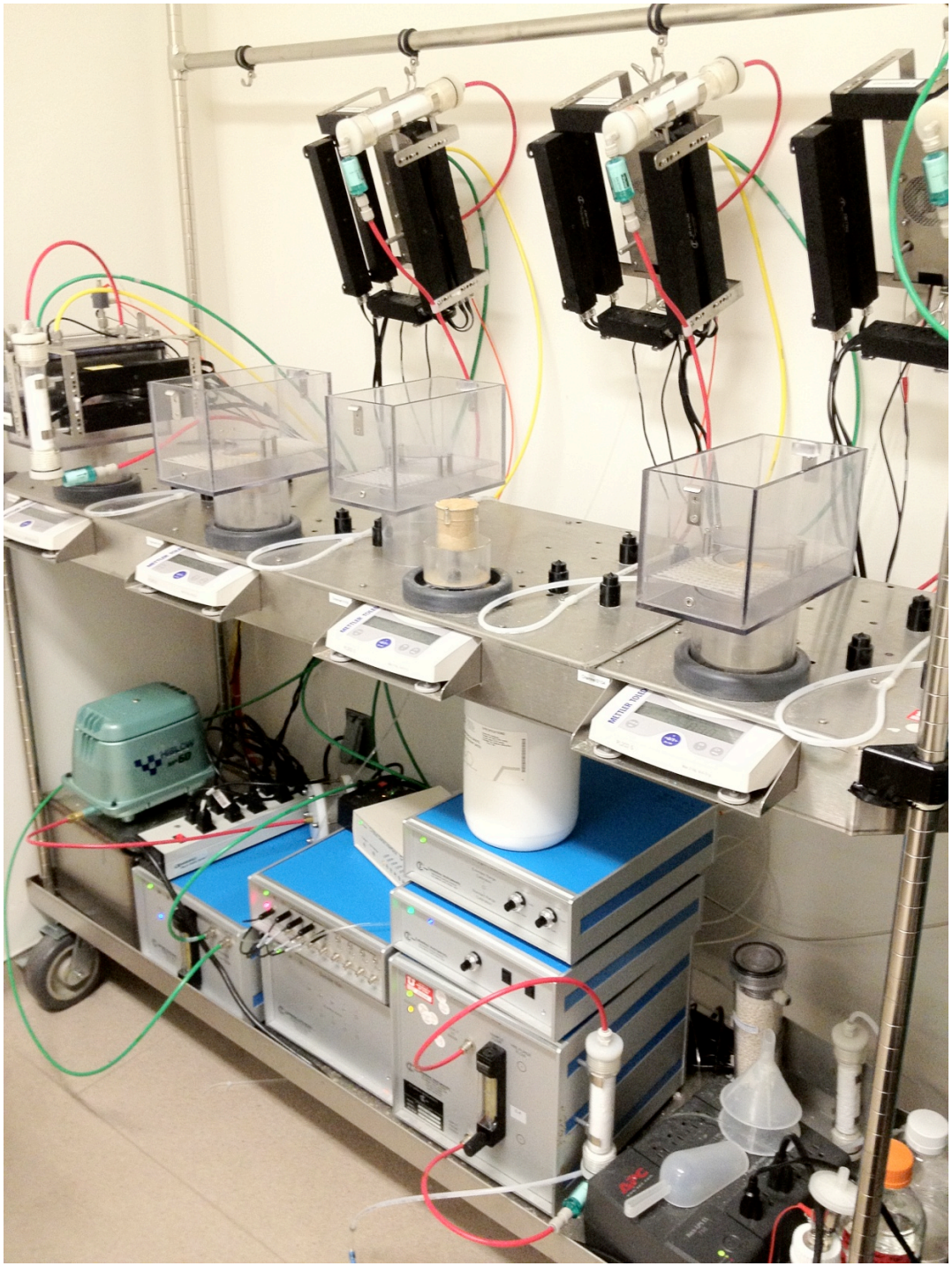
Rationale

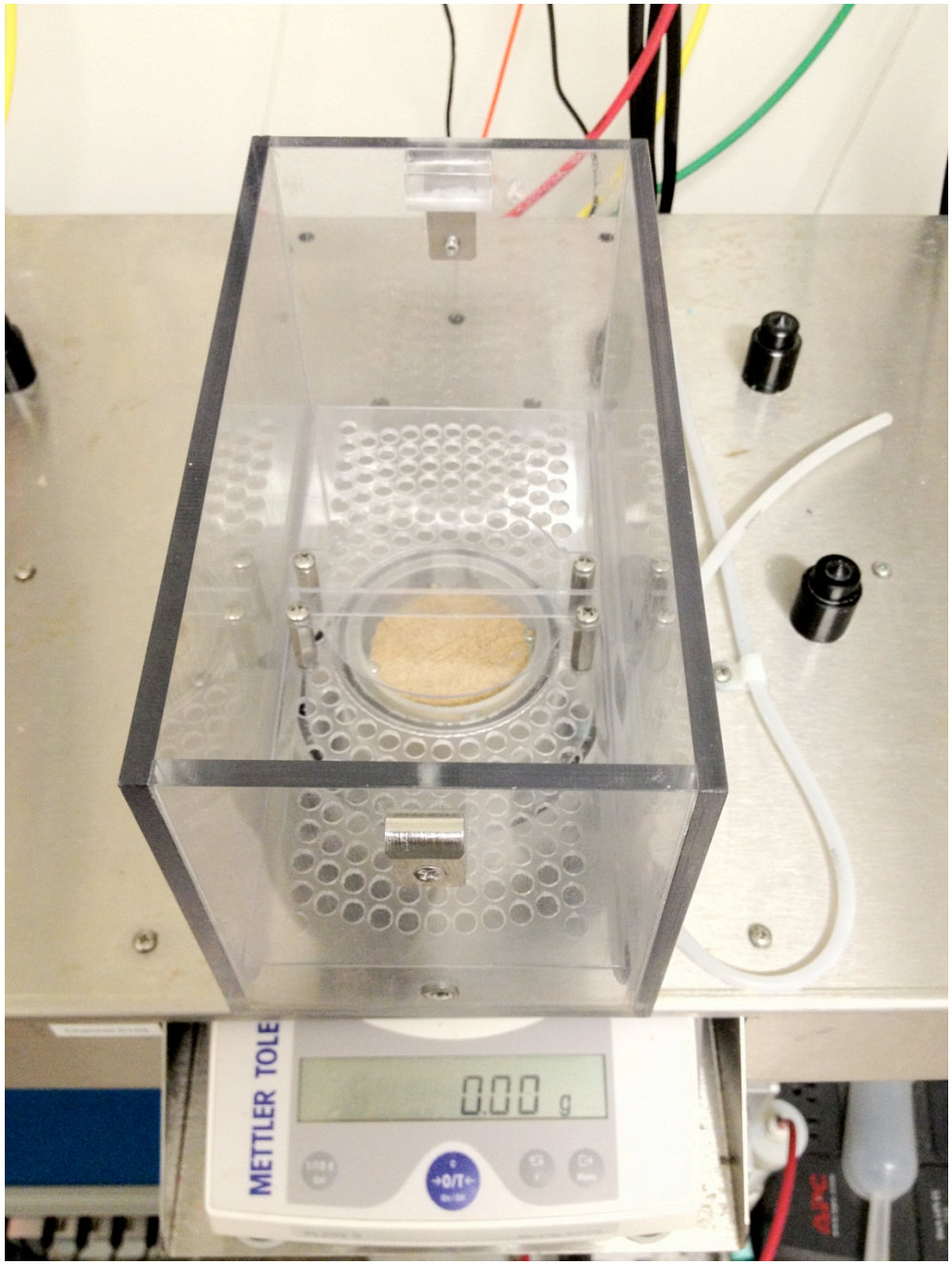
- Weight differences exist between mice with genetically modified Atxn2 mice (KO↑, BAC Q72↓, BAC Q22 – may be down in older animals).

- Open cage behavior suggests that genetically modified Atxn2 mice are more active.

Methods

- N of 2 for each group
- Mice were ~10-11 weeks old
  - This age point is slightly younger than the average age at which we observe circling behavior in the Q72 mice
