THE PENNSYLVANIA STATE UNIVERSITY
MILLENNIUM SCHOLARS PROGRAM

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

USING WING-SPECIFIC DRIVERS TO ASSESS THE IMPORTANCE OF CNV'S ON TISSUE DEVELOPMENT

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SPRING 2018

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry and Molecular Biology

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ABSTRACT

Genes that have been established as important for nervous tissue development could have further implications in general tissue development. In order to assess this possibility among gene regions with established relationships to neurodevelopmental disorders, this study sought to test individual genes from these regions within the wing pouch tissue of *Drosophila melanogaster.* As part of this high throughput study, 10 such regions encompassing 58 genes were analyzed, and lines of the genes totaled 117. While many tests assaying the success and efficiency of the RNAi knockdown should be conducted on these lines, the results of this study indicate that there are 16 genes that definitely contribute to the development of non-nervous tissue. This number likely would increase once qPCR would reveal the knockdown success (or lack thereof) in genes with discordant lines.

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ACKNOWLEDGEMENTS

Several people were instrumental in encouraging me to complete this body of work. My parents, Hemant and Neela Yennawar, for their knowledgable guidance, sister Madhumita Yennawar for her understanding and support, and my dearest friends for keeping my spirits high.

I am also extremely grateful for the patience support of my advisor Santhosh Girirajan and reviewer Scott Selleck, and for all the resources provided by the Millennium Scholars Program.

Chapter 1

Introduction

In the field of neuroscience, the genetic etiology neurodevelopmental disorders have been the subject of many studies. Large regional deletions or duplications encompassing multiple genes, or copy number variations (CNVs), have been implicated in the onset and severity of such disorders, namely autism spectrum disorders (ASDs), epilepsy, and schizophrenia. A statistically significant increase of CNVs is seen in diseased patients as compared to healthy individuals but targeting specific CNVs in order to study them and observe their effects is made difficult by the phenotypic heterogeneity characteristic of genomic disorders.

In this study, we have conducted a high-throughput study of several regional deletions implicated in neurodevelopmental disorders. The deletions were simulated using RNA interference, which reduce the levels of mRNA of the targeted gene, specifically within the wing-pouch region of *Drosophila melanogaster* in order to isolate candidate genes that may be influencing tissue development besides that of the nervous tissue. *Drosophila melanogaster*, commonly known as the fruit fly, has emerged as an efficient model to study such neurodevelopmental defects, as their genome possess representative orthologs of over 75% of human disease-causing genes (Pandey et al.).

Chapter 2

Materials and Methods

Fly stocks & Rearing conditions

All stocks used in this study were obtained from Vienna Drosophila Stock Center (VDRC), with the exception of the overexpression lines as well as the MS1096 driver stock, both of which were obtained from Dr. Zhichun Lai's lab. The stocks from used from VDRC are indicated below, categorized by region of genome they belong to, or by the molecular pathway they encode proteins for. As part of this high throughput study, 10 regions containing 58 genes were tested. Across the 58 genes, 117 lines were subjected to analysis. Line numbers are assigned arbitrarily by VDRC, so the human and fly genes being targeted are indicated as well in the following lists. Different lines targeting the same gene target different areas of the given gene. The control used for this experiment was a stock called w[VDRC].

Table 1: Stocks used from selection of Core Genes

Stock	***Human Gene***	***Fly Gene***
10328	*CHRNA7*	*nAcRα-34E*
10330		
101820		
39411		
104775	*SCN1A*	*para*
6131		
6132/TM3		
6132/Tm6b		
100130	*UBE3A*	*Ube3a*
45876		
45875		
103592	*SHANK3*	*Prosap*
21218/TM3		

101554	*SUCLG2*	*Sucb*
106638	*SLC6A1*	*Gat*
109414	*CHD8*	*Kis*
50200	*FOXP1*	*FoxP*
25291	*LRRN2*	*CAPS*
25292		
104393	*TBX1*	*org-1*

Table 2: Stocks used from Microcephaly Genes

Stock	***Human Gene***	***Fly Gene***
14194	*CEP135*	*Cep135*
14195		
106051	*CENPJ*	*Sas-4*
17975		
108586	*TUBGCP6*	*Dgrip163*
27482		
6005	*SLC25A19*	*Tpc1*
21066	*MCPH1*	*MCPH1*
28098		
2910	*ASPM*	*Asp*
2911		
52548	*KIF11*	*Klp61F*
52549		
108279	*RRBP1*	*alt*
49919		

Table 3: Stocks used from the ß-Catenin pathway

Stock	***Human Gene***	***Fly Gene***
6545	*Eph1*	*Eph*
27236		
4771		
105360	*LGR5*	*rk*
904		
4735		
29931		
29932		
107344	*CTNNB1*	*arm*
7767		
36328	*NRXN1*	*Nrx-1*
36326		
4306		
35731	*PTEN*	*Pten*

101475		

Table 4: Stocks used from CNV region 15q13.3

Stock	Human Gene	Fly Gene
17576	*MTMR10*	*CG14411*
17579		
33670	*TRPM1*	*Trpm*
30609		
30610		
33669		

Table 5: Stocks used from CNV region 1q21.1

Stock	***Human Gene***	***Fly Gene***
101452	*FMO5*	*Fmo-2*
42829		
42830		
105874	*PRKAB2*	*alc*
5694	*BCL9*	*lgs*

Table 6: Stocks used from CNV region 15q11.2

Stock	***Human Gene***	***Fly Gene***
29073	*TUBGCP5*	*Grip128*
29074		
34907	*CYFIP1*	*Sra-1*
34908		
110180	*NIPA2*	*spict*
106123	*HEC2P2*	*CG7420*
46316		

Table 7: Stocks used from CNV region 16p13.1

Stock	***Human Gene***	***Fly Gene***
100713	*NDE1*	*nudE*
29788		
105419	*ABCC6*	*MRP*
106964	*KIAA0430*	*CG17018*
32810		

Table 8: Stocks used from CNV region 3q29

Stock	***Human Gene***	***Fly Gene***
106488	*ZDHHC19*	*App*
108502	*SLC51A*	*CG6836*
100575	*PCYT1A*	*Cct1*
18628		
108037	*LRRC33 //NRROS*	*CG7869*
907		
36340		
36343		
837		*CG5819*
836		
7997		
27076		
100110		*atk*
31044		
101805	*PIGX*	*PIG-X*
49267		
7362		
108937	*PAK2*	*Pak*
107112	*NCBP2*	*Cbp20*
50433		
106747	*PIGZ*	*PIG-Z*
5236	*MELTF*	*Tsf2*
30337	*BDH1*	*CG8888*
30336		
107115	*TCTEX1D2*	*CG7276*
104357		*CG5359*
21565		
107315	*FBX045*	*Fsn*
26577		
41136	*DLG1*	*Dlg1*
109274		

Table 9: Stocks used fromt the CNV region 16p11.2 distal

Stock	***Human Gene***	***Fly Gene***
101326	*CCDC101*	*Sgf29*
41739		
103646	*SH2B1*	*Lnk*
32892		
34956	*ATXN2L*	*Atx2*
108065	*TUFM*	*EfTuM*
48981		
48982		
107617	*RABEP2*	*CG34030*
10562	*SPNS1*	*Spin*
3229		
46030		

Table 10: Stocks used from CNV region 16p12.1

Stock	***Human Gene***	***Fly Gene***
100818	*UQCRC2*	*UQCR-C2*
26404		
26405		
51696	*POLR3E*	*Sin*
52094		
108941		
33444	*CDR1*	*Cen*
5370	*CG14182*	*C16orf52*
5371		

These 117 lines (the UAS construct lines) were crossed individually with Gal4 construct flies. In this experiment, the MS1096 driver contained the Gal4 construct, meaning that the Gal-4 was designed to be coexpressed with MS1096, a gene expressed in the wing-pouch. Therefore, when crossed with the UAS lines, the Gal-4 prompted the UAS construct in the designated line to promote the transcription of a particular inverted repeat, creating a double-stranded RNA. This molecule activates an RNAse molecule that targets the specific mRNA transcribed from the gene

of interest. leading to the RNA interference of the desired gene occurred only in the wing pouch (due to the coexpression with MS1096), in order to simulate a functional knockdown of the gene in this tissue. It is also important to note that there are several issues associated with the RNAi mechanism. Not all constructs have the same amount of knockdown activity, and in addition, some can have off-target effects. Thus, more tests are warranted before making definitive conclusions about these genes; studies with RNAi simply give potential guidance about which genes could be important for further study. This being said, while positive results from RNAi - such as concordant developmental phenotypes resulting from these functional knockdowns among several lines - do prove a gene-product's involvement in development, the alternate is not true. It is not possible to conclude the lack of involvement of a gene in wing development due to a lack of observed phenotype when knocked-down using RNAi.

