THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOLOGY

PHENOTYPIC VARIABILITY ASSOCIATED WITH THE 15q11.2 CNV REGION IN A *DROSOPHILA MELANOGASTER* MODEL FOR NEURODEVELOPMENTAL DISORDERS

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ABSTRACT

Discovering genes within copy-number variant (CNV) regions that contribute to neurodevelopmental disorders can help lead to mechanistic understanding of etiology that can lead to potential therapies. In order to determine the effects of altered expression of three target developmental genes within the specific 15q11.2 CNV region of *Drosophila melanogaster*, RNA interference was used to knockdown the genes, *CYFIP1*, *NIPA2*, and *TUBGCP5*. The resulting variable phenotypes were qualitatively and quantitatively measured. These three genes were determined to contribute to phenotypes that include eye roughness, increased lethality during development, and wing defects. An important finding is that knocking down one gene was sufficient to produce robust phenotypic variations. Thus, this experiment explored a one hit model of gene knockdown that demonstrated altered single gene expression within the 15q11.2 CNV region critically contributes to developmental outcome. Further research is needed to determine if interaction between these genes leads to a more severe phenotype.

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Chapter 1

Introduction

Neurodevelopmental disorders classify a broad range of conditions. For some neurodevelopmental disorders, like autism, the prevalence seems to be increasing.[4] This rise in autism has been contributed in part to increased awareness and broader diagnostic criteria.[4] With increased awareness and better diagnosis of neurodevelopmental disorders, like autism, it is important to also study the etiology of neurodevelopmental disorders. Many neurodevelopmental disorders have high comorbidity with other disorders, for example autism has a high comorbidity with intellectual disability.[4] With increasing prevalence and high comorbidity between neurodevelopmental disorders, it is important to understand the causes behind these wide ranges of phenotype variation. Genetic etiology of these neurodevelopmental diseases has shown that there are common molecular etiologies that cause neurodevelopmental disorders.[4] By studying the genetic causes of neurodevelopmental disorders, researchers can better understand how neurodevelopmental defects arise and how genetic interactions may produce variable phenotypes.

Copy-number variants (CNV) are deletions or duplications within sections of the genome.[3] CNVs effectively change the dosage of genes within a specific CNV region depending on whether it contains deletions of the genes or duplications of the genes.[3] In humans, the alteration of gene dosage from these CNVs are associated with a number of neurodevelopmental disorders including autism, intellectual disability, and schizophrenia.[3] While there are many identified rare CNV regions that have been associated with developmental disorders, the 15q11.2

region is particularly interesting. In human patients, the BP1-BP2 microdeletion in the 15q11.2 region has been associated with psychiatric and neurobehavioral issues and developmental and language delay.[2] While the occurrence of autism, seizures, and schizophrenia is low, the clinical features of patients has a wide range of phenotypic variability of intellectual disability.[2] Whereas not everyone with the microdeletion was affected, a majority of people had developmental and speech delays, writing and reading difficulties, memory problems, low IQ scores, and general behavioral problems.[2] The cause of variability of intellectual disability phenotypes associated with the 15q11.2 CNV region is thought to be related to gene dosage and possible gene x gene interaction. The 15q11.2 CNV region is located on chromosome 15 in humans and non-allelic homologous recombination during meiosis can produce deletions or duplication of gene segments.[2] Offspring can inherit affected chromosomes that contain such a deletion, which results in differential gene dosage.[2] Specifically for 15q11.2 region, deletions cause a decreased level of gene expression, which results in low levels of gene dosage that is reminiscent of being haploid at the locus.[2] The lack of diploid expression at the locus has been associated with intellectual disability.[2] Understanding how changes of gene dosage in the 15q11.2 region affect development will aid in identifying genetic etiology that produces intellectual disabilities in humans.

Neurodevelopmental disorders, like intellectual disability, have been studied in various models in order to better understand the genetic etiology. *Drosophila melanogaster* is an effective model to examine CNV regions in the genome of humans. The reason *Drosophila* is a great model system is because there is conserved homology of neurodevelopmental genes between human and flies.[3] Specifically, there is conservation of developmental processes and signaling pathways.[3] These homologous regions extend beyond the scope of neurodevelopmental

genes, since over 75% of human disease genes are homologous between humans and *Drosophila.*[3] While neurodevelopmental disorders, like intellectual disability, cannot be measured in flies the same way humans are diagnosed, examining the gene dosage effects on specific tissues and development in *Drosophila* provides insight into CNV gene dosage effects in humans. In addition, *Drosophila* have a short life span, short genome, and reproduce quickly. That along with the small space and limited resources *Drosophila* need to survive makes them a model organism. Using a model organism like *Drosophila* can help improve understanding of the molecular mechanisms underlying how these genes can cause neurodevelopmental defects. Furthermore, the applications of this research can help to identify potential targets for therapies.

In this study, we looked at the genes located within the 15q11.2 region to understand how they contribute to phenotypic variability in *Drosophila*. The human genes associated with this rare CNV region are *CYFIP1*, *NIPA2*, and *TUBGCP5*. We hypothesized that these genes in the 15q11.2 region will be associated with neurodevelopmental defects and we quantified the severity of these phenotypes for each gene. Using *Drosophila* as a model system, we evaluated the phenotypes associated with decreased dosage of these genes. In order to study the effects of these genes, we used RNA interference (RNAi) to knockdown the genes. RNAi is a method that utilizes small RNA molecules that recognizes specific messenger RNA sequences and destroys them. Therefore, RNAi is a way to silence a gene without editing the genome and removing that gene. In the fly model, RNAi is used to approximate the effects of CNV microdeletions in humans. In *Drosophila*, the *UAS-GAL4* system allows for RNAi knockdown of gene expression in specific tissues of interest.[3] Using the *UAS-GAL4* system to set up crosses simulates how one parent can pass on a CNV microdeletion, which leads to lower levels of expression of that gene in the offspring. To decrease the dosage of these genes, we used tissue-specific drivers that

include ubiquitous (*Da-GAL4*), pan neuronal (*Elav-GAL4*), eye (*GMR-GAL4*), and wing (*MS1096-GAL4*). Although, not every construct and tissue specific driver will achieve the same level of gene knockdown. For the eye tissue specific model, *GMR-GAL4* was used with and without *UAS-Dicer2*. Since *GMR-GAL4* is temperature dependent, the knockdown efficiency can be modulated by *UAS-Dicer2*.[5] Utilizing *UAS-Dicer2* creates varying doses of *GMR-GAL4* RNAi knockdown.[5] Previously we have used the fly eye as well as the wing to study genes associated with neurodevelopmental disorders. We have used the fly eye phenotype as a screening tool to identify genes of interest and we also used other tissues to look into different phenotypes that includes, overall gross defects, lethality, wing defects, and neuronal defects.

Chapter 2

Material and Methods

Fly Stocks & Rearing Conditions

The stocks used in this study were bought from Vienna *Drosophila* Stock Center (VDRC). The stocks used for studying the 15q11.2 region are listed in Table 1 below, organized by stock number, human gene, fly genotype, fly chromosome, and the corresponding fly gene.