- Individually housed and monitored in open-circuit metabolic chambers for 72 hrs.

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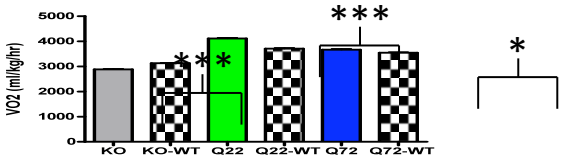
Statistical Analysis

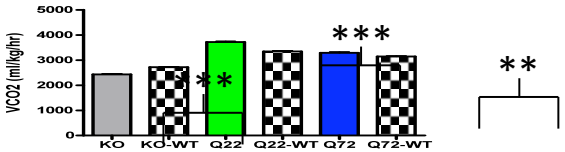
- One-way ANOVA followed by Bonferroni post-hoc tests

Results – Respiratory Function

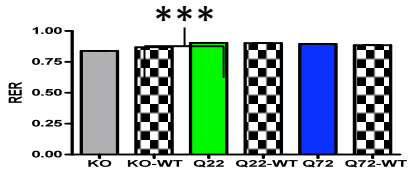
O_2 Consumed



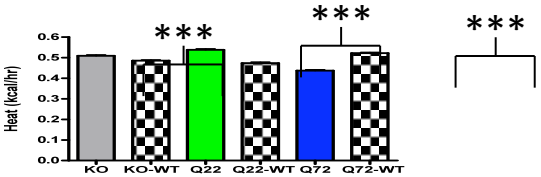
CO_2 Produced



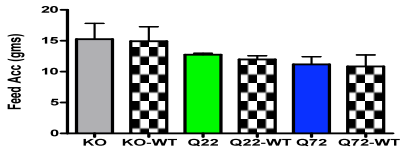
Respiratory exchange ratio (VCO_2/VO_2)