Crosses were incubated at 25°C. Adult progeny were isolated on day 0-1, but remained at 25°C until day 2-5, at which point they were frozen at -80°C. From here, they were phenotyped and analyzed. Samples in the freezer for longer than one month were moved to -20°C. Approximately 20-25 female *Drosophila melanogaster* wings were mounted, imaged, and analyzed per line.

Imaging

Female wings were analyzed after being imaged under a light microscope. All images were taken at 40X. Slides were prepared by plucking the wings from the frozen progeny and aligning them on the slide. Cover slips were sealed onto the slide using clear nail polish.

Qualitative Analysis

The wings were also visually analyzed. Common landmark changes were categorized and noted. These included changes to the vein structure and/or to the wing texture. The phenotypes were visually categorized (Figure 1). Wrinkled texture in the wings was attributed to improper tissue development as a result of the knockdown. Wings that showed more than one phenotype among these categories were automatically considered 'severe'.

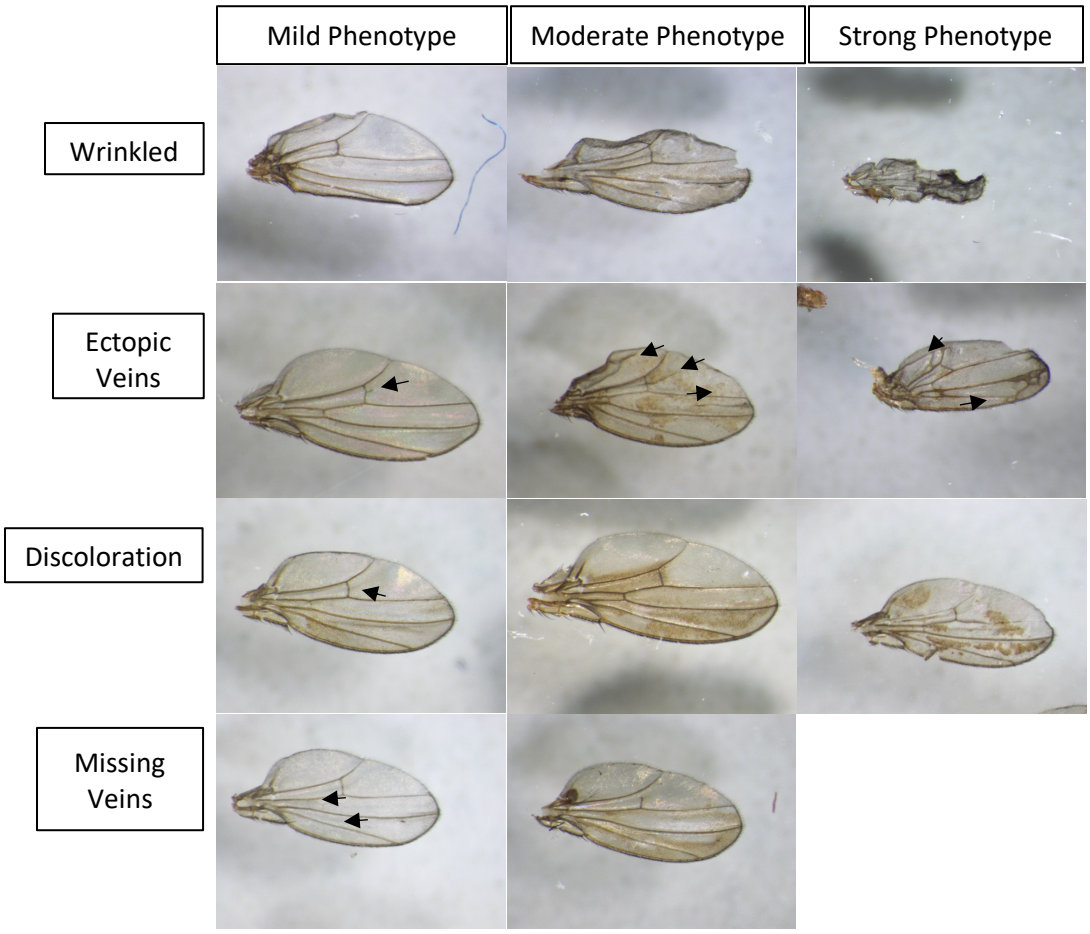

Figure 1: Categorization of Phenotypes

Quantitative Analysis

The images were processed using image analysis software ImageJ. A ruler was used to set the image ratio at the 40X scale to 0.785 pixels to 1 mm, so measurements are recorded in millimeters. The L2, L3, L4, and L5 veins of each wing were then manually measured using the segmented-line tool measured to be used as an indication of the size of the wing (Figure 2). The wing in Figure 2 is also a representative image of a control wing for this experiment.

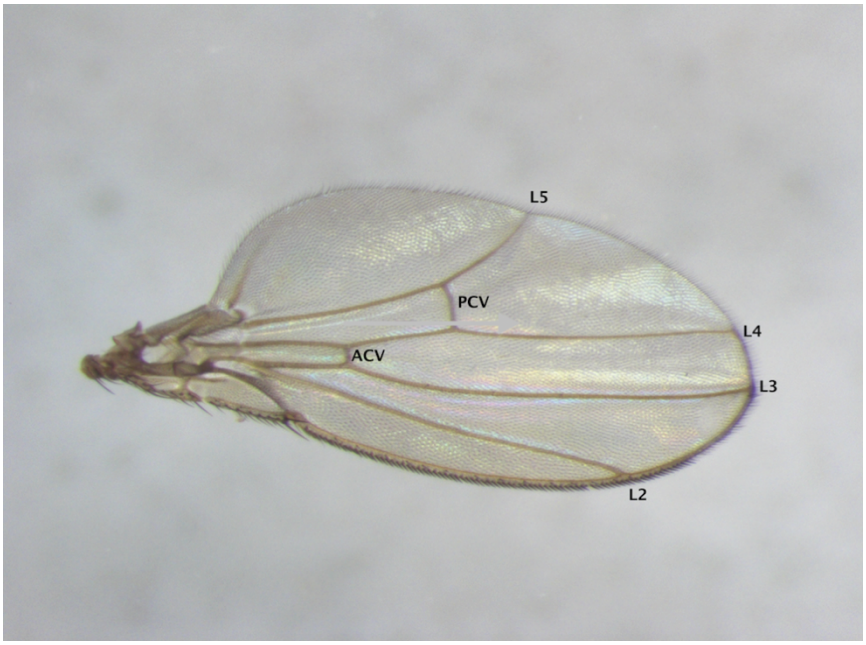

Figure 2: Diagram of control wing, with landmark veins labeled.

Chapter 3

Results

Control

The control line (w[VDRC]) was first subject to analyses in order to establish what the phenotype of a normal wing was. Quantitative measurements were automatically included in each measurement graph, and the labeled wing diagram above is a representative image of the control wings (Figure 2). There was a baseline phenotype of ectopic veins present in the control wings (2 wings out of 91), so a low level of mild ectopic veins observed in other lines were considered normal (Figure 3).

