# COVER SHEET

TITLE:

AUTHOR'S NAME: Katie Tontillo

MAJOR: Genetics

DEPARTMENT: Genetics

MENTOR: Barry Ganetzky

DEPARTMENT: Genetics

MENTOR(2): Daniel Miller

DEPARTMENT(2): Genetics

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Katie Tontillo
Barry Ganetzky Lab

# Pilot Screen for Second Site Suppressors of *wstd*

## Abstract

Triosephosphate isomerase (TPI) loss of function (*wstd*), in flies has been shown to have increases in methylglyoxal and advanced glycation end products (AGEs) which have also been found in increased levels in diseases such as Parkinson's and Alzheimer's disease. By showing that an increase in AGEs leads to neurodegeneration in drosophila, then the accumulation of AGEs might also be the cause of neurodegeneration in human diseases. This experiment is a pilot version of a screen looking for second site modifiers that interact with the TPI gene. A TPI rescue construct was performed from a cross with mutated male drosophila with a null allele for TPI with females deficient for TPI. The experiment selected for non-balancer phenotypes and restored viability in the F1 generation of the cross. The cross resulted in a low number of offspring, and no rescue phenotypes were observed. A fertility test showed that one possibility for the low number of offspring produced is that the mutant male drosophila had low fertility.

## Introduction

Drosophila are a useful tool in forward genetics which have enabled researchers to look for certain phenotypic mutants and link them to specific neurological functions, neurological disorders, or the proteins or pathways involved in those disorders (1). Many genes affecting neurodegeneration have been found using this method (2-8). In this experiment we used this method to look for second site modifiers that interact with the Triosephosphate isomerase (TPI) gene.

Wasted away (*wstd*) is a mutation in TPI and results in shortened life span, progressive motor impairment, and neurodegeneration in drosophila (1)(Figures 1,2). These phenotypes make drosophila a great model in studying neurodegeneration because they can be easily tested or screened. However, only the mutations in TPI, out of all of the enzymes in glycolytic pathway, are associated with neurodegeneration in drosophila. Loss of function of TPI is responsible for the accumulation of dihydroxyacetone phosphate (DHAP) and advanced glycation end products (AGEs) associated with neurodegeneration. This mechanism might be similar to the mechanism involved in neurodegenerative diseases (9).

*Wstd* causes glucose to not be broken down properly, and there is an increase in the amount of DHAP. DHAP then accumulates and converts to methylglyoxal. High levels of methyglyoxal result in the accumulation of AGEs, which impair neural function by oxidative damage, DNA modification, and protein modification (1, 10-13) (figure 3).

Human neurodegenerative diseases like Parkinson's and Alzheimer's are associated with the accumulation of AGEs and show similar phenotypes of *wstd* drosophila, which are neurological dysfunction, and a decreased life span (14, 15). These

diseases have clinical phenotypes of tremors, uncontrollable movement, cognitive loss, and loss of neurons in the brain, along with many other symptoms (9).

If AGEs are shown to be the cause of neurodegeneration in flies, AGEs also might also be the cause of neurodegeneration in humans (1). Our experiment examines the hypothesis that the accumulation of AGEs is associated with neurodegeneration by using second site modifiers to identify gene interactions with TPI. The results from this experiment might disprove the AGEs hypothesis, but the gene identified will give some insight to an alternate mechanism involved with neurodegeneration.

In the screen, male flies that had a null allele for TPI were collected and mutagenized by putting Ethyl Methane-Sulphonate (EMS). We created a random mutation in the male drosophila in order to find a second site modifier that would interact with the TPI gene. The males were also isogenized, so that the X chromosomes in the males would all look the same except for the new second site modifier. This would make it easier to identify a gene interaction.

The *wstd* males were crossed with attached X virgin female flies with a TPI deficiency. Homozygosity of this deficiency results in lethality. The attached X was used to ensure that the mutated X would stay within the males so that we could identify any second site modifiers.

The screen used looked for recessive repressors on the X chromosome of male drosophila. This experiment used a rescue type recovery in which a lethal genotype, (the null over the deficiency), is saved by a second site modifier which suppresses lethality of *wstd*. This allowed us to screen for surviving flies in the F1 generation.

Any male surviving Offspring of the F1 generation of the cross with non-balancer phenotypes would be collected and the X chromosome would be sequenced. These offspring would be J1/ def and lack all TPI function. Any changes on the X chromosome of the males would be unique in the mutant, making it easier to sequence and locate where the gene interaction was occurring.