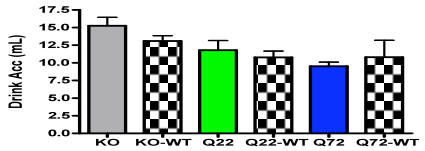


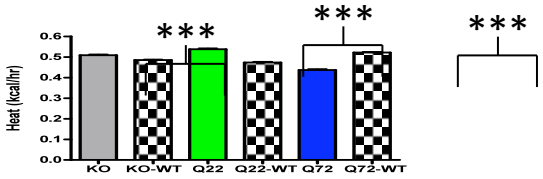
Energy Expenditure

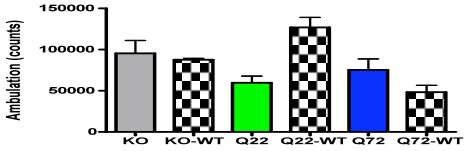


Results – Intake, Thermal Output, and Ambulation







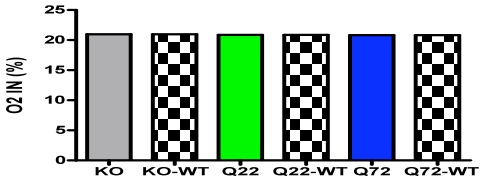


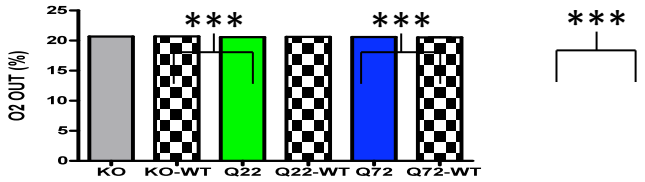
Conclusions (N = 2)

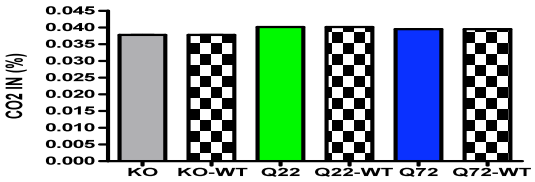
- The difference in RER between KO and WT littermates suggests that the KO mice could be metabolizing more fat.

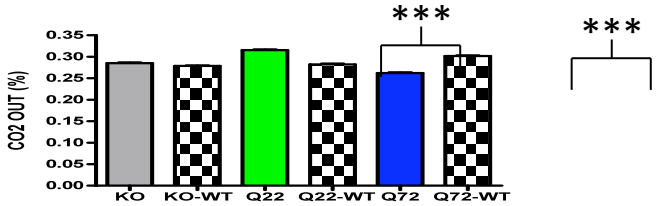
- The 2 SCA2 KO mice and the 2 BAC Tg Q22 mice burned calories at a slightly higher rate than WT littermates. However, the 2 BAC Tg Q72 burned calories at a lower rate compared to WT littermates. This trend was not in agreement with total movement (ambulatory counts) for the BAC animals (ambulatory counts were not significant between any groups due to small sample size).

Results – Respiratory Function

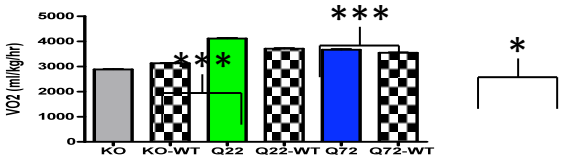


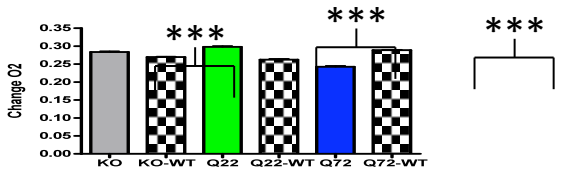


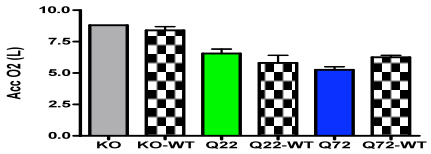


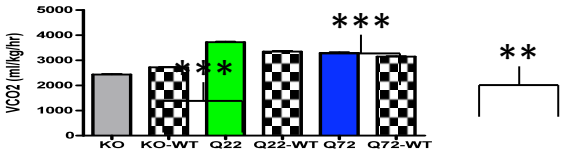


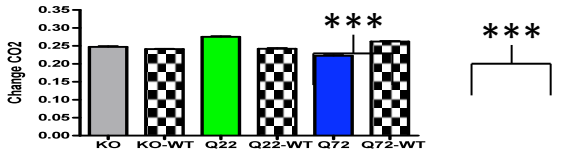
Results – Respiratory Function

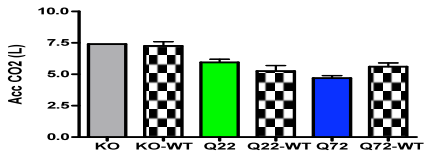




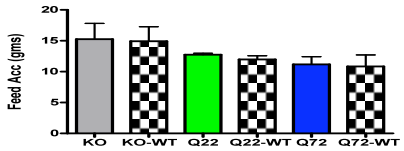


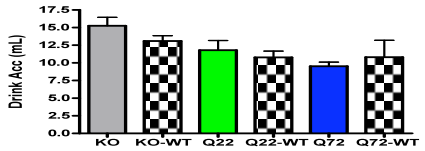


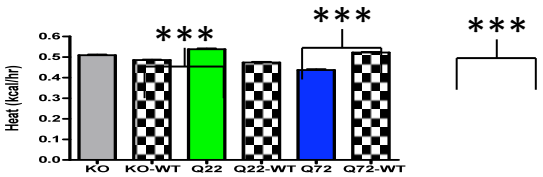


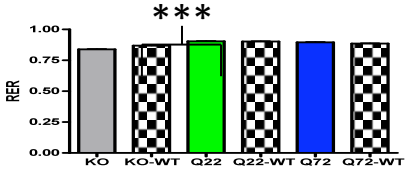


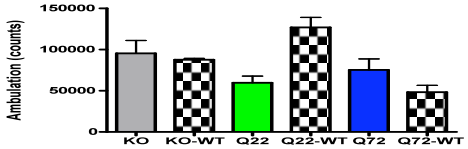
Results – Intake, Thermal Output, and Ambulation