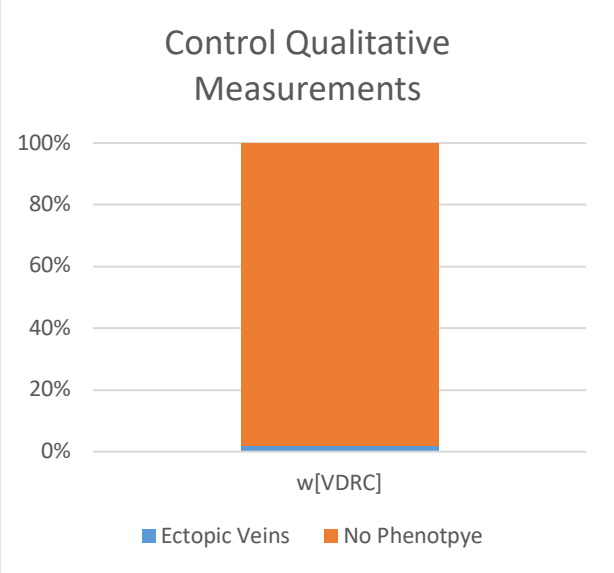

Figure 3: Control Qualitative Observations

Core Genes

The genes studied that were categorized as core genes were studied due to their known involvement in neurodevelopmental disorders. Testing the phenotype with knockdowns of these genes in the wing pouch was intended to elucidate the importance of these genes in development of tissues that were purely nervous

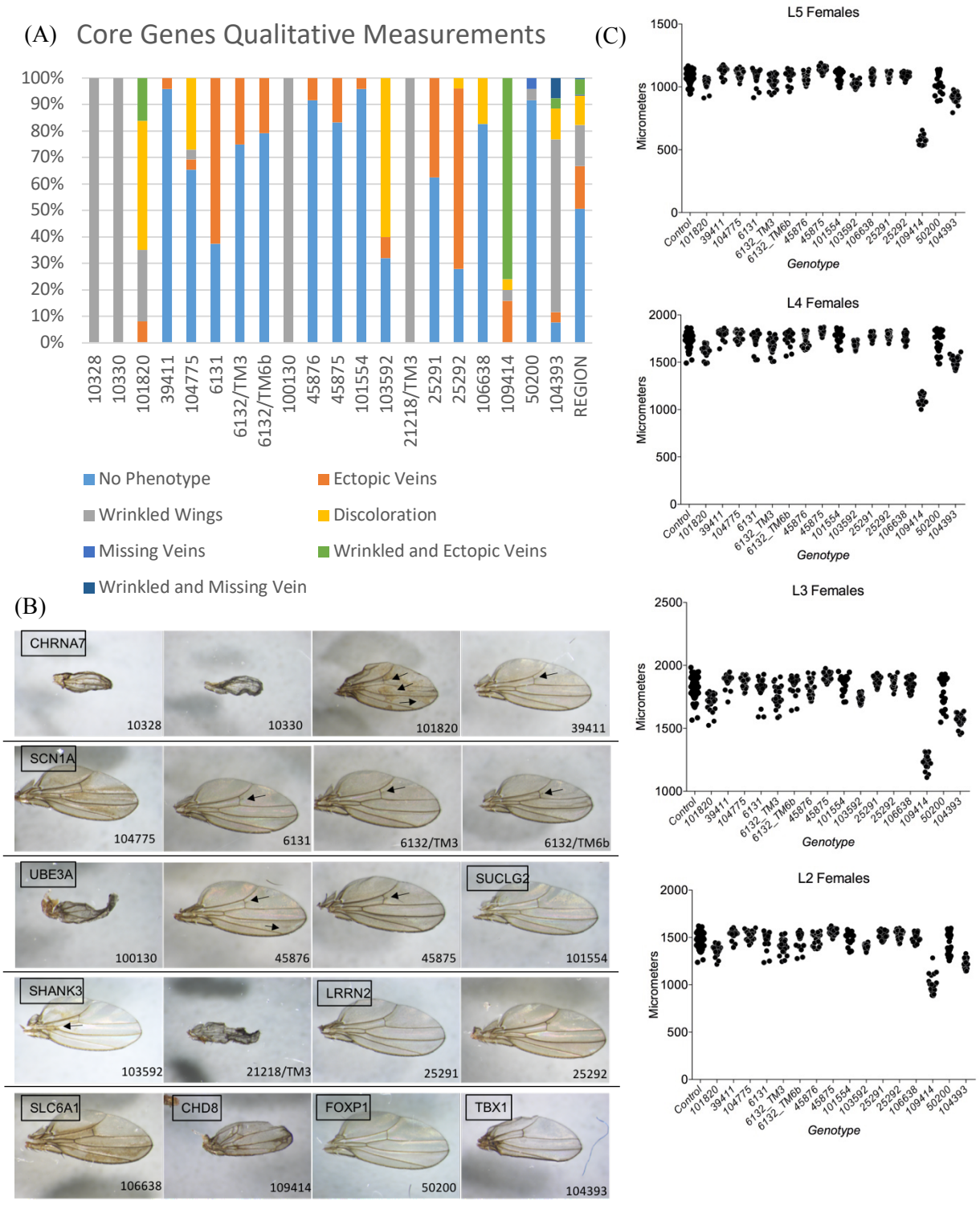

Figure 4: Qualitative and quantitative analysis of Core Genes; female adult *Drosophila melanogaster* wings.
(A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured.

system. The genes as part of this category were *CHRNA7, SCN1A, UBE3A, SHANK3, SUCLG2, SCL6A1, CHD8, FOXP1, LRRN2*, and *TBX1*. Female adult flies were the focus of the analyses.

The four lines from gene *CHRNA7* showed variable concordance. There were two lines, (10328,10330) that showed consistently wrinkled wings. Another line (39411) showed primarily no phenotype. The fourth (101820) showed a higher severity of developmental phenotypes due to some wings showing combined phenotypes, such as being wrinkled and developing ectopic veins. The presence of 3 lines with phenotypes (10328, 10330, 101820) out of 4 would indicate that *CHRNA7* does play an important role in wing tissue development. *CHD8* showed signs of a severe combined phenotype, but was only tested with one line (109414), so there is no validation for this result (Figure 4A, 4B). Similarly, the single line (104393) for gene *TBX1* showed a wrinkled phenotype, without a second line for validation (Figure 4A, 4B). While the lines for CHD8 for TBX1 were not too damaged to be measured for quantitative data, they did show a decrease in vein length, indicating a smaller wing (Figure 4C). There were three lines tested in *UBE3A*. There were two lines (45876 and 45875) that showed a lack of developmental phenotypes concordantly. The third phenotype however, 100130, showed consistently wrinkled wings across the line. This lack of concordance is likely attributed to the lack of successful knockdown for lines 45876 and 45875. However, this is speculation, and with only one out of three lines showing a phenotype, it is difficult to validate and say with certainty that *UBE3A* is implicated in improper wing development. This is the same case for *SHANK3*, where one line (103592) shows varied mild phenotypes, and the other (21218/TM3) shows consistently wrinkled wings (Figure 4A, 4B).

The four lines of *SCN1A* showed a high level of concordance in that they showed little to no developmental phenotypes as compared to the control. When compared to the control, ectopic veins are not abnormal. In addition, the discoloration was mild (Figure 4B). Three genes (*SUCLG, SLC6A1* and FOXP) were studied using one line each, but none of these showed a distinct phenotype. In addition, both lines studied for gene *LRRN2* (25291 and 25292) showed little to no developmental phenotypes (Figure 4A, 4B).

Thus, the genes in the Core genes category that are likely contribute to tissue development besides nervous tissue include *CHRNA7, CHD8,* and *TBX1*. While *UBE3A* and *SHANK3* may be also part of this category, it is impossible to say without assessing the success of the knockdown in the line lines. Assessment of RNAi knockdown success can be done using qPCR, and this should be conducted in the future for all genes to gain confidence in these conclusions, but especially those with non-concordant data or data that wasn't validated using more than one line. Genes where more research needs to be done into their involvement in a developmental phenotype are *SCN1A*, *SUCLG2, SLC6A1*, *FOXP1,* and *LRRN2.*

Microcephaly Genes

A common indicator of neurodevelopmental disorders is microcephaly. A set of genes is known to contribute to this craniofacial abnormality, and some of these genes were tested in this study. There were eight genes studied: *CEP135, CENPJ, TUBGCP6, MCPH1, ASPM, KIF11, RRBP1*, and *SLC25A19*.