Table 1: List of Genes in the 15q11.2 Region

Stock	***Human Gene***	***Genotype***	***Fly Chromosome***	***Fly Gene***
v34907	*CYFIP1*	w1118;; UAS-sra-1 RNAi[34907]	3	*Sra-1*
v34908	*CYFIP1*	w1118;; UAS-sra-1 RNAi[34908]	3	*Sra-1*
v29073	*TUBGCP5*	w1118; UAS-grip128 RNAi[29073]	2	*Grip128*
v29074	*TUBGCP5*	w1118; UAS-grip128 RNAi[29074]	2	*Grip128*
v110180	*NIPA2*	y,w1118; UAS-spict RNAi[110180]	2	*spict*

One region, 15p11.2, containing three human gene orthologs were tested. Across the three fly genes, five lines numbers were analyzed. Line numbers are assigned to the human and fly genes by VDRC and are indicated by the stock. Different lines can target the same gene by focusing on different areas of that same gene, as in the case of *TUBGCP5* and *CYFIP1*. The controls used for this experiment were GD and KK lines which are listed in Table 2. *NIPA2* is a KK RNAi line and so the corresponding control is the KK control. The two other genes, *CYFIP1* and *TUBGCP5*, are GD RNAi lines and thus use the GD control. All stocks and crosses were maintained with standard media of cornmeal, sucrose and dextrose with yeast at 25℃ or 30℃, as specified by the cross.[1]

Table 2: List of Controls

Stock	***Control***	***Genotype***
V60000	Control (GD)	w1118
V60100	Control (KK)	y,w1118

RNAi knockdown was achieved by using the *UAS-GAL4* system, which allows for tissue specific decrease of a target gene.[3] All the *GAL4* driver lines used in the crosses are listed in Table 3 below. The five lines were crossed individually with *GMR-GAL4,* which is a tissue specific driver for studying eye phenotype. For the eye phenotype, *GMR-GAL4* with and without *UAS-Dicer2* was used. *UAS-Dicer2* varies the knockdown efficiency of *GMR-Gal4*, thus creating two levels of gene expression for the eye model.[5] These crosses were incubated at 30°C. The five lines were also crossed individually with *Da-GAL4,* which is a ubiquitous driver; *Elav-GAL4,* which is a pan neuronal driver; and *MS1096-GAL4,* which is a wing driver. Except for the lethality assay, using the ubiquitous knockdown, which was incubated at 25°C, all of the crosses were incubated at 30°C.

Table 3: List of *GAL4* Driver Lines

Fly Gene	***Genotype***	***Tissue Specific Driver***
GMR-GAL4	w;dCad-GFP,GMR-GAL4/CyO	eye
GMR-GAL4 with UAS-Dicer2	w1118;GMR-GAL4;UAS-Dicer2	eye
Da-GAL4	w;da-GAL4;+	ubiquitous
Elav-GAL4	w;;elav-GAL4, UAS-Dicer2	pan neuronal
MS1096-GAL4	MS1096-GAL4/FM7c;;UAS-Dicer2/TM6B	wing

Eye and Wing Imaging

The adult progenies were collected from the crosses and isolated on day 0-1 and remained at 30℃ until day 2-5. The collected progenies were then frozen at -80℃ before being moved to -20℃ where they were stored until they were imaged under a light microscope. Approximately 20 female progenies were collected and imaged per line for both eye and wing phenotypes. For eye imaging, the frozen progenies were mounted on a slide covered in sticky tack, Blu-tac (Bostik Inc, Wauwatosa, WI, USA).[3] An Olympus BX53 compound microscope with LMPLan N 20X air objective using 477 a DP73 c-mount camera at 0.5X magnification (Olympus Corporation, Tokyo, Japan) and CellSens 478 Dimension software (Olympus Corporation, Tokyo, Japan) were used to produce the eye images.[3] The eye images were taken as 20 consecutive optical z-sections with a z-step size set to 12.1 1μm.[3] The wings were imaged by removing the wings from the frozen progeny, placing them on the slide, and securing coverslips in place with clear nail polish. Wing imaging utilized a light microscope, Zeiss Discovery 452 V20 stereoscope (Zeiss, Thornwood, NY, USA), with a ProgRes Speed XT Core 3 camera and 453 CapturePro v.2.8.8 software (Jenoptik AG, Jena, Germany) at 40X magnification.[3]

Lethality Assay

The lethality assay utilized ubiquitous knockdown RNAi. Any resulting lethality or defects were recorded for the developing progenies. The crosses from the five lines were examined for embryonic lethality, larval lethality, pupal lethality, wing defects, and overall gross defects. The embryonic, larval, and pupal lethality were measured by observing the development of the crosses. Embryonic lethality is indicated by the lack of development into larvae, larval

lethality is indicated by the death of larvae and lack of development into pupae, and pupal lethality were measured by death of pupae in the pupal cases and lack of development into adults. Any dead larvae and pupae were counted to measure the phenotype in addition to measuring the progression of each stage of development of the crosses. Wing defects were qualitatively measured in adult *Drosophila* along with any other gross defects.

Negative Geotaxis Assay

The negative geotaxis assay was conducted daily for ten days with ten progenies from each cross. The progenies were collected and isolated on days 0-1 and then the assay began on day 1-2. Progenies for the negative geotaxis assay were collected in the same manner as for eye and wing imaging. The progenies were first transferred to the experimental vials and allowed to recover for 60 seconds. After being tapped to the bottom of the vial, the progenies were recorded for ten trials to measure the number of progenies that climbed up past the eight-centimeter mark on the vials. The trials were consistently timed so that the number of flies that made it past the eight-centimeter mark after 10 seconds were recorded. The flies then had 60 seconds to recover before the next trial began.

Survival Assay

The final assay was a survival assay that measured the percentage of progenies that survive to 100 days. Survival assays were counted every 2-3 days and the percentage of progenies alive were recorded. The progenies were collected in a similar manner to the eye and wing imaging. Adults were collected and isolated on days 0-1 and then the assay was started on

day 1-2. For each cross, a total of 120 progenies were collected and measured for the survival assay. Due to subject loss during the transferring of flies into new vials, the data was based only on 100 subjects.

Qualitative Analysis

The lethality assay and wing phenotypes were visually analyzed. For the lethality assay, embryonic lethality, larval lethality, and pupal lethality were visually measured. Additionally, the presence of wing defects and overall gross defects were also visually assessed. For wing phenotypes, variation from common landmarks were classified as mild, moderate, or strong. For wings that displayed more than one phenotype, they were identified as severe. In addition, phenotypes were categorized by wrinkled texture (WR), ectopic veins (EV), missing veins (MV), bristle polarity (BR), and discoloration (DC).