.Screens that test the life span of flies, or the temperature sensitivity of flies involve a lot more work, and it is harder to identify flies that live longer or survive better at higher temperatures. An advantage to this experiment is that it was more of a selection for phenotypes rather than a screen. Flies that completely lost TPI function never make it to adulthood, and died at the late larval stage. This made the selection easier because the second set modifier is marked by survival. The fact that the flies were able to survive to the late larval stage suggested the possibility that the flies had the ability to survive into adulthood. This suggested that a rescue of *wstd* was possible.

## Methods

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### Female deficiency flies

Female flies Df(3R)Exel6214/TM3,Ser ,with a TPI deficiency were produced by using an attached X and a Shi-ts loose X. This ensured that only the males would carry the X that was Shi-ts.

Cross: (attached) XX/Y /$X^{shi-ts}$; ; Def 6214/TM3

Shi-ts is temperature sensitive and caused all males carrying it to be unable to survive to adult form when incubated at 28 °F for 16 hours. This process made it easier to collect deficiency female virgins. These deficiency female virgins were then crossed to the sc,w/Y ; J1/TM3 males.

**Mutant males**

sc,w/Y ; J1/TM3 males were isogenic so that any new suppressor mutation would be able to be identified. 100 males were selected for the cross. To produce mutant males, Ethyl Methane-Sulphonate(EMS), a known mutant, was put into the food vial of the drosophila for 48 hours. The males were then removed from the EMS food, and put on normal food for a day until transferred into vials containing the crosses. The males varied in age and were randomly distributed throughout the crosses.

**Cross Setup**

The female virgins containing deficiencies were crossed with the mutant males in 20 bottles containing around 10 males and 10 female virgins. Any male offspring without balancer phenotypes that were able to survive to adult form were collected because they suppressed *wstd* from being lethal.

## Results

**Proof of Principle, Rescue of TPI Null Lethality**

Before the experiment could be performed, we needed make sure that the null allele was lethal, and whether it could be rescued with chromosomal TPI. To test whether J1/J1 was lethal, we performed the cross: w; J1/TM3 x w; ChrTPI, J1/TM3

Table 1: cross w; J1/TM3 x w; ChrTPI, J1/TM3

| | J1/TM3 | J1/J1 |
|---|---|---|
| number of offspring | 33 flies | 0 flies |

In table 1, J1/J1 was unable to produce any offspring which shows that J1/J1 is lethal and could not be saved by chromosomal TPI. J1 is put over a deficiency instead of J1/J1, because J1 carries additional lethal mutations on it besides *wstd*, so that it can not be rescued. However, J1 could have a second lethal mutation that is also missing by the deficiency, making it impossible to rescue J1/Def. To test whether J1/ Def could be rescued, we used the cross w; Df(3R)Exel6214/m3 x w; ChrTPI, J1/TM3.

Table 2: The cross w; Df(3R)Exel6214/m3 x w; ChrTPI, J1/TM3

| | J1/Def | Def/TM6, sb | J1/TM6 | TM6/TM6 |
|---|---|---|---|---|
| Number of offspring | 38 flies | 43 flies | 34 flies | 0 flies |

Table 2 shows that when J1/def, it is able to produce offspring. Therefore, the J1/Def can be saved with chromosomal TPI.

**F1 offspring: suppressor**

No suppressors were found since there were only about 5-7 offspring per bottle per cross. This offspring production is too low to find any suppressors of the *wstd*.

**Fertility Test setup**

Since there was such a low production of offspring, fertility of the mutant males by crossing EMS mutant males and wild type males to females of two different genotypes. Crosses were made so that the ratio of males to females were the same for each cross comparing wild type males to mutant males. The ratios consisted of the following:

Females: 33 w/ 118;+;+ x Males: 19 sc,w/Y ; J1/TM3

Females: 33 w/ 118;+;+ x Males: 19 mutated sc,w/Y ; J1/TM3

Females: 24 Canton S x Males: 19 sc,w/Y ; J1/TM3

Females: 24 Canton S x Males: 19 mutated sc,w/Y ; J1/TM3

The parent generation was removed from the cross vials, and the offspring were collected until the F1 offspring hatched to completion. These crosses were made in four different food vials. The parental generation was flipped into new food twice, to produce more offspring. Males and female offspring were separated, counted, and these values were recorded and totaled over the three different vials of the crosses.