There was a high severity associated with *KIF11,* and this is seen in both lines tested (52548 and 52548) (Figure 5A, 5B). All *KIF11* line wings were wrinkled to the extent that they could not be quantitatively measured (Figure 5A, 5B). In addition, the functional *KIF11* knockdown showed partial lethality. Whether female progeny were also susceptible to lethality was not assessed, but there was lethality at the larval, and pupal stages in one line (52548). There was full male lethality for line 52549, and despite these being male, the severe phenotype among female *Drosophila* supports that *KIF11* is highly important for the development of wing tissue. In addition, *RRBP1* showed a strong phenotype in both lines tested with 108279 yielding a slightly more severe phenotype than 49919 (Figure 5A, 5B). T

The two lines measured for gene *CENPJ* (106051 and 17975) showed discordant phenotypes. The 106051 wings were wrinkled with a few exceptions, while 17975 didn't show an abnormal phenotype (Figure 5A). It is therefore not possible to conclude that *CENPJ* is important in wing without running more analyzing the efficiency of the knockdown and/or running more tests.

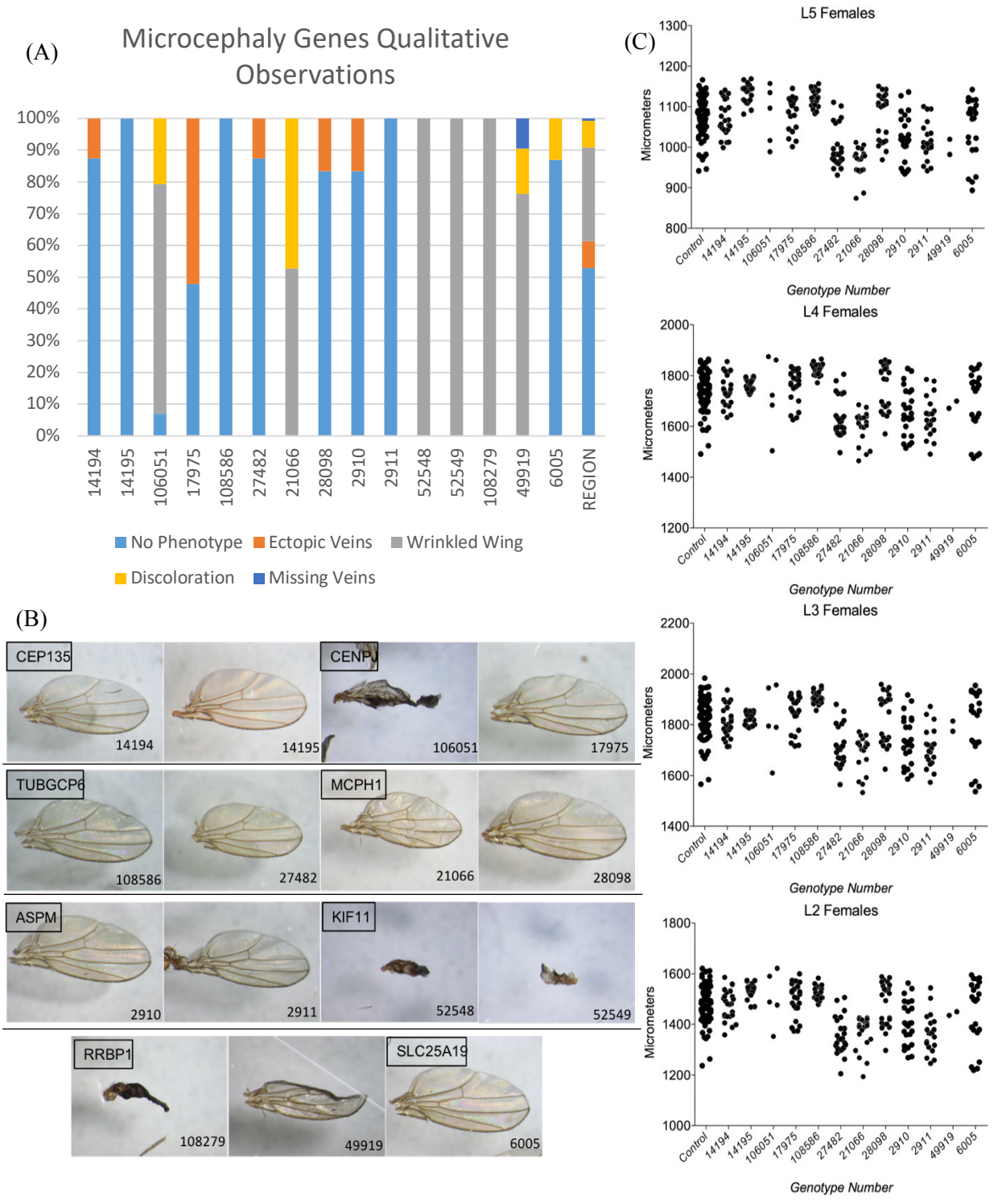

Figure 5: Qualitative and quantitative analysis of Microcephaly Genes; female adult *Drosophila melanogaster* wings
(A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

The gene *CEP135* showed no phenotype in either line tested (14194 and 14195). The mild ectopic veins are seen in control females as well and is thus not considered an abnormal phenotype. This is the same for genes *TUBGCP6* and *ASPM*; both lines for each gene – 108586 and 27482 for *TUBGCP6* and 2910 and 2911 for *ASPM* - show normal phenotypes (Figure 5A, 5B). For gene *SLC25A19,* only one line was analyzed (6005). This single line showed no phenotype, but if the knockdown of this line didn't work properly, this could be a false-negative (Figure 5A, 5B). More tests, such as qPCR would need to be done to either confirm that the knockdown worked and still led to no phenotype, or if it didn't work, more lines of this gene would need to be part of the study.

In summary, these analyses of the Microcephaly genes indicate that *KIF11* and *RRBP1* are only the genes from this region that likely have a strong effect on the development of the wing. Gene *CENPJ* may have some part in development, but the results within this study are inconclusive. Genes *CEP135, TUBGCP6, ASPM,* and *SLC25A19* all yielded negative results, meaning they may or may not be involved with wing development, but more tests of a different kind must be run to make this conclusion.

ß-Catenin Genes

Genes involved in creating parts of the ß-catenin pathway are an important part of cellular metabolism, and thus can be linked to the growth and development thereof. Candidate genes that contribute to the pathway that have been implicated in neurodevelopmental disorders include the

genes *LGR5, EPHB1, CTNNB1, NRXN1,* and *PTEN*. These were studied in the context as part of this study.

CTNNB1 was the only gene which showed concordant data supporting its involvement in the development of the wing tissue. Both lines used to analyze this gene (107344 and 7767) showed severe wrinkled phenotypes (Figure 6A, 6B).

Several others showed discordant qualitative phenotypes. Three of the lines (105360, 4735, 29931) representing a knockdown in gene *LGR5* showed wrinkled phenotypes, with two of them being much more severe (4735, 29931) (Figure 6A, 6B). There were a few measureable wings for line 4735, but they showed consistently smaller vein lengths than the control wings (Figure 6C). However, the two other lines (904, 29932) for gene *LGR5* yielded either no abnormal phenotypes (Figure 6A, 6B). Similarly, one line for gene *NRXN1* led to a developmental phenotype (4306), while the other two (36328, 32326) maintained normal phenotypes (Figure 6A, 6B). These genes require further testing to assess their involvement in wing tissue development.

For gene *EPHB1*, two of the lines (6545 and 4771) showed no phenotypes, while the third line (27236) yielded a few wings that were mildly improperly developed, though most showed no phenotypes (Figure 6A, 6B). This goes for *PTEN* lines as well, where the phenotypes are primarily normal. Both lines (35731 and 101475) show a couple of wings with mild phenotypes, but most are normal (Figure 6A, 6B). However, previously published literature has alluded to the importance of the *Pten* gene in *Drosophila melanogaster* in the cell size and development in flies, so it likely does contribute in ways unobservable based on the limits of this study or based on the RNAi lines used in this study (Scanga et al).