Quantitative Analysis

The eye images were visually analyzed using the software *Flynotyper*. *Flynotyper* is an analysis tool that gathers quantitative measures about the severity of the phenotype from the images taken with the light microscope. Images taken of the eyes on the light microscope were stacked using Zerene Stacker software (Zerene Systems LLC, Richland, 480 WA, USA).[3] *Flynotyper* then measures the patterns and spacing of the ommatidia, the optical units of fly eyes, to score the severity of the phenotype from the stacked eye image.[1] There is normally a symmetrical, ordered hexagonal arrangement of the ommatidia in the eye.[1] In an eye that has phenotypic defects, there is an irregular hexagonal pattern of the ommatidia.[1] *Flynotyper*

measures the disorganization of the ommatidia and calculates a score that is a quantitative value reflection of the phenotype observed.[1] For the wing images, the analytic software Fiji ImageJ was used.[3] The Measure Area tool was used to calculate the wing area and the landmark veins, L2, L3, L4, L5, and anterior and posterior crossveins (ACV and PCV) were then manually measured using the Segmented Line tool.[3]

Chapter 3

Results

Robust Eye Phenotypes from Gene Knockdown within the 15q11.2 Region

For examining eye phenotype in progenies, the eye specific tissue driver, *GMR-GAL4*, was used in the crosses with the five experimental lines. The two control lines for imaging the eye phenotype in the progenies were GD and KK lines. In Figure 1, the eye image for each line and the controls illustrates the qualitative differences present in eye phenotypes. The more severe disruption of the eye phenotype is designated as a 'rough eye' phenotype. The rough eye phenotype is particularly exemplified in the *TUBGCP5* 29073 line in Figure 1.

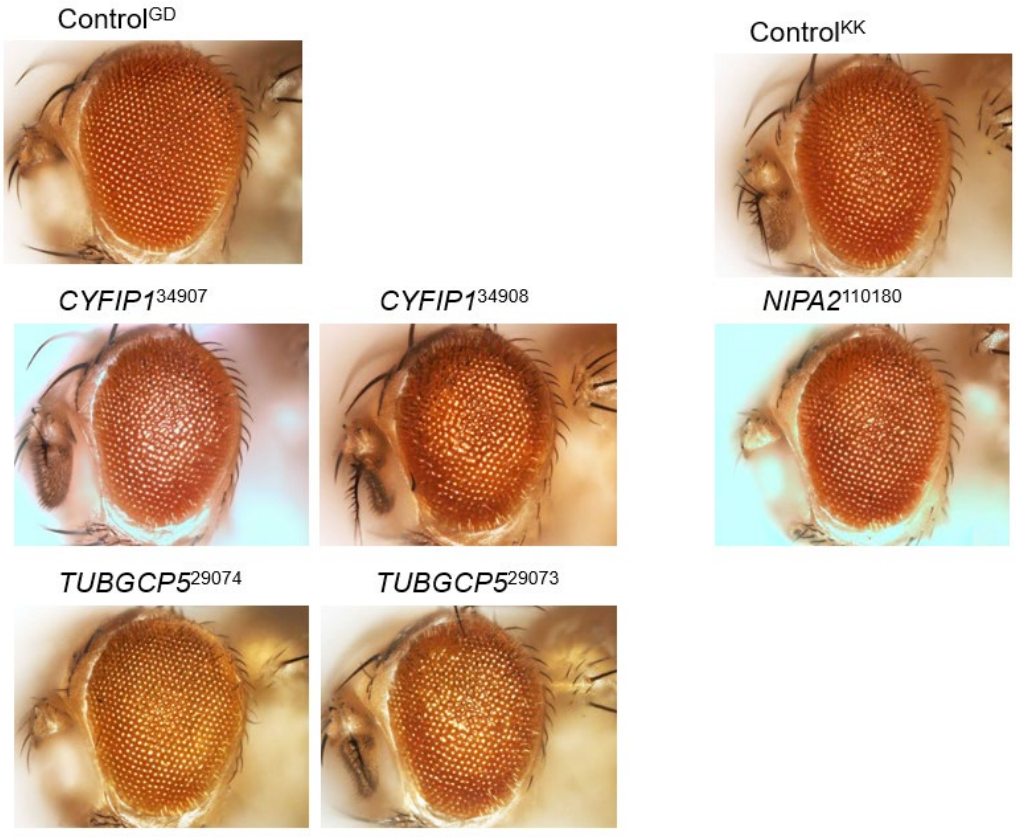

Figure 1: 15q11.2 Region Eye Phenotype *GMR-GAL4*

These images illustrate the phenotypic variability observed within the eye. The Control^{GD} had a normal, smooth eye where the ommatidia have a symmetrical, ordered hexagonal arrangement. The *TUBGCP5*^{29073} had irregular hexagonal pattern of ommatidia that is characteristic of a rough eye phenotype.

In a cross with *GMR-Gal4* with *UAS-Dicer2*, the progenies of the five experimental lines had their eye phenotype quantitatively measured by using *Flynotyper*. For all five experimental lines, *CYFIP1* 34907 and 34908, *TUBGCP5* 29073 and 29074, and *NIPA2* 110180, the average *Flynotyper* score was above both of the controls. (Figure 2). A higher *Flynotyper* score indicates a more severe eye phenotype because *Flynotyper* scores the hexagonal patterns of the ommatidia and the level of defective hexagonal arrangement in the eyes.[1] The disruption of the cells in the ommatidia indicates developmental issues caused by decreased dosage of certain genes.

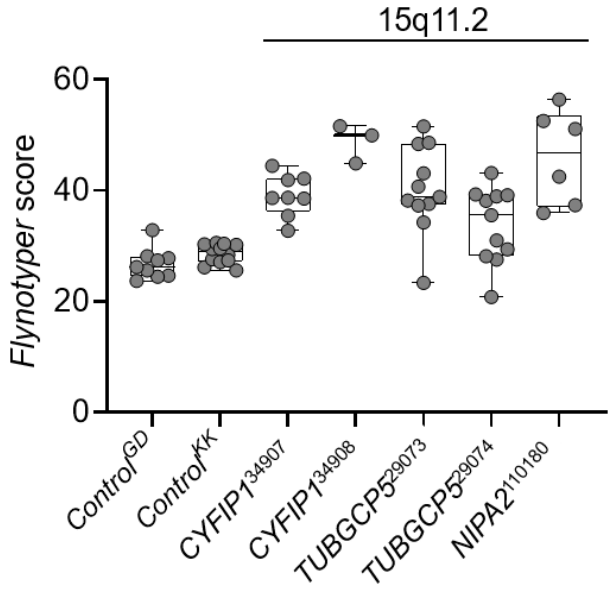

Figure 2: 15q11.2 Region *Flynotyper* Score *GMR-GAL4* with *UAS-Dicer2*

The *Flynotyper* score of Control^{KK} is significantly lower compared to the *Flynotyper* score of *NIPA2*^{110180}, since the error bars do not overlap. Compared to the Control^{GD}, only *CYFIP1*^{32908} has a significantly higher *Flynotyper* score. The three remaining lines have an average *Flynotyper* score higher than the Control^{GD}, but the error bars overlap, making them not significant.