There was about 48% more offspring produced with the wild type males than the mutant males when crossed to females of genotype 33 w; 118;+;+. There was about 22% more offspring produced with the wild type males than the mutants when crossed with the Canton S. females (table 3).

This shows that there is a significant decrease in fertility in the mutant male flies. The results also show that this test is repeatable, since we got a decrease in fertility with mutant males with females of two different genotypes.

Table 3: Cross testing fertility. See crosses listed previously. The following genotypes are abbreviated: 33 w; 118;+;+ (33W), 19 sc,w/Y ; J1/TM3 ( 19 M) , 24 Canton S (24 CS), 19 mutated sc,w/Y ; J1/TM3 (19 MM)

| 33 W, 19 M | 33 W, 19 | 24 C.S, 19 | 24 C.S., 19 |
| --- | --- | --- | --- |

| | MM | M | MM | |
|---|---|---|---|---|
| female offspring | 190 | 74 | 132 | 72 |
| male offspring | 233 | 76 | 143 | 106 |
| total offspring | 423 | 150 | 275 | 178 |

## Discussion

This experiment was set up as a pilot screen for future rescue mutation experiments. We hoped to find second site modifiers that were involved with suppressing the lethality of *wstd*. The screen was set up in a way that it would be easy to identify the second site modifier because of the isogenic males. Unfortunately, the low production of offspring made it hard to identify any modifiers of *wstd*.

Table 3 showed that there is a significant decrease in the amount of offspring produced when the males are mutated by EMS in both of the female genotypes. From these results, we can attribute some of the cause of the low offspring production to the low fertility rate in mutated male flies.

However, when comparing the mutated male offspring production (>100) compared to the screened cross (5-7 offspring), there is too big of a difference in the number of offspring to contribute the low offspring production to mutant male fertility alone. The fertility rate could also be very low for the females used in the screen. Another contributing factor could have been the food used for the crosses. The food used in the experiment contained a sugar and molasses mix, but eggs lay better in food that does not contain molasses. The rarity of finding a suppressor, or that that it is not possible to find a

suppressor of loss of function TPI might be other possibilities to explain why no suppressor phenotypes were found.

Future experiments will use much more expanded crosses, with more males and virgin females to make up for the low fertility rates. It will be necessary to use food that will help provide the best environment for the flies to lay their eggs in. Rescue mutation experiments will be more successful with higher amounts of offspring produced.

Finding second site modifiers that suppress lethality in a null TPI genotype will demonstrate that AGEs have a role in the neurodegenerative phenotypes of drosophila. This will suggest that the increased levels of AGEs might have a major role in neurodegenerative diseases such as Alzheimer's and Parkinson's.

## Credit

This experiment was done under the guidance of Professor Barry Ganetzky, Daniel Miller, and R. A. Kreber, Dept. of Genetics, UW Madison.

## References

1. **Gnerer, J. P., R. A. Kreber, and B. Ganetzky.** 2006. wasted away, a Drosophila mutation in triosephosphate isomerase, causes paralysis, neurodegeneration, and early death. Proc. Natl. Acad. Sci. U. S. A. **103:**14987-14993.

2. **Botella, J. A., J. K. Ulschmid, C. Gruenewald, C. Moehle, D. Kretzschmar, K. Becker, and S. Schneuwly.** 2004. The Drosophila carbonyl reductase sniffer prevents oxidative stress-induced neurodegeneration. Curr. Biol. **14:**782-786.

3. **Finley, K. D., P. T. Edeen, R. C. Cumming, M. D. Mardahl-Dumesnil, B. J. Taylor, M. H. Rodriguez, C. E. Hwang, M. Benedetti, and M. McKeown.** 2003. Blue Cheese Mutations Define a Novel, Conserved Gene Involved in Progressive Neural Degeneration. J. Neurosci. **23:**1254-1264.

4. **Min, K. T. and S. Benzer.** 1999. Preventing neurodegeneration in the Drosophila mutant bubblegum. Science **284:**1985-1988.

5. **Min, K. T. and S. Benzer.** 1997. Spongecake and eggroll: two hereditary diseases in Drosophila resemble patterns of human brain degeneration. Curr. Biol. **7:**885-888.