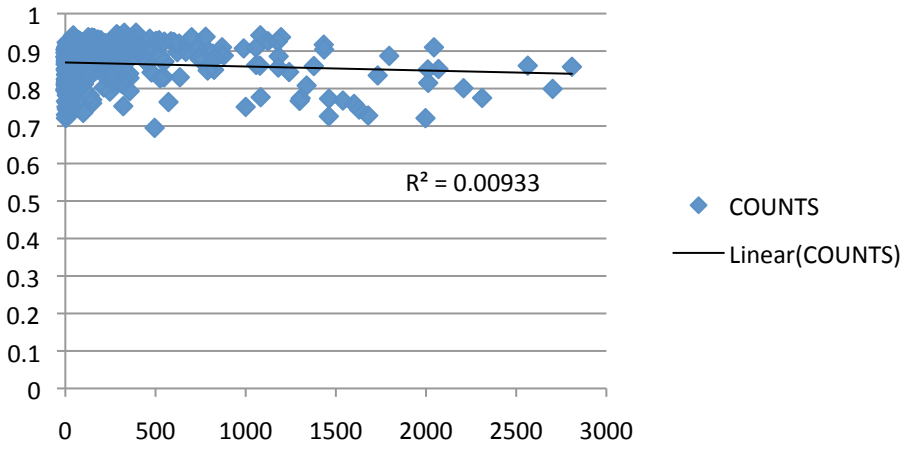




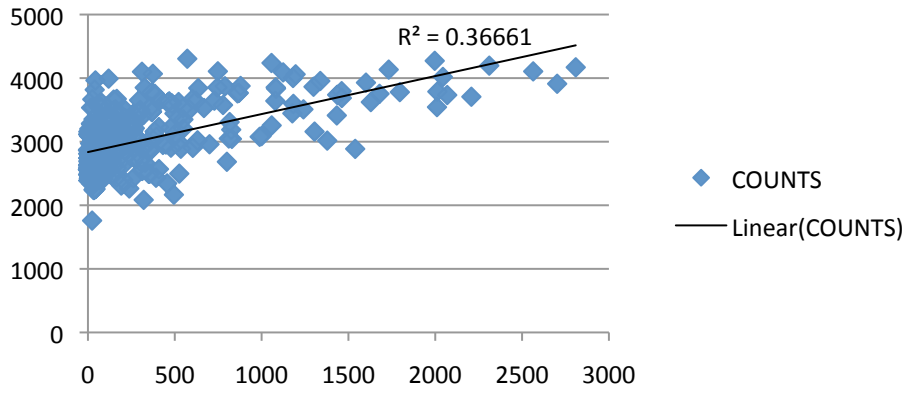




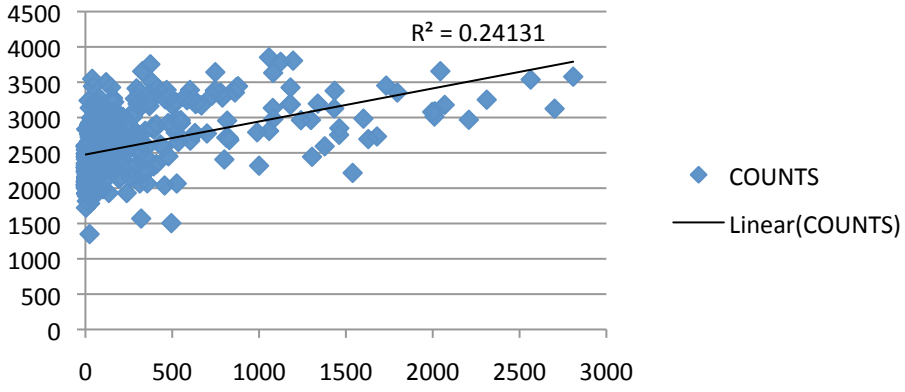
Ambulation vs RER (KO WT 42)



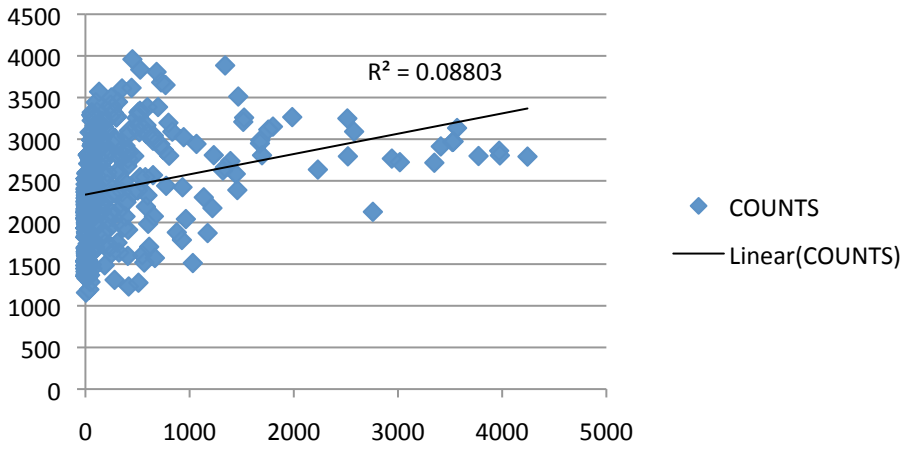
Ambulation vs VO2 (KO WT 42)