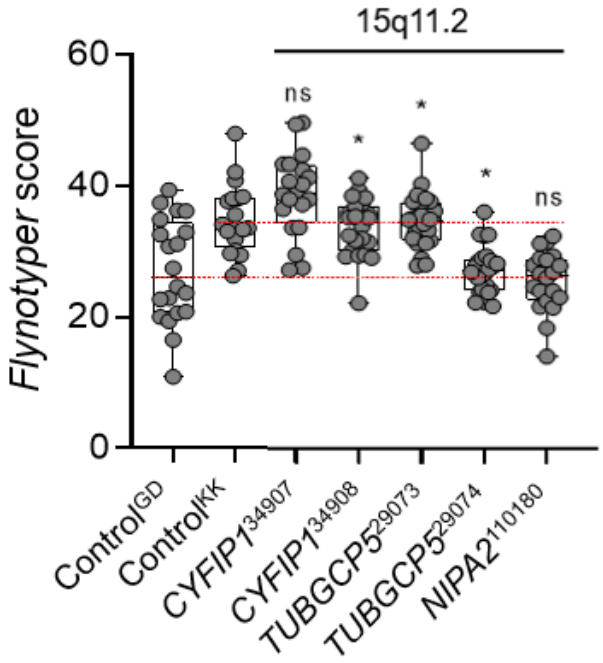

Figure 3: 15q11.2 Region *Flynotyper* Score *GMR-GAL4* without *UAS-Dicer2*

The stars above the plots indicate significance, ns above the plot indicates no significance, and the red line is the average *Flynotyper* score of the control. The *Flynotyper* score for *NIPA2*^{110180} is not significantly different than the Control^{KK}. However, the Control^{KK} has a higher *Flynotyper* score than *NIPA2*^{110180}. The *Flynotyper* scores for *CYFIP1*^{34908}, *TUBGCP5*^{29073} and *TUBGCP5*^{29074} are significantly higher than the Control^{GD}.

The *GMR-GAL4* driver without *UAS-Dicer2* was crossed with genes in regions associated of 15q11.2. For this experiment, five lines in the 15q11.2 region were specifically examined. The progenies from crosses with the *GMR-GAL4* driver and the five lines were imaged and their eye phenotype was scored in *Flynotyper*. In the 15q11.2 region of the *CYFIP1* line 34907, there was no significant difference in *Flynotyper* score compared to the GD control. In contrast, the *CYFIP1* line 34908 had a significantly higher *Flynotyper* score. Both of the *TUBGCP5* lines

29073 and 29074 had significantly higher *Flynotyper* scores than compared to the GD control. The *NIPA1* 110180 line was not significantly different than the KK control.

Lethality Assay Shows Phenotypes in Ubiquitous Gene Knockdown

For the lethality assay, the ubiquitous *Da-GAL4* driver was used to cross the five lines. The progenies of the five lines in the 15q11.2 region did not have any embryonic lethality or wing defects. The two lines for the *TUBGC5* gene and the one line for the *NIPA2* gene showed no gross defects. Both lines for the *CYFIP1* genes showed lethality, however the 34908 line had larval lethality and the 34907 line had pupal lethality (Figure 4).

Lethality Assay at 25 °C					
Gene	Embryonic lethality	Larval lethality	Pupal lethality	Wing defects	No gross defects
CYFIP1^{34907}			■		
CYFIP1^{34908}		■			
NIPA2^{110180}					■
TUBGCP5^{29073}					■
TUBGCP5^{29074}					■

Figure 4: 15q11.2 Region Lethality Assay

Larval lethality was observed for *CYFIP1*^{34908} and pupal lethality was observed for *CYFIP1*^{34907}. No gross defects were observed for *NIPA2* or *TUBGCP5*.

The lethality assay of both lines of *CYFIP1* indicate that this gene plays a crucial role in the development during the larval and pupal stages. Therefore, decreasing the dosage of the *CYFIP1* gene leads to a lethal phenotype.

Negative Geotaxis Phenotypes Show Pan Neuronal Knockdown Affects

The negative geotaxis assay tested the progenies from a pan neuronal driver *ELAV-GAL4* crossed with the *CYFIP1* line 34908, the *NIPA2* line 110180, and the *TUBGCP5* lines 29073 and 29074. The four lines and KK control showed the same general negative trend where a fewer percentage of progenies were crossing the eight-centimeter mark across the ten days. The *NIPA2* 110180 line significantly underperformed compared to the KK control. For both *TUBGCP5* lines, there was a lower percentage than the control on days 2-6 and 8-9. The *CYFIP1* line had a lower percentage compared to the control, but only day 9 was significantly lower (Figure 5).

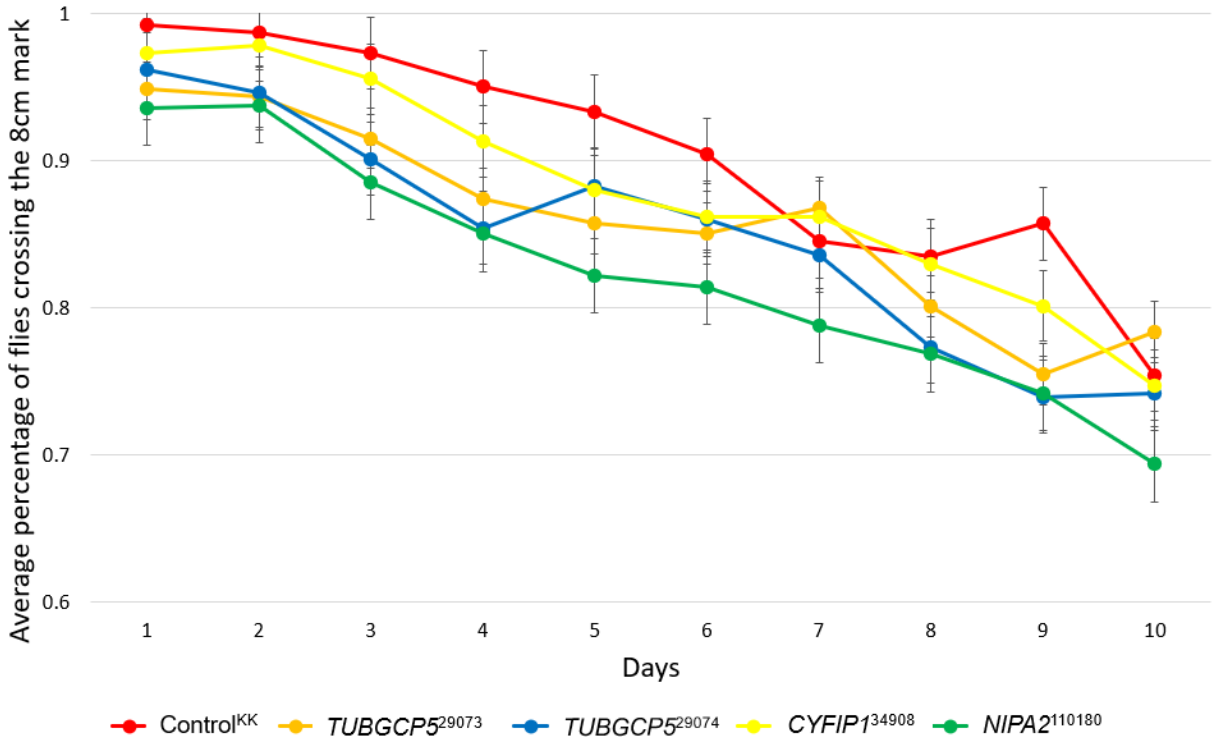

Figure 5: 15q11.2 Region Negative Geotaxis

The negative geotaxis assay produced significantly different trend lines for *NIPA2*^{110180} compared to the Control^{KK}. Both lines for TUBGCP and CYFIP1^{34908} showed significantly poorer performance compared to the control, but these were isolated occurrences across the 10 days.

