Center for Human Genetics

A Drosophila Model for Sanfilippo Syndrome

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Abstract

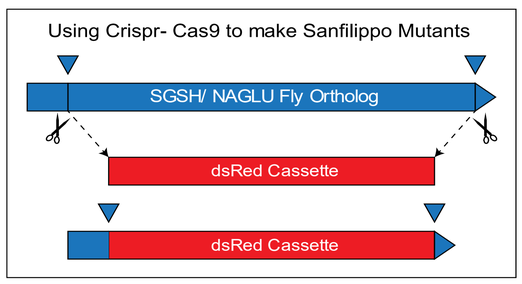
Sanfilippo syndrome (Mucopolysaccharidosis Type III, MPS III) is a rare, autosomal recessive disorder, caused by a deficiency in enzymes involved in the breakdown of heparan sulfate. The disease is characterized by the accumulation of heparan sulfate in lysosomes, which leads to degeneration of the central nervous system. Patients exhibit developmental regression, hyperactivity, sleep irregularity, coarse features and a reduced lifespan. The genome of *Drosophila melanogaster* contains conserved orthologs of human disease genes. We obtained Drosophila lines with CRISPR-Cas9 generated deletions of fly orthologs of *SGSH* and *NAGLU*, mutations in which cause MPS IIIA and MPS IIIB. A deletion in *CG14291*, which is orthologous to *SGSH*, displayed phenotypes reminiscent of human Sanfilippo Syndrome. Flies of this line showed reduced lifespan (-28.8%, males; -15.1%, females) and reduced productivity (-78.7% at 2 weeks old) compared to the control. Males of this line exhibited a significant increase in average activity ($p<0.0001$) and showed impaired phototaxis compared to the control. Thus, the *CG14291* deletion line can serve as a model for Sanfilippo syndrome. However, the same mutation in an independently obtained mutant did not replicate these phenotypes, suggesting the existence of genetic modifiers in the strain used to generate the deletions. Naturally segregating epistatic partners that affect penetrance of the mutation can be identified by crossing the *CG14291* deletion line to lines of the *Drosophila melanogaster* Genetic Reference Panel and screening for the observed phenotypes.

Experimental Design

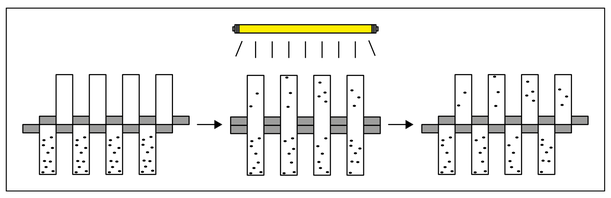
Our research focused on the disease genes of MPS IIIA and MPS IIIB, which account for 90% of Sanfilippo patients.

Human Ortholog	*Drosophila* Gene
NAGLU (MPS IIIB)	*CG13397* (CG13)
SGSH (MPS IIIA)	*CG14291* (CG14)

NB: CG13_1 and CG13_2 are independently generated but genetically identical lines of flies. Similarly for CG14_1 and CG14_2.

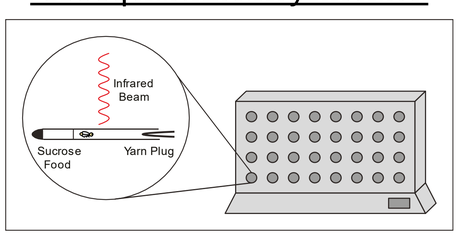


Positive Phototaxis



- The assay was performed in a dark room, using a counter-current distribution apparatus.
- Apparatus was shifted to expose flies to light for 20 s.
- Vials were shifted back to start position.
- The number of flies which moved to a new vial i.e. towards the light were counted, out of a total of 20 flies

Drosophila Activity Monitor



- An infrared beam crossed the tube and the number of times the fly crossed the beam was recorded.
- Sucrose plus agar food does not allow larvae to survive (which might occlude the readings) .

Lifespan/ Productivity Assay

- Vials set up with 3 males and 3 females of the same line.
- Flies tipped onto new food 3 times a week.
- Vials monitored daily for dead or missing flies.
- Fly productivity was scored weekly.

Sanfilippo Syndrome

Sanfilippo Syndrome or Mucopolysaccharidosis III (MPS III), is an autosomal recessive disease of lysosomal storage.