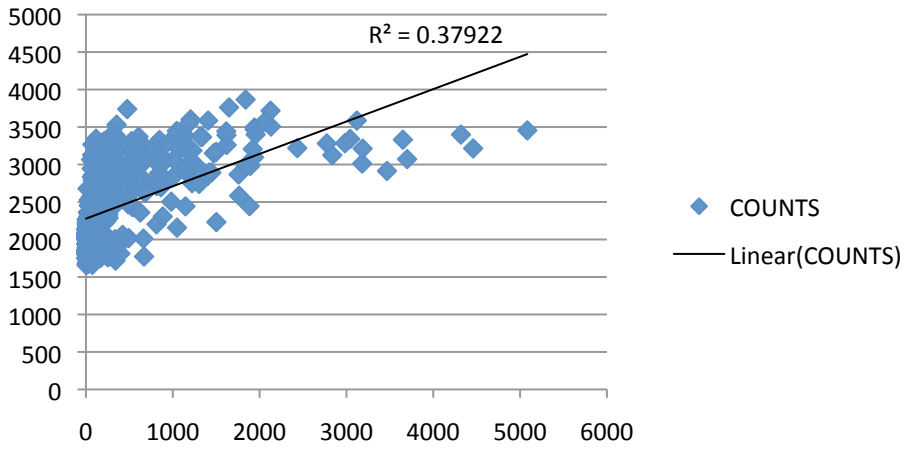
Ambulation vs VCO2 (KO WT 42)



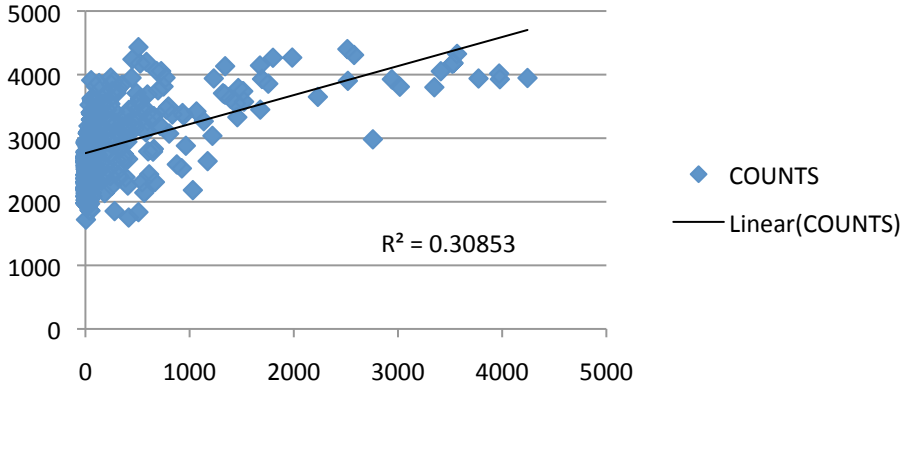
Ambulation vs VCO2 (KO 62)



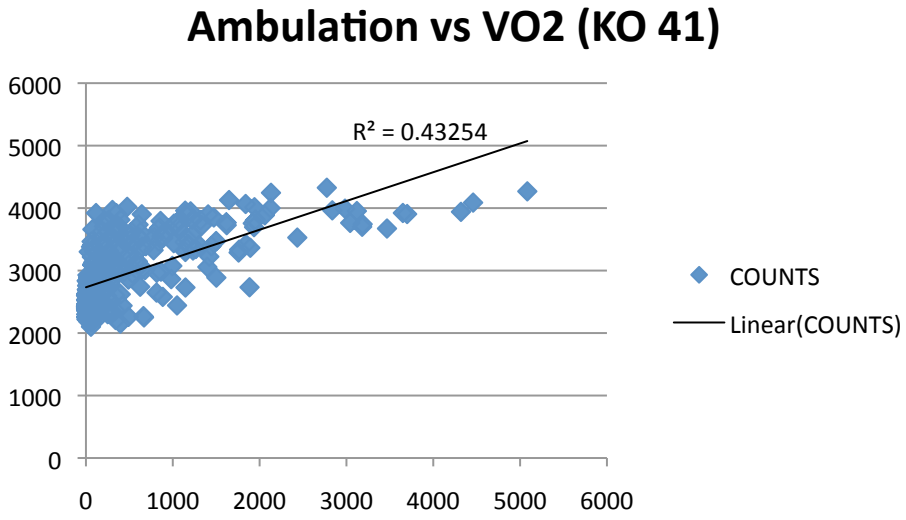
Ambulation vs VCO2 (KO 41)



Ambulation vs VO2 (KO 62)



Ambulation vs VO2 (KO 41)