Pan Neuronal Gene Knockdown Longevity Phenotypes in Survival Assay

The progenies used for the survival assay were from a pan neuronal tissue specific driver. The driver *Elav-GAL4* was crossed with four lines: *TUBGCP5* 29073 and 29074, *NIPA2* 110180, and *CYFIP1* 34908. After 40 days, survival differences in lines and the controls appeared. (Figure 6)

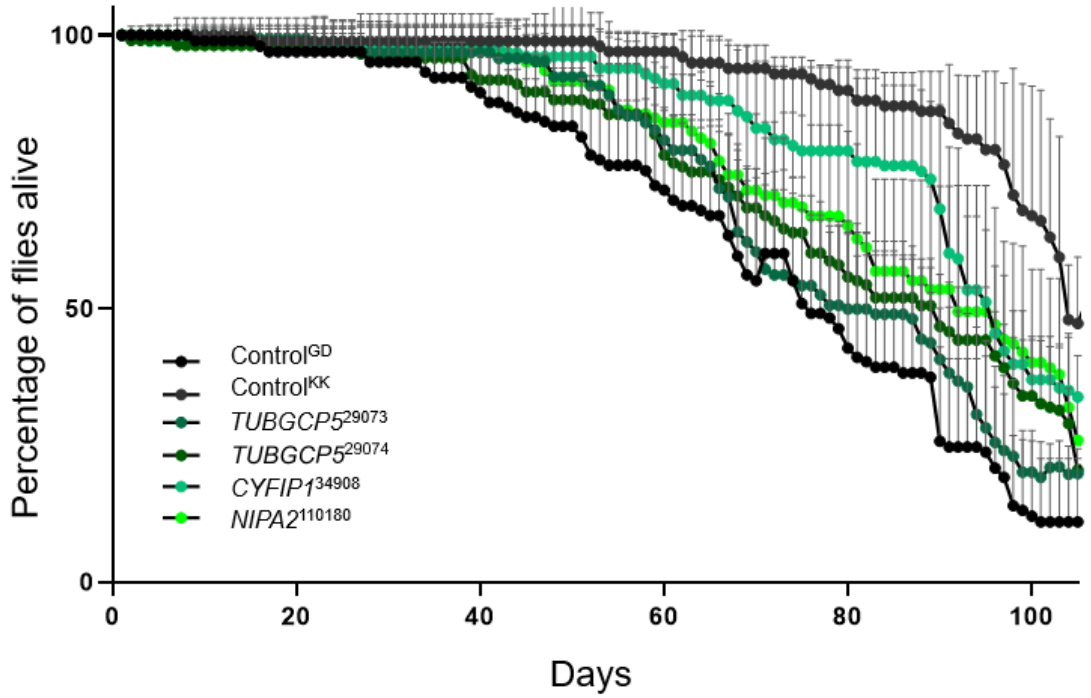

Figure 6: 15q11.2 Region Survival Assay

The top dark trend line is the Control^{KK} which corresponds to the experimental *NIPA2*^{110180}. *NIPA2*^{110180} had significantly lower percentage of flies alive compared to the control after ~70 days. The bottom dark trend line is the Control^{GD}. *CYFIP1*^{34908} had a significantly higher percentage of flies alive after ~55 days.

The progenies from both *TUBGCP5* lines 29073 and 29074 showed similar survival outcomes with the *NIPA2* 110180 line. The *CYFIP1* line 34908 had a higher survival rate compared to the GD control after approximately 55 days until around 90 days where the mortality steeply increased. The *NIPA2* line 110180 had a significantly smaller percentage of flies alive than the KK control after approximately 70 days.

Robust Wing Phenotype Gene Knockdown within the 15q11.2 Region

The female and male progenies from the crosses of wing tissue specific driver *MS1096* and the five lines *CYFIP1* 34908 and 34907, *NIPA2* 110180, and *TUBGCP5* 29074 and 29073 were qualitatively phenotyped for wing structure. There were differences in severity of phenotype between males and females for the *CYFIP1* gene in both lines. Males had stronger phenotypes of missing veins, discoloration, wrinkled texture, and ectopic veins. The *CYFIP1* lines both produced a stronger phenotype in males, except the 34907 line only caused moderate ectopic veins in males. The *CYFIP1* genes caused a mild missing vein phenotype in females, but had a strong missing vein phenotype in males. There was a difference in females between the two *CYFIP1* lines. The *CYFIP1* 34907 line caused a more moderate phenotype for discoloration, wrinkled texture, and ectopic veins in females. The *CYFIP1* 34908 line only caused moderate discoloration. The *NIPA2* and *TUBGCP5* genes and lines show similar mild phenotypes in both males and females. (Figure 7).