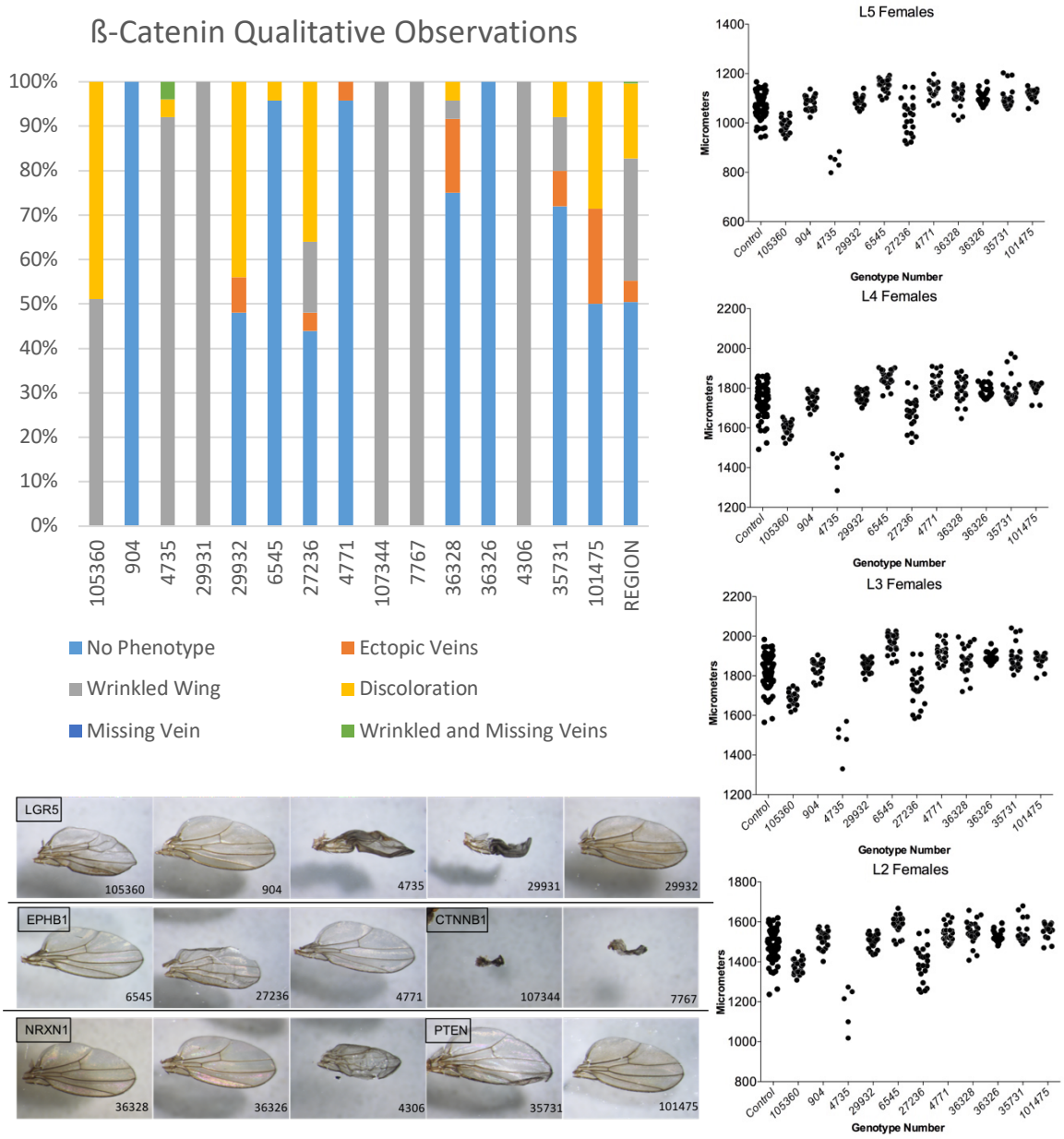

Figure 6: Qualitative and quantitative analysis of ß-catenin Genes; female adult Drosophila melanogaster wings (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

In summary, *CTNNB1* is the only gene that appears to be involved in wing development, while genes *LGR5* and *EPHB1* need more tests to confirm their involvement. *NRXN1* and *PTEN* must be analyzed using different assays in order to make a conclusion.

15q13.3

Several CNV regions have been implicated in neurodevelopmental disorders, and elucidating which genes among these broad deletions are responsible for tissue development is important. One CNV region analyzed in this study was 15q13.3. The genes within this region include *TRPM1* and *MTMR10.* Both showed primarily normal phenotypes. For *TRPM1*, one line (107537) showed mild discoloration (Figure 7A).

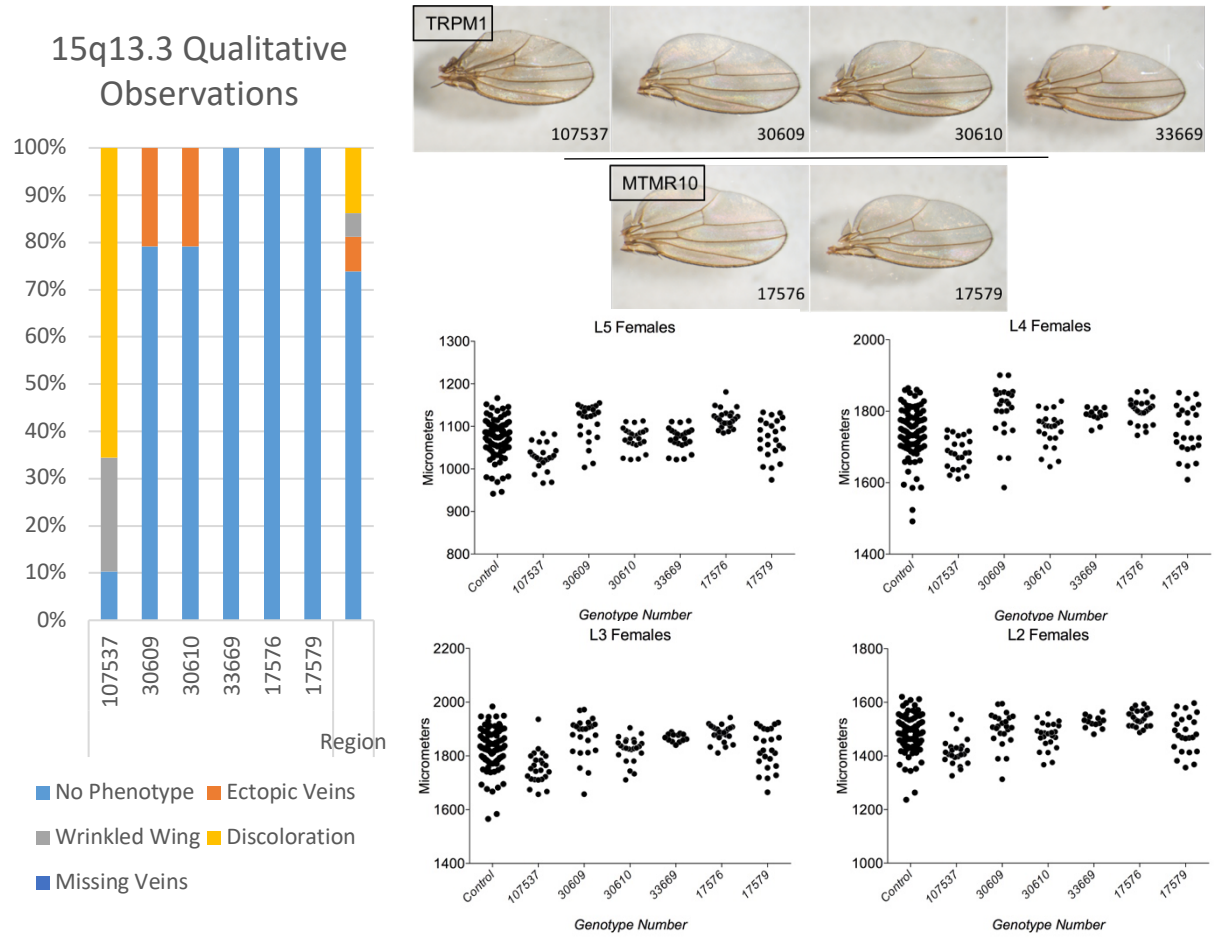

Figure 7: Qualitative and quantitative analysis of CNV region 15q13.3; female adult Drosophila melanogaster wings (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

However, the representative image indicates that this was a mild phenotype, and did not severely affect the integrity of the wing (Figure 7B). Two other lines (30609 and 30610) had ectopic veins, but these are normal compared to the control. The last line for *TRPM1* (33669) had no notable features (Figure 7A). As for *MTMR10*, neither line (17576 and 17579) yielded progeny with any phenotypes on the wing tissue (Figure 7A). Thurs, neither *TRPM1* nor *MTMR10* yielded conclusive results pertaining to their involvement in wing development. Functional assays for the knockdowns could provide further validation for these results.