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Gene expression profiles in the cerebellum of transgenic mice over expressing the human *FMR1* gene with CGG repeats in the normal range

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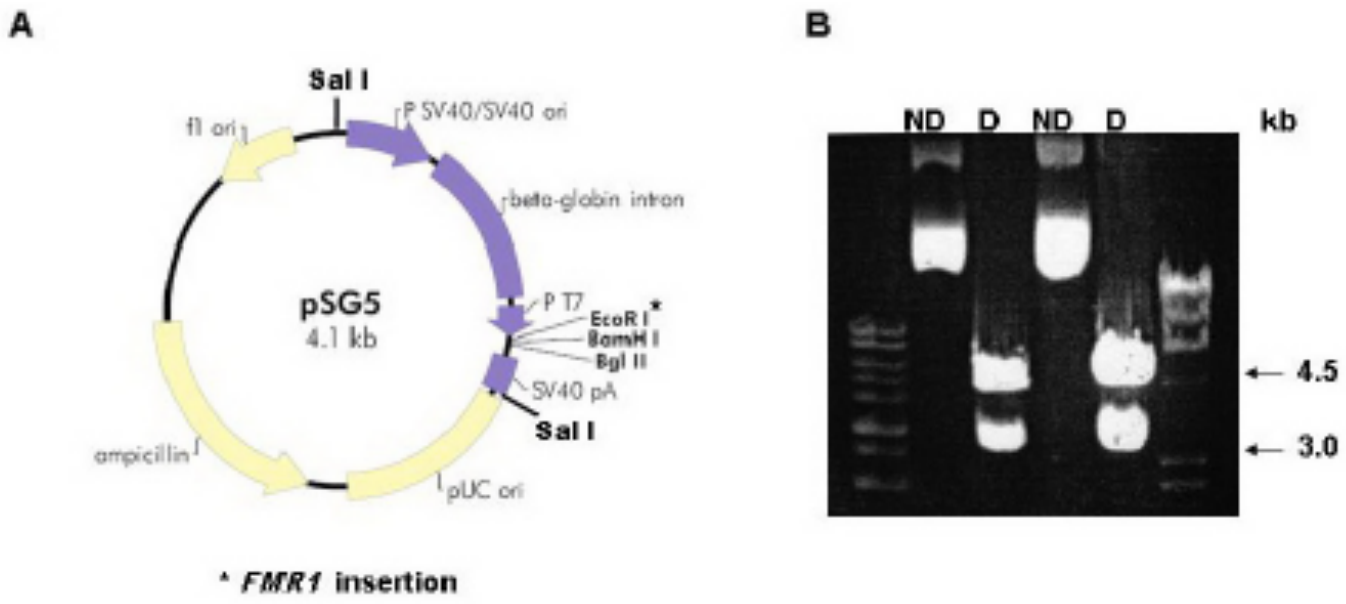
Their Hypothesis

- Increased levels of FMR1 mRNA of normal CGG repeats has no effect on the GABAergic pathway which is believed to be the pathway most disrupted in fragile X syndrome and fragile X-associated tremor ataxia syndrome.

Methodology

- Create a transgenic mouse that overexpresses normal FMR1.
- Characterize expression patterns using microarray analysis



- 4.5 kb fragment containing FMR1 cDNA with 29 CGG repeats

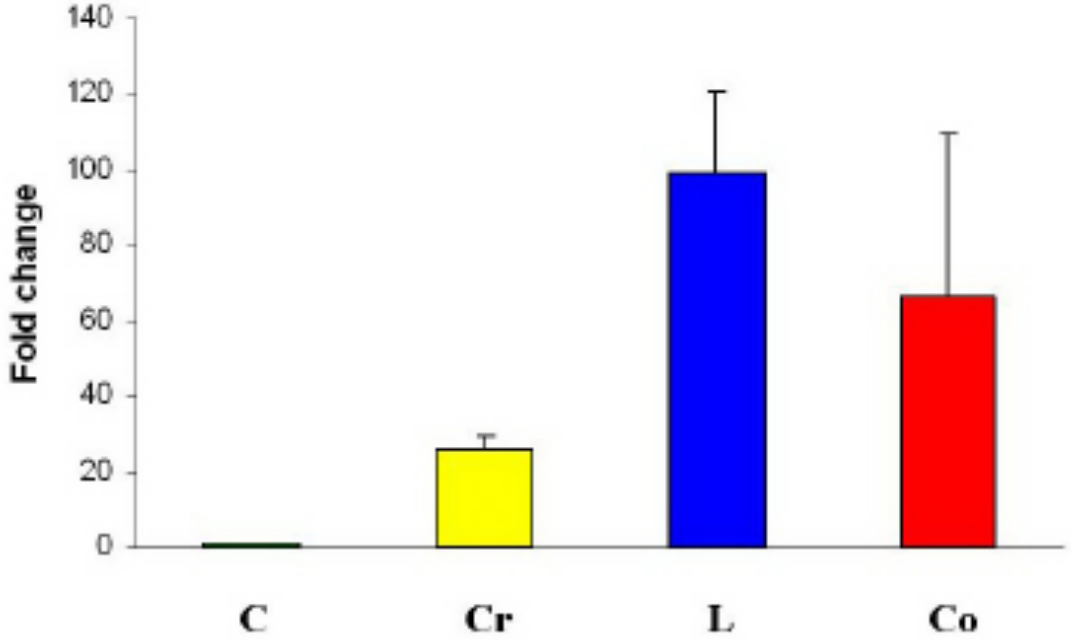


Figure 2. Quantitative RT-PCR of m*FMR1* in transgenic mice. Cerebellum (Cr), liver tissue (L) and cortex (Co) were dissected from transgenic and wild-type mice. RNA was isolated and reverse transcribed as indicated in Material and Methods. Real-time PCR showed that the expression of human *FMR1* was 20 to 100 times (fold change) higher than the values in control littermates, which were normalized to one (C). The results are reported as means ± SD of three different experiments from line B.