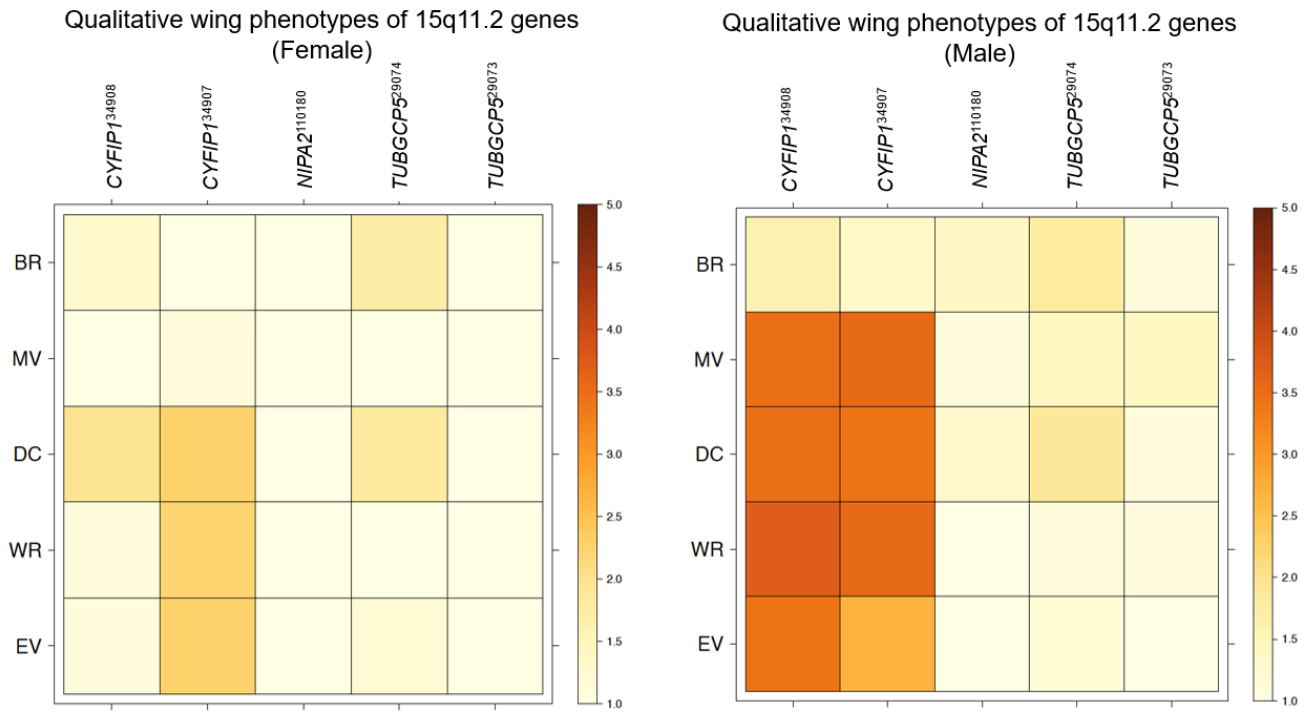

Figure 7: 15q11.2 Region Wing Phenotype

The abbreviations of the wing phenotypes are as follows: BR= bristle polarity, MV= missing veins, DC= discoloration, WR=wrinkled texture, and EV= ectopic veins. In females, *CYFIP1*^{34907} produced a medium level of discoloration, wrinkled texture, and ectopic veins. *CYFIP1*^{34908} produced mild discoloration and *TUBGCP5*^{29074} produced mild discoloration and bristle polarity in females. In males, both *CYFIP1*^{34908} and *CYFIP1*^{34907} produced a high level of missing veins, discoloration, and wrinkled texture. *CYFIP1*^{34908} also produced a high level of ectopic veins, but *CYFIP1*^{34907} only produced a medium level of ectopic veins. In males, *TUBGCP5*^{29074} also produced a mild level of discoloration and bristle polarity.

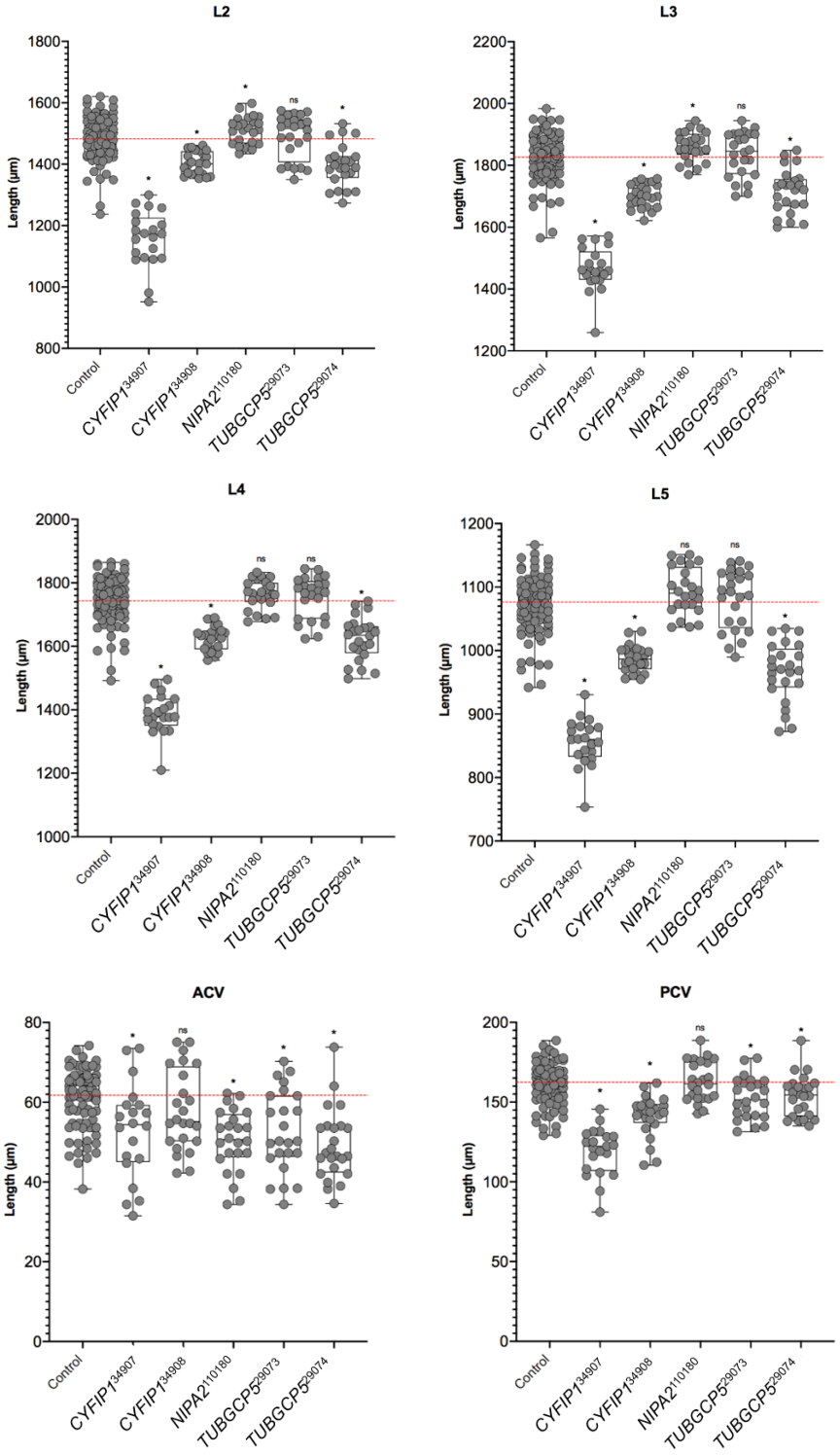

Figure 8: 15q11.2 Region Female Veins Wing Phenotype

The stars above the plots indicate significance, ns above the plot indicates no significance, and the red line is the average vein length of the control. For the L2 and L3 veins, *CYFIP1* both lines and *TUBGCP5*^{29074} had significantly shorter lengths than the control, but only *NIPA2*^{110180} was significantly longer than the control. For L4 and L5 veins, *CYFIP1* both lines and *TUBGCP5*^{29074} had significantly shorter lengths than the control. For the ACV vein, all lines except *CYFIP1*^{34908} were significantly shorter than the control. For the PCV vein, both lines of *CYFIP1* and *TUBGCP5* were shorter than the control.

The measurements of six landmark veins were collected from the female progenies of crosses with all five lines and *MS1096*. These measurements were compared to the control progenies. The *CYFIP1* line 34907 had significantly shorter veins for all six of the landmark veins, while the other *CYFIP1* line 34908 only had significantly shorter L2, L3, L4, L5, and PCV veins. For *CYFIP1* line 34908, the ACV vein was similar to the control. For the *NIPA2* 110180 line, it was significantly longer than the control for the L2 and L3 veins, but significantly shorter than the control for the ACV vein. The *TUBGCP5* line 29073 was only significantly shorter than the control for the ACV vein and the PCV vein. Yet, the *TUBGCP5* line 29074 was significantly shorter than the control for all six of the veins. (Figure 8). The variation in significance between the *TUBGCP5* lines indicates different targets within the gene are affected by the decreased dosage of parts of that gene.