6. **Tschape, J. A., C. Hammerschmied, M. Muhlig-Versen, K. Athenstaedt, G. Daum, and D. Kretzschmar.** 2002. The neurodegeneration mutant lochrig interferes with cholesterol homeostasis and Appl processing. EMBO J. **21:**6367-6376.

7. **Kretzschmar, D., G. Hasan, S. Sharma, M. Heisenberg, and S. Benzer.** 1997. The swiss cheese mutant causes glial hyperwrapping and brain degeneration in Drosophila. J. Neurosci. **17:**7425-7432.

8. **Buchanan, R. L. and S. Benzer.** 1993. Defective glia in the Drosophila brain degeneration mutant drop-dead. Neuron **10:**839-850.

9. **Bilen, J. and N. M. Bonini.** 2005. Drosophila as a model for human neurodegenerative disease. Annu. Rev. Genet. **39:**153-171.

10. **Lo, T. W., M. E. Westwood, A. C. McLellan, T. Selwood, and P. J. Thornalley.** 1994. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. J. Biol. Chem. **269:**32299-32305.

11. **Kalapos, M. P.** 1999. Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicol. Lett. **110:**145-175.

12 . **Ramasamy, R., S. J. Vannucci, S. S. Yan, K. Herold, S. F. Yan, and A. M. Schmidt.** 2005. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology **15:**16R-28R.

13. **Ramasamy, R., S. F. Yan, and A. M. Schmidt.** 2006. Methylglyoxal comes of AGE. Cell **124:**258-260.

14. **SCHNEIDER, A. S., W. N. VALENTINE, M. HATTORI, and H. L. HEINS Jr.** 1965. Hereditary Hemolytic Anemia with Triosephosphate Isomerase Deficiency. N. Engl. J. Med. **272:**229-235.

15. **Schneider As, Valentine WN, Hattori M, Heins HI, Jr** (1965) N Engl J med 272:229- 235.

**Figures**

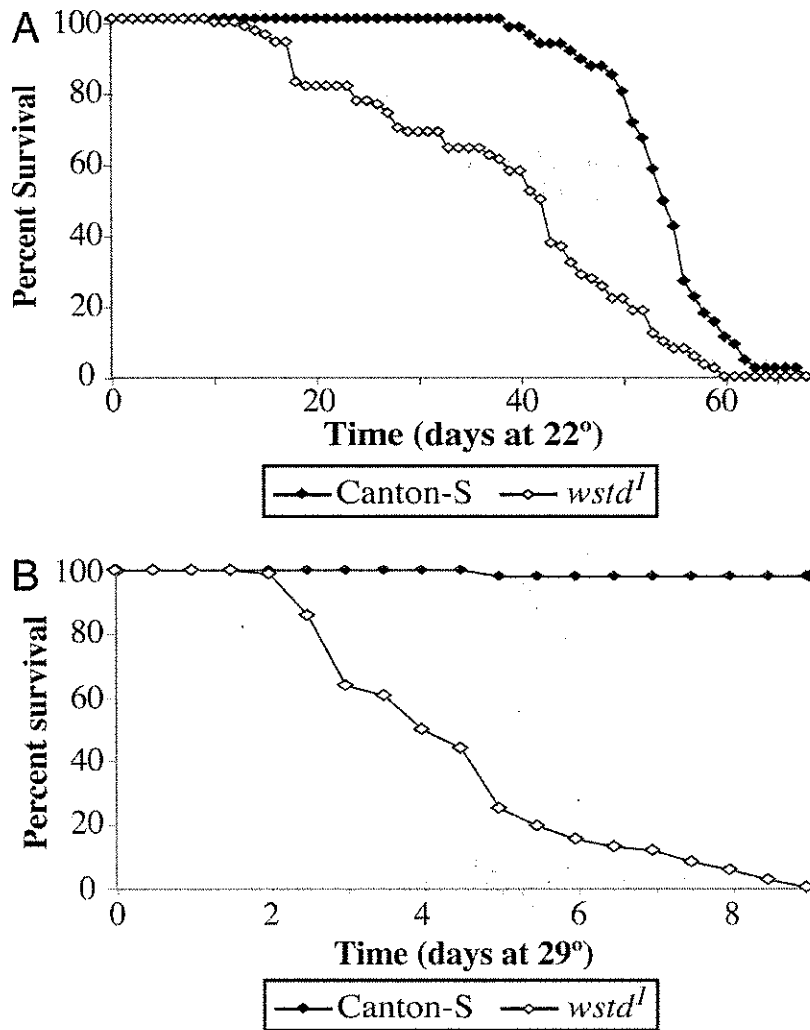


Figure 1(1): This figure shows how the *wstd* produces flies with a shorter lifespan than wild type flies. The mean of six samples is labeled as a point on the *wstd* graph. For

the Canton S, each point represents the mean of three samples. The (Graph A) flies were raised at 22 ° C. The (Graph B) flies were aged at 29° C. (1)