Table 1. Assessment of exploration and activity.

	Wild type		Transgenic	
	Distance	PT%	Distance	PT%
7 months	13883 ± 5377 (6)	13.57 ± 5.7 (6)	12187 ± 2561 (6)	16.04 ± 7.43 (6)
14 months	11923 ± 4777 (4)	23.2 ± 4.73 (4)	11928 ± 2770 (4)	15.79 ± 11.18 (4)

Distance covered is reported in cm and permanence time (PT%) in percent of the total time spent in the periphery of the arena. Data are reported as means ± SD. Number of animals analyzed is indicated in parentheses.

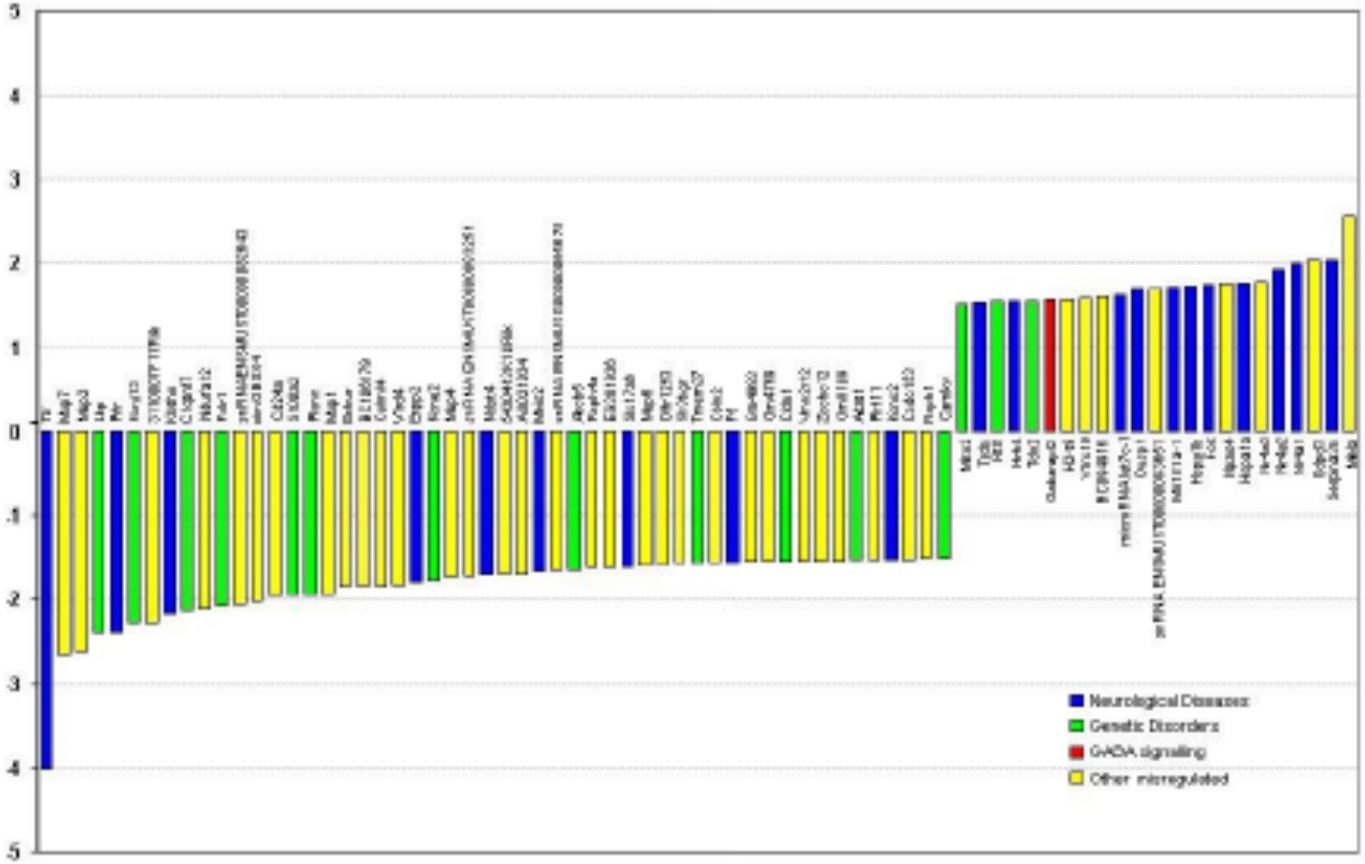
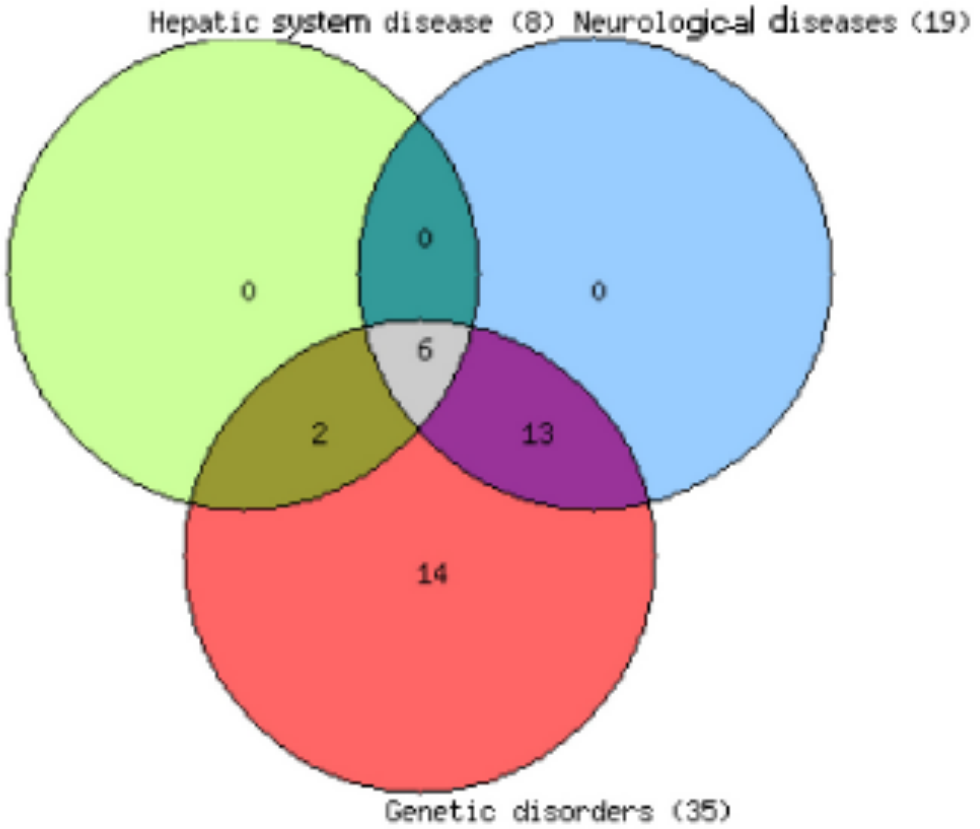