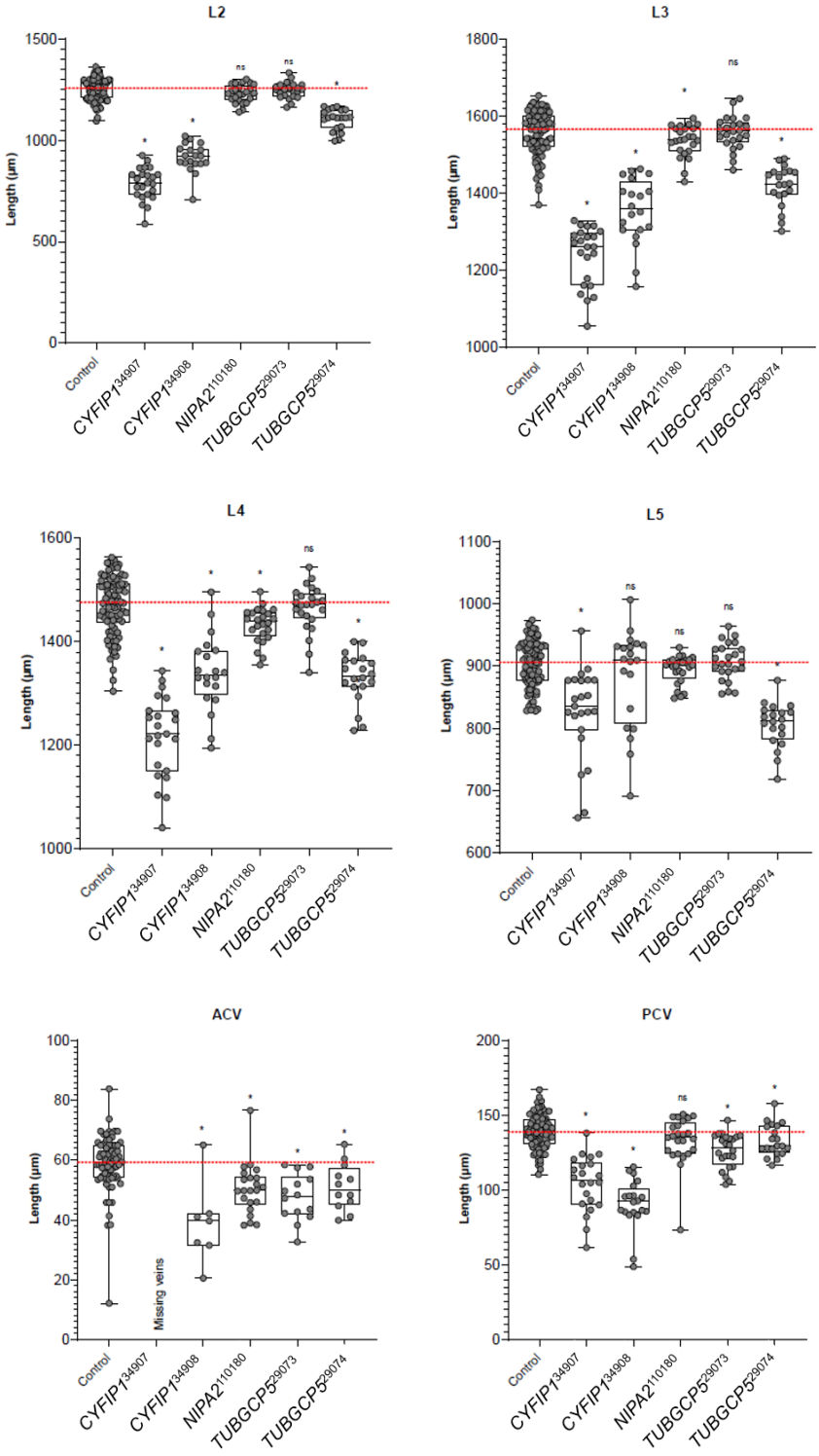

Figure 9: 15q11.2 Region Male Veins Wing Phenotype

The stars above the plots indicate significance, ns above the plot indicates no significance, and the red line is the average vein length of the control. For L2 and L3 veins, both lines of *CYFIP1* and *TUBGCP5*^{29074} were significantly shorter than the control, but in L3 veins *NIPA*^{110180} is also significantly shorter. In L4 veins, all lines but *TUBGCP5*^{29073} were significantly shorter than the control. For L5 veins, only *CYFIP1*^{34907} and *TUBGCP5*^{29074} were significantly shorter than the control. For ACV veins, all lines were significantly shorter and *CYFIP1*^{34907} was missing ACV veins. In PCV veins, all lines except *NIPA2*^{110180} were significantly shorter than the control.

Similar to the measuring of six landmark veins (L2, L3, L4, L5, ACV and PCV) in the female progeny of the five lines and *MS1096*, male progenies were also measured and compared to the control. For both *CYFIP1* lines, 34907 and 34908, there was a significant decrease in the length of all six landmark veins. However, for the *CYFIP1* line 34907, the ACV veins were missing and thus could not be measured and quantitively compared to the control. The *NIPA2* 110180 line was only significantly shorter than the control veins for the L3, L4, and ACV veins. There were differences between the *TUBGCP5* lines as well as differences when compared to the control. The *TUBGCP5* line 29073 was only significantly shorter than the control for the ACV and PCV veins. Yet, the other *TUBGCP5* line 29074 was significantly shorter for all six landmark veins when compared to the control. (Figure 9).

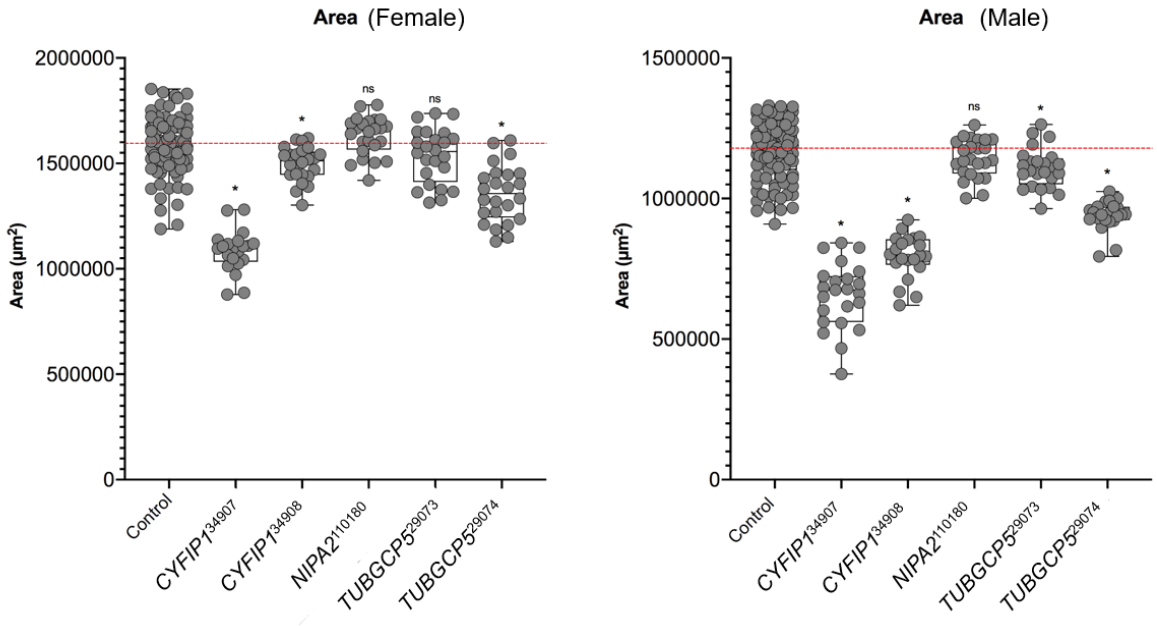

Figure 10: 15q11.2 Region Wing Areas Wing Phenotype

The stars above the plots indicate significance, ns above the plot indicates no significance, and the red line is the average area of the control. For females, both lines of *CYFIP1* and *TUBGCP5*^{29074} had significantly smaller wing area than the control average. For males, every line except *NIPA2*^{110180} had significantly smaller wing area compared to the control average.