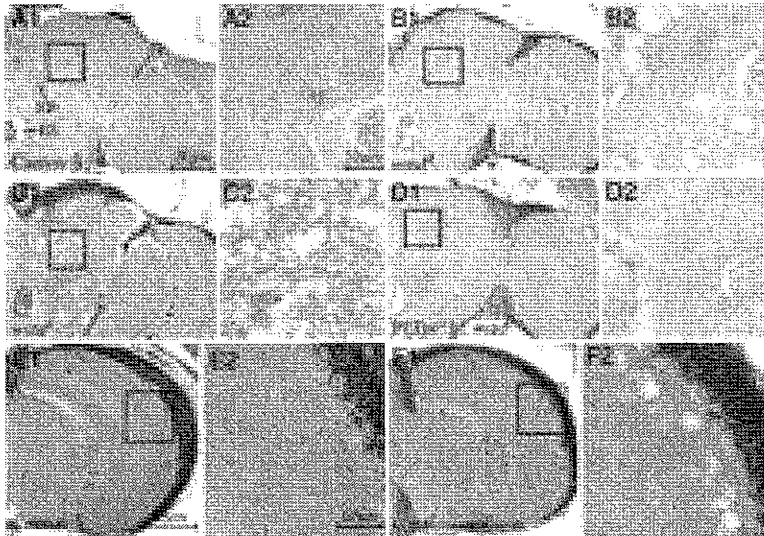

Figure 2(1): This figure shows how *wstd* results in neurodegeneration. A1, B1, C1, D1 are frontal sections at around the midbrain. A1 and A2 are Canton S. B1 and B2 are *wstd* and have vacuolar-like lesions (neurodegeneration). C1 and C2 are the extreme cases of neurodegeneration in a smaller portion of the *wstd* flies, giving a sponge like appearance. D1 and D2 are *wstd* flies that have a transgene of TPI, and those brain sections appeared the same as the wild type Canton S. brain sections. Flies used in these brain sections were aged for 1-4 days at 22° C, and then at 29° C for 3 days before they were sectioned (1).

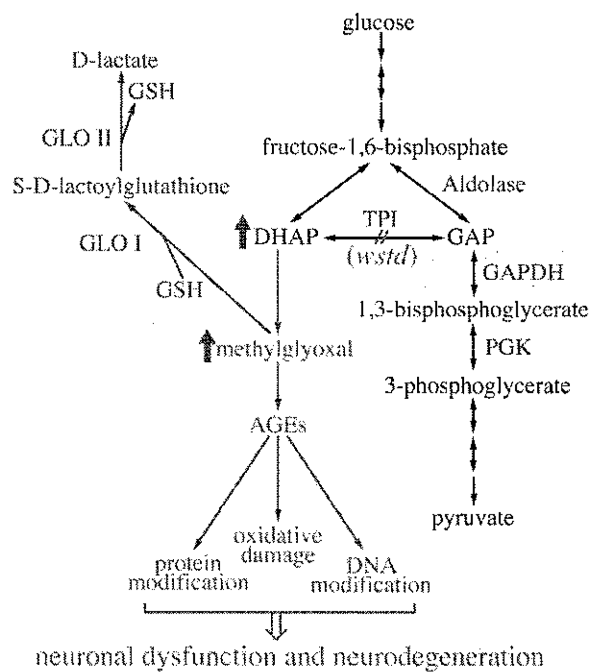


Figure 3(1): This figure shows the glycolytic pathway, and the how *wstd* leads to accumulation of DHAP. DHAP nonenzymatically converts to methylglyoxal, which then converts to AGEs. These AGEs cause oxidative damage, DNA modification, and protein modification. The single arrow heads move in one direction, while the double headed arrow can flow in either direction (1).