1q21.1

This CNV region includes the genes *FMO5* and *BCL9*. For gene *BCL9,* both lines show a very wrinkled phenotype (Figure 8A, 8B). According to this assay, *BCL9* is therefore linked to proper wing tissue development. Gene *FMO5,* on the other hand, had one line (101452) which yielded a milder wrinkled phenotype, and two others (42829, 42830) that showed no phenotype (Figure 8A, 8B). When quantitatively analyzed, line 101452 yields wings that are also slightly smaller than the control, while the other two *FMO5* genes do not. This discordance indicates that more tests to assess the success of the knockdown should be performed to conclude a relationship between *FMO5* and wing tissue development.

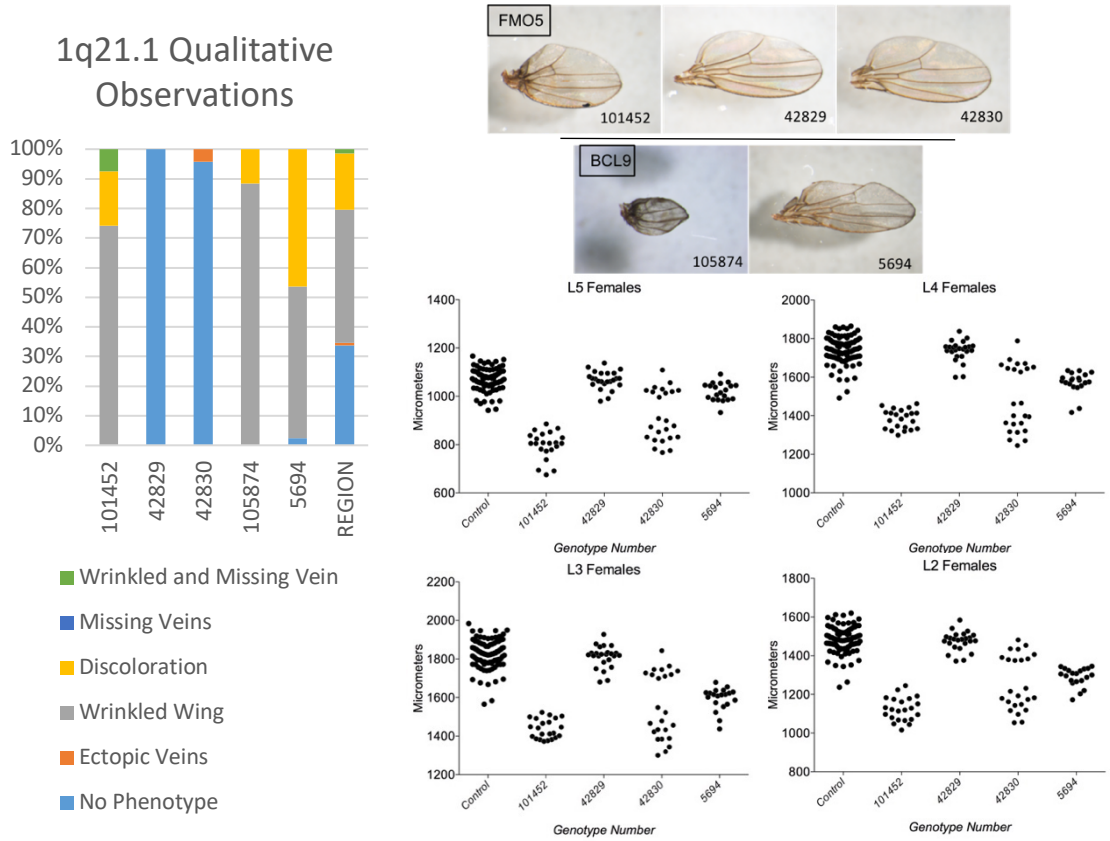

Figure 8: Qualitative and quantitative analysis of CNV region 1q21.1; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

15q11.2

The third CNV region studied was 15q11.2. This region encompasses genes including *TUBGCP5*, *CYFIP1*, *NIPA2*, and *HERC2P2*.

Of these genes, *CYFIP1* has two concordant phenotypes between the two lines analyzed (34907 and 34908). Ectopic veins, discoloration, and wrinkled wings are seen in both lines (Figure 8A). The images indicate that these are mild/moderate phenotypes (Figure 9B). Thus, *CYFIP1* likely has a role in wing tissue development.

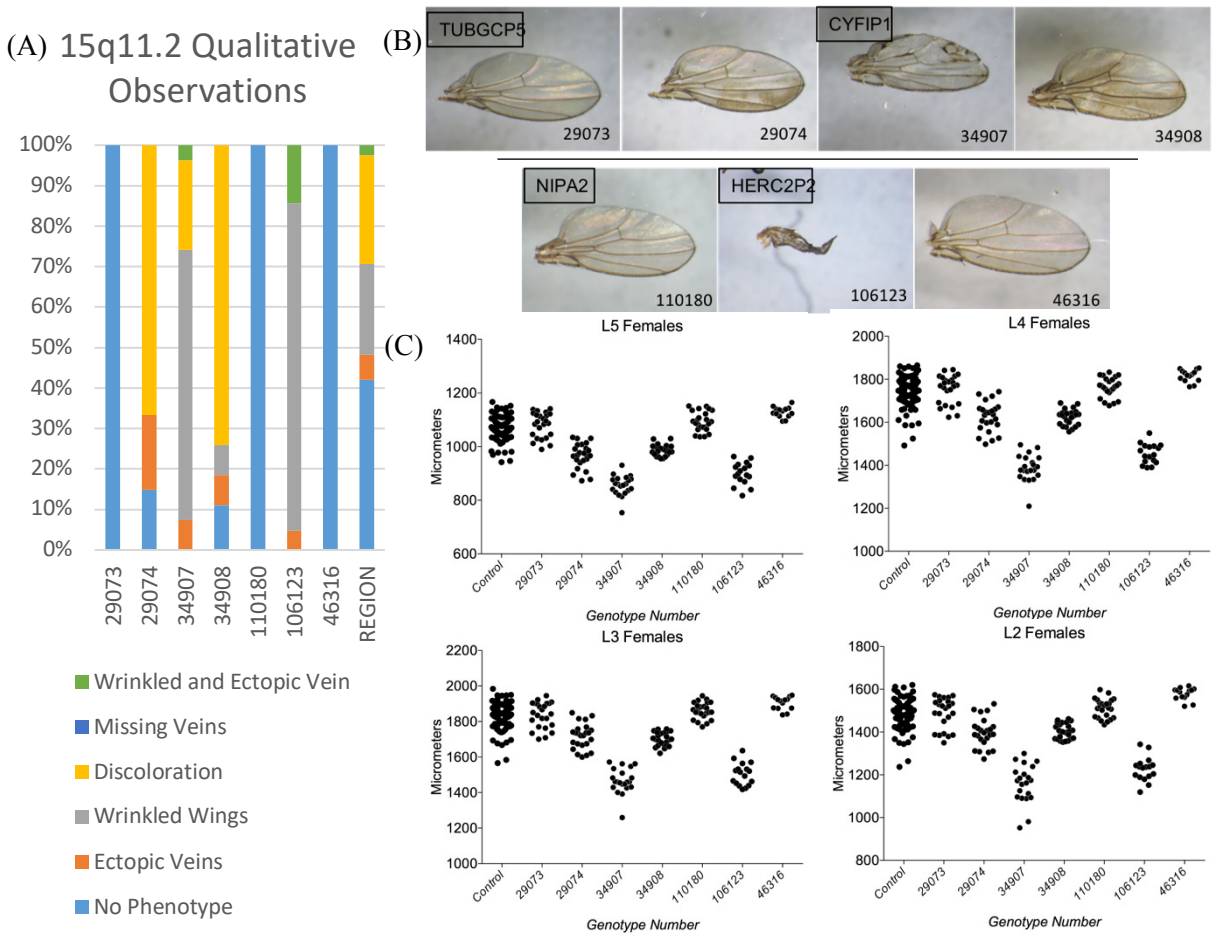

Figure 9: Qualitative and quantitative analysis of CNV region 15q11.2; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