MPS III patients have a deficiency in one of the enzymes involved in the degradation of the sugar molecule heparan sulfate (HS). These defects cause the build up of HS in lysosomes, which disrupts cell function.

The primary effect of MPS III is neurodegeneration in the central nervous system, which ultimately leads to developmental regression, behavioural issues, sleep abnormalities, hyperactivity and vision problems.

There are 4 main subtypes of MPS III, each accounting for a different enzyme involved in the degradation of HS.

Subtype	Human Gene	Enzyme
MPS IIIA	SGSH	Heparan N-Sulfatase
MPS IIIB	NAGLU	Alpha- N-Acetylglucosaminidase
MPS IIIC	HGSNAT	Acetyl-CoAlpha-Glucosaminide Acetyltransferase
MPS IIID	GNS	N-Acetylglucosamine 6- Sulfatase

1. Positive Phototaxis

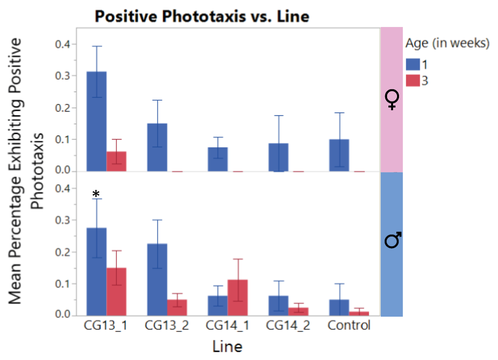


Fig 1. Results taken at ages 1 week vs 3 weeks.
CG13_1 males showed a significant increase in positive phototaxis compared to the control at age 1 week, indicating a possible increase in light sensitivity.
The results were analysed separately by sex with a fixed-effects ANOVA for a line effect, followed by a post-hoc Dunnett's test. Significance is indicated with an asterisk (*)

2. Lifespan and Productivity

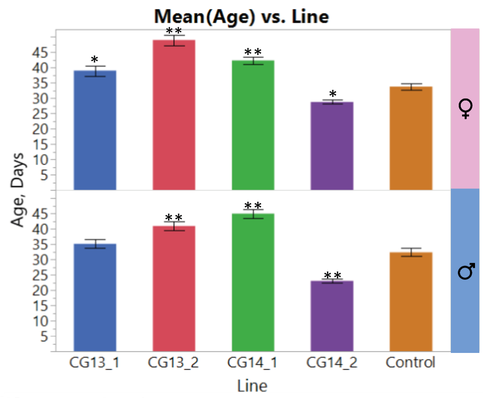


Fig 4. Mean lifespan in days.
CG14_2 showed the lowest lifespan in both males and females.
Surprisingly, CG13_2 and CG14_1 both had a significantly higher lifespan than the control in both males and females.
The results were analysed separately by sex with a fixed-effects ANOVA for a line effect, followed by a post-hoc Dunnett's test. Significance is indicated with an asterisk (*)

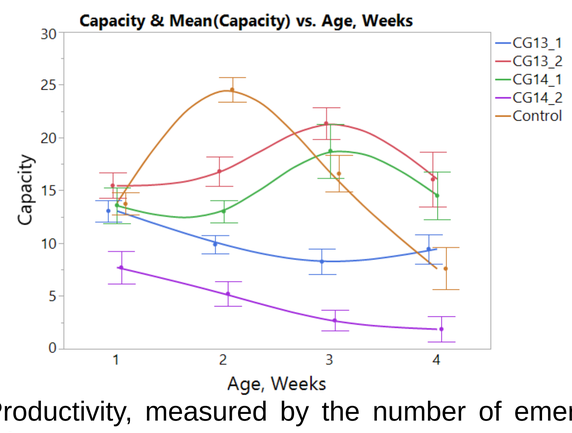


Fig 5. Productivity, measured by the number of emerged flies, per female.
CG14_2, which had reduced lifespan, showed reduced productivity at each age compared to the control, -78.7% at 2 weeks old.
The results were analysed separately by sex with a two-way fixed-effects ANOVA for a line x week interaction effect, followed by a post-hoc Dunnett's test.