Figure 3. Gene expression profile in the cerebellum from transgenic mice. Cerebellar tissue was dissected and RNA was extracted as described in Material and Methods. cDNA was obtained by reverse transcription and expression was analyzed by "GeneChip Mouse ST 1.0 Array" manufactured by Affymetrix. The diagram shows the 75 well-characterized genes that present changes equal to or above 1.5 times with respect to control littermates and with a P value <0.1. From these genes 70% were inhibited (down in the graphic) and 30% were increased (up in the graphic). Genes related to neurological diseases are represented in blue, genes related to known genetic disorder in green and, other misregulated genes in yellow. With the exception of *GabarapL2* (red column) no known gene from the GABAergic pathway was altered.



Venn diagram. Intersection of genes from different groups. The criteria used to define candidate differentially expressed genes are indicated in Material and Methods. Genes from "Other misregulted" group (yellow columns in Figure 3) are not described.

Their conclusions

- The GABAergic pathway is not greatly affected by increasing expression levels of the normal FMR1 gene.
- No cerebellar phenotype.
- Therefore, gain-of-function in FXTAS must be caused by expanded CGG repeats rather than mRNA toxicity… maybe.
- They claim RNA toxicity may affect other genes, such as Ttr and Serpina3, and work in an indirect manner.

Genes shared with our mouse models

4wk Tg	8wk Tg	4wk KO	8wk KO		FMR1 Study
				Gene	
			1.2037193	Nr4a3	Up in FMR1
			-2.1029937	Tdo2	Up in FMR1
			1.5316557	Nr4a1	Up in FMR1
	-1.12778		2.188433	Fos	Up in FMR1
		-1.68246	2.0662901	Serpina3n	Up in FMR1
			2.3754296	Calml4	Down in FMR1
	1.773136		2.7462626	Folr1	Down in FMR1
	1.607576		2.3995326	Kl	Down in FMR1
			1.7464721	Lbp	Down in FMR1
	1.730603		2.991229	Ttr	Down in FMR1
			1.4559321	Dusp1	down in FMR1
			2.0903482	Cldn1	down in FMR1
			3.0470924	Kcne2	Down in FMR1
	1.064627		1.7399819	Prlr	Down in FMR1
	1.117159		1.9205033	Enpp2	Down in FMR1
			2.202564	Kcnj13	Down in FMR1
	1.686241		2.3837333	F5	Down in FMR1
			1.5592657	Nr4a2	Up in FMR1
1.352534				Zcchc12	Down in FMR1
1.333437				Slc17a6	Down in FMR1
1.720725				Meis2	Down in FMR1
1.176866				Kcnc2	Down in FMR1

steroid-thyroid hormone-retinoid receptor

differentiation and maintenance of (mdDA) neurons

Neuron-derived orphan receptor