Another element of wing phenotype is the area of the wing. To measure the effects of decrease dosage of genes from RNAi knockdown of expression, the total area of the wings in the progenies from the crosses with *MS1096-GAL4* were measured. The area of the wings was measured for both female and male progenies. Both of the *CYFIP1* lines, 34907 and 34908, were significantly smaller than the average wing area of the control progenies for both male and female progenies. There also was a difference in the severity of decreased wing area between females and males. The average area of male wings was lower than that of females. The *NIPA2* 110180 line was not significantly different from the area of the control for either males or females. For the *TUBGCP5* line 29073, there was a significant decrease in the area of male wings, but the average area of female wings was not significantly lower than that of the control.

The remaining *TUBGCP5* line 29074 was significantly smaller than the control in both female and male progenies. (Figure 10).

qPCR Confirmation of Gene Knockdown within the 15q11.2 Region

Several genes have been confirmed for reduce expression using quantitative PCR (qPCR).[5,6] *NIPA2* had a knockdown percent of 24.39% and *CYFIP1* had a knockdown percentage of 55.94% with both genes having significant results. Multiple lines were tested for each knockdown and concordance of results would suggest gene knockdown.

Chapter 4

Discussion and Future Experiments

The experiments with tissue specific knockdown in expression for the five lines in the 15q11.2 region helps provide insight in the influence these genes have in normal development. While previous studies have examined how these genes play a role in neuron development and are associated with neurodevelopmental disorders, understanding how these genes affect other tissue development can help understand the broader effects these genes might have in general development. For eye tissue development, the genes *TUBGCP5* and *CYFIP1* showed a significant phenotype. Specifically, the *CYFIP1* line 34908 and both *TUBGCP5* lines 29074 and 29073 had a significant *Flynotyper* score. *Flynotyper* measures the severity of disruption of the ommatidia in eye, so high a *Flynotyper* score mean there is a more severe phenotype and defect.[1] To examine if any of these five genes were lethal, a ubiquitous driver was used and both lines of *CYFIP1* showed lethality. However, the two lines of the *CYFIP1* gene were lethal in different developmental stages. The *CYFIP1* line 34907 was lethal for pupae, but the *CYFIP1* line 34908 was lethal for larvae. The results for the pan neuronal driver in the negative geotaxis and survival experiments were discordant and had fewer significant results than the other experiments. However, *NIPA2* gene showed significant decrease compared to the control for the negative geotaxis and survival experiments. Lastly, the experiments on the effects of wing phenotype showed some interesting differences between males and females. The *CYFIP1* line 34907 showed some wing phenotype in females, but both *CYFIP1* lines showed a more severe phenotype in males. There were differences as well between the five lines and different genes when looking between males and females for wing phenotype of veins and area. The qPCR results confirmed reduce expression and the concordance of results would suggest gene

knockdown. Therefore, the RNAi model in flies produced a similar level of low gene dosage to that of microdeletions in humans within the 15q11.2 CNV region.

Future experiments are needed to examine whether there are any gene x gene interaction between any of the lines of *TUBGCP5*, *CYFIP1*, and *NIPA2* or between the genes themselves. Understanding any potential interactions these genes may have upon each other would help better model and predict phenotypic variability. Furthermore, creating a two-hit model of gene expression knockdown could further examine differences seen between lines with significant results from the one-hit model, like for *TUBGCP5* and *CYFIP1* lines in the eye phenotype, lethality, and wing phenotype experiments. In addition, further research into the apparent differences in wing phenotype severity for different *CYFIP1* lines might help in potentially understanding possible molecular mechanisms behind the sex bias observed in these experiments. Since sex bias is prominent in human neurodevelopmental disorders, like autism, continuing research on *Drosophila* might lead to an understanding of why there are stark differences in phenotypic variability between males and females. These future experiments can help further examine phenotypic variability in genes in the 15q11.2 region associated with neurodevelopmental disorders. This research can lead to findings that focus therapies to specific developmental genes.

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EDUCATION

The Pennsylvania State University | Schreyer Honors College
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LEADERSHIP & ACTIVITIES

Vaccinate America **University Park, PA**
Founding Secretary and Vice President 2017 – 2020
- Promoted education of vaccinations in local communities
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Remote Area Medical **University Park, PA**
Member and Volunteer 2019 – 2020
- Volunteered on a RAM Clinic trip to Ashtabula, OH in April 2019

Science LionPride **University Park, PA**
Member and Mentor 2017 – 2020
- Acted as an Ambassador for the Eberly College of Science and gave tours to prospective students
- Engaged in philanthropy to support THON which supports families of children with pediatric cancer
- Mentored a junior member of the organization

Schreyer Honors College Orientation **University Park, PA**
Mentor 2017
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Global Brigades Medical Chapter **University Park, PA**
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RESEARCH EXPERIENCE

Girirajan Laboratory at Pennsylvania State University
Undergraduate Research Assistant 2018 – 2020
- Set up genetic crosses, phenotyped progeny, and maintained drosophila stocks
- Ran survival & negative geotaxis assays and imaged eyes

Publications: *Manuscript in preparation*: Tanzeen Yusuff*, Matthew Jensen*, Sneha Yennawar*, Lucilla Pizzo, Siddharth Karthikeyan, Dagny J. Gould, Avik Sarker, Yurika Matsui, Janani Iyer, Zhi-Chun Lai, and Santhosh Girirajan. High-throughput analysis of human copy-number variant genes shows global and non-neuronal developmental defects in Drosophila melagnogaster.

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VOLUNTEERING

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AWARDS

Phi Beta Kappa (Honors Society) | *2019*
University Nominated Candidate for the Marshall Scholarship – 2019
Dean's List | *Fall 2016, 2017, 2018, 2019 and Spring 2017, 2018*
The Schreyer Honors College – Academic Excellence Scholarship
Schreyer Honors College Grant – recipient for semester exchange in Germany 2018 and for Fieldwork in Tanzania 2019
Eberly College of Science Global Experience Scholarship – recipient for Fieldwork in Tanzania 2019
Penn State University Student Engagement Network Grant – recipient for Fieldwork in Tanzania 2019
Phi Eta Sigma (Freshman Honor Society) | *2017*