3. Activity

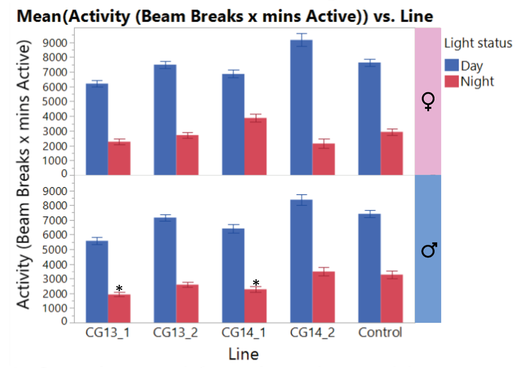


Fig 2. CG14_2 males and females showed increased activity vs. control during the day. These results suggest hyperactivity, however were not significantly different. CG13_1 males showed significantly reduced night activity vs. control.
The results were analysed separately by sex and light-status with a fixed-effects ANOVA for a line effect, followed by a post-hoc Dunnett's test. Significance is indicated with an asterisk (*)

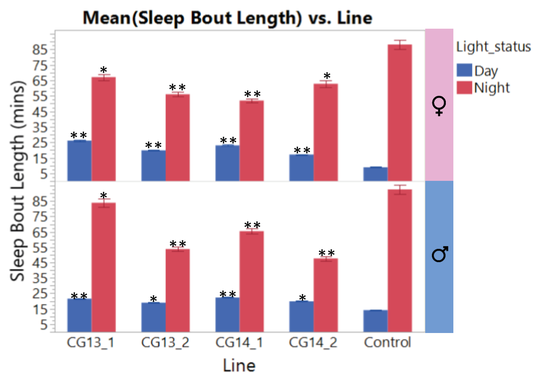


Fig 3. All Sanfilippo mutants showed abnormal sleep patterns.
Sleep bout lengths were significantly increased compared to the control during the day in all Sanfilippo mutants in both sexes.
Sleep bout lengths were significantly decreased at night across all Sanfilippo mutants for both sexes.
The results were analysed separately by sex and light-status with a fixed-effects ANOVA for a line effect, followed by a post-hoc Dunnett's test. Significance is indicated with an asterisk (*)

4. Lifespan: SF Lines x DGRP Line
A Screen for Genetic Modifiers

In most of the behavior assays performed on the knockouts, we observed different results in the independent mutants, which are supposed to be genetically identical (confirmed locally around the knockout by Sanger sequencing).
Hence, we decided to cross the SF mutants with 8 lines from the Drosophila Genetic Reference Panel (Mackay *et. al.*, 2012, Nature) in order to understand whether making the knockout heterozygous in variable genetic backgrounds results in changes in the phenotypes.

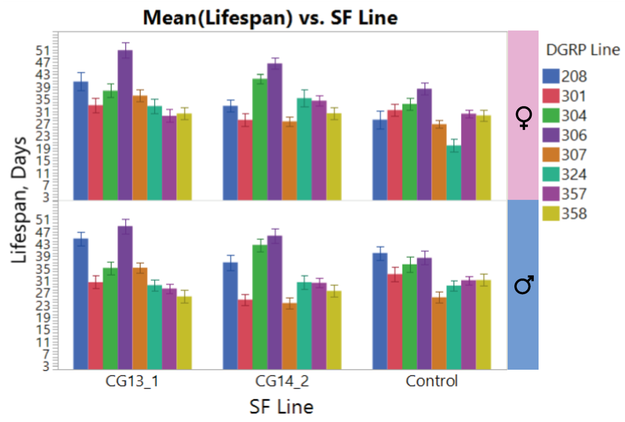


Fig 6. Lifespan was scored for all the SF x DGRP crosses as in the knockout experiment, except that we only scored until each line reached 50% death for both sexes. The time to reach 50% death is highly correlated with full lifespan.
The results were analysed separately by sex with a two-way fixed-effects ANOVA for a SF line x DGRP line interaction effect, followed by a post-hoc Dunnett's test. The interaction term was significant for both sexes indicating that genetic background plays a role. Further analysis and experiments need to be done.

Conclusion

We observed that the knockout of the SGSH fly ortholog shows phenotypes matching some of the human symptoms. Hence, this mutant could be used to model Sanfilippo syndrome.
The independently generated knockouts of the same gene showed significant differences in multiple phenotypes leading us to think that the mutants were not genetically identical.
Hence, we are currently performing a screen for genetic modifiers by generating heterozygotes for the SF knockouts in various DGRP backgrounds. We have observed that genetic background influences lifespan.

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