Among the other genes, there are two with discordant genes. One is *TUBGCP5*, in which one line (29073) shows no phenotypic alteration due to the knockdown, while the other line (29074) shows mild discoloration (Figure 9A, 9B). Although the change appears mild, more lines should be assessed to gauge the involvement of *TUBGCP5* on wing tissue development, especially tests determining the effectiveness of the knockdown. The gene with a level of higher discordance is *HERC2P2*. In this gene, one line yielded a wrinkled phenotype (106123), and the other appeared to have no phenotype (46316) at all (Figure 9A, 9B). This is a drastic difference between lines,

and ultimately no conclusion can be made about *HERC2P2* until a validation of the knockdown is completed.

Lastly, *NIPA2*'s singular gene tested yielded wings with no phenotype. As with most genes with a single line being tested, this should be verified using a second line or validation of a successful knockdown before concluding that it is not involved in the development of the wing tissue.

In conclusion, while *CYFIP1* appears to be involved, more tests need to be done to assess the involvement of *TUGGCP5* and *HERC2P2* on wing development. Gene *NIPA2* must be analyzed using other assays in order to make a conclusive statement about its involvement.

16p13.1

The next CNV region analyzed was 16p13.1, containing the genes *NDE1, ABCC6*, and *KIAA 430*.

The gene *NDE1* shows mixed phenotypes between lines. In 100713, there is no abnormal phenotype, while 29788 shows mild wrinkling and discoloration (Figure 10A, 10B). There is also shrinkage associated with 29788 (Figure 10C). However, due to the discordance, it is impossible to conclude what *NPE1*'s role is in the development of wing tissue. Gene *KIAA430* however shows concordant data between lines (106964 and 32810) that there is no phenotype (Figure 10A). It would be helpful still to assess the knockdown success in order to confirm this result. However, it would be necessary to confirm for the line testing the involvement of ABCC6 (105419). This line shows no abnormal phenotype, but as it was the only line analyzed for this gene, it is important to confirm that the knockdown was successful.

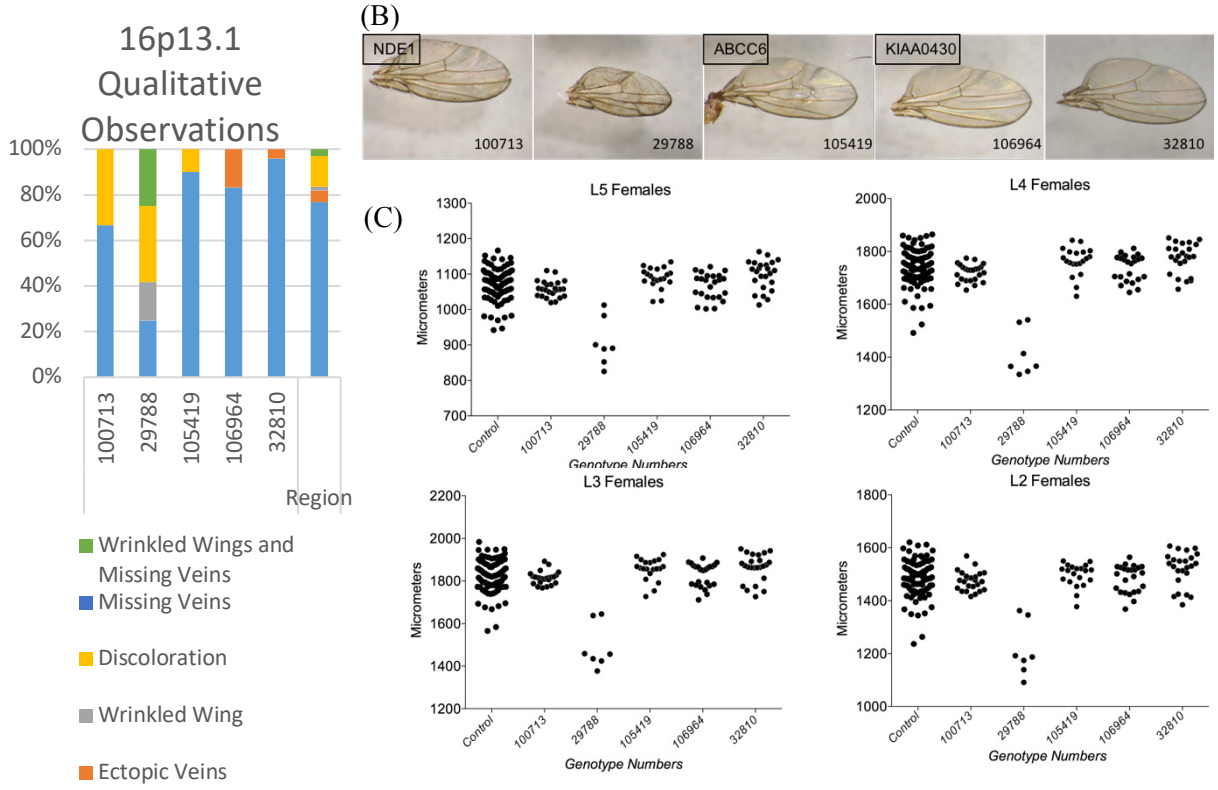

Figure 10: Qualitative and quantitative analysis of CNV region 16p13.1; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

3q29

There were 13 genes tested as part of the CNV region 3q29, including *LRRC33, ZDHHC19, SLC51A, PCY21A, PAK2, PIGX, NCBP2, PIGZ, MELTF, TCTEX1D2, BDH1, FBX045,* and *DLG1*.

Genes *PAK2* and *MELTF* were among the genes that showed developmental phenotypes, but only one line was tested for each. The line tested for *PAK2* (106488) showed a moderately wrinkled phenotype, while the line for *MELTF* (5326) showed a more severe wrinkled phenotype

(Figure 11B). More tests should be run to confirm that genes*PAK2* and *MELTF* are involved in wing development.

Gene *LRRC33* showed primarily normal development in many lines (907, 36340, 36343, 837, 836, 7997, 100110), and in others showed mild developmental phenotypes (27076, 31044) (Figure 11A, 10B). In one line (108037), a more severe developmental phenotype was observed (Figure 11A, 11B). However, since this was the only line of gene *LRRC33* displaying severe phenotypes of this nature, tests to assess the viability of the RNAi are necessary to make conclusions. However, the presence of mild and severe phenotypes could indicate some involvement in wing development. A similar case exists for gene *PCY21A*. One line (100575) shows a few varied phenotypes, such as wrinkled wings (Figure 11A). These are mostly mild, but a few wings expressed combination phenotypes, which for the sake of the experiment were considered a more severe phenotype (Figure 11A, 11B). However, the other line for gene *PCY21A* (18628) showed very few wings that expressed an abnormal phenotype (Figure 11A). Similarly, for gene *NCBP2*, the two lines analyzed (107112 and 50433) displayed varied phenotypes. While one line (107112) was severely wrinkled, the other (50433) showed no phenotype. Gene *TCTEX1D2* showed three lines (107115, 104357, 21565) with different phenotypes (Figure 11A). While two lines (107115, 21565) had mild phenotypes, the third (104357) displayed a moderate wrinkled phenotype close to the root of the wing (Figure 11A, 11B). Gene *BDH1* was analyzed with two phenotypes (30337, 30336), one of which (30337) showed a severe phenotype, and the other (30336) showed varied milder phenotypes (Figure 11A, 11B). Similarly, gene *FBX045* displayed one phenotype (107315) with a moderate phenotype, while the other (26577) showed no phenotype. The most drastically varied gene, however, was gene *DLG1*. Two lines were tested

(41136, 109274), and while line 109274 showed no

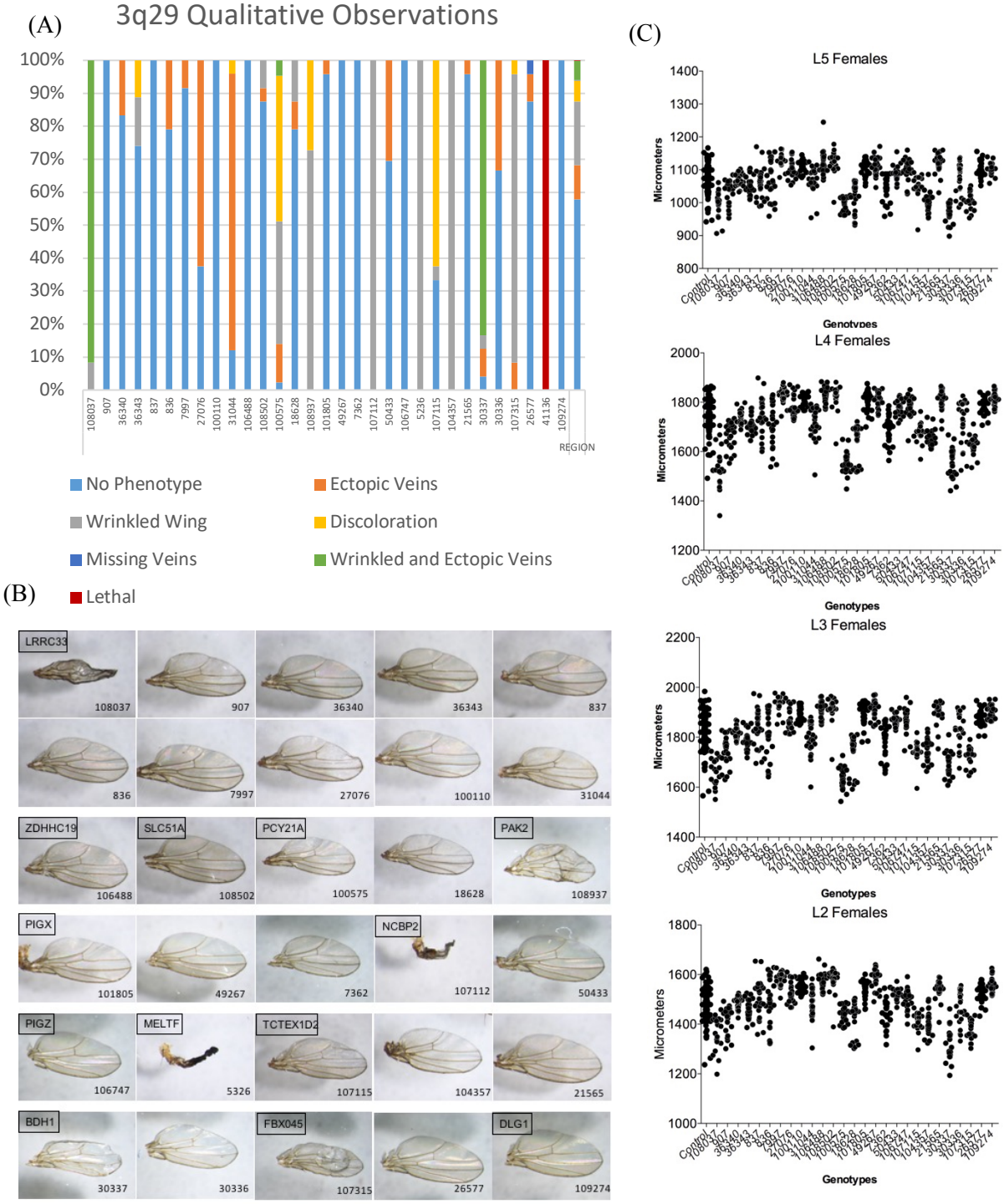

Figure 11: Qualitative and quantitative analysis of CNV region 3q29; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

phenotype, the knockdown due to line 41136 led to lethality in all progeny (Figure 11A, 11B). These genes (*LRRC33, PCY21A, NCBP2, TCTEX1D2, BDH1, FBX045,* and *DLG1)* should all be tested with more lines, or the existing lines should be assayed for successful RNAi knockdowns in order to confirm a phenotype.

Genes *ZDHHC19*, *SLC51A,* and *PIGZ* were all analyzed using one line each. The lines used to analyze gene *ZDHHC19* (106488) and *PIGZ* (106747) showed no phenotype throughout the wings analyzed (Figure 11A). However, the gene used to analyze *SLC51A* (108502) showed a few minor lines that are negligible due to the control showing similar mild phenotypes. Therefore, these genes are likely not associated with wing phenotypes. For gene *PIGX*, three lines were tested (101805, 49267, 7362), and none showed any developmental phenotypes (Figure 11A, 11B). Assaying the success of the RNAi knockdown may be a good way to confirm these knockdowns, but the negative results mean the genes need to be assayed differently in order to make conclusions about their involvement

In conclusion, based on the experiments conducted thus far, the CNV region 3q29, contains two genes that are likely involved in wing development: *PAK2* and *MELTF*. Several other genes, such as *LRRC33, PCY21A, NCBP2, TCTEX1D2, BDH1, FBX045*, and *DLG1* showed mixed results, and will definitely need more tests before making any conclusions. The remaining genes (*ZDHHC19, SLC51A, PIGZ,* and *PIGX*) showed no phenotypes, indicating further study must be done.

16p11.2 distal

In the CNV region known as 16p11.2 distal, six genes were analyzed. These were *CCDC101, SH2B1, ATXN2L, TUFM, RABEP2,* and *SPNS1*.

Gene *SH2B1* was analyzed using two lines (103646, 32982), and both showed mild phenotypes (Figure 12A, 12B). One line (103646) showed mild ectopic veins, and the other (32892) showed mild discoloration. This indicates that gene *SH2B1* likely has mild influence on wing development. Gene *TUFM* was analyzed using three lines (101856, 48981, 48982), two of which (101856, 48982) were severely wrinkled (Figure 12A, 12B). However, the third line (48981) did not lead to a phenotype (Figure 12A, 12B). However, the concordance between the two lines (101856, 48982) shows that gene *TUFM* is most likely strongly associated with a developmental phenotype. Genes *ATXN2L* and *RABEP2* were both genes that were analyzed with only one line each. The line for *ATXN2L* (34956) led to a very severe wrinkled phenotype (Figure 12A, 12B). On the other hand, the line for *RABEP2* (107617) led to a much milder, but present phenotype (Figure 12A, 12B). Both of these genes are thus likely associated with a wing development phenotype, but being that they were tested with a single line, more tests should be run to further confirm these conclusions.

Gene *CCDC10* was tested with two lines (101326, 41739), and neither showed a phenotype (Figure 12A, 12B). Similarly, gene *SPNS1* was tested with three lines (105462, 3229, 46030), and none of them led to a phenotype (12A, 12B). Thus, alternate assays are needed to confirm whether *CCDC10* and *SPNS1* are involved with wing development.

Thus, *SH2B1, TUFM, ATXN2L,* and *RABEP2* are likely to be involved in wing development, while it is impossible to make a conclusion about *CCDC10* and *SPNS1* from this study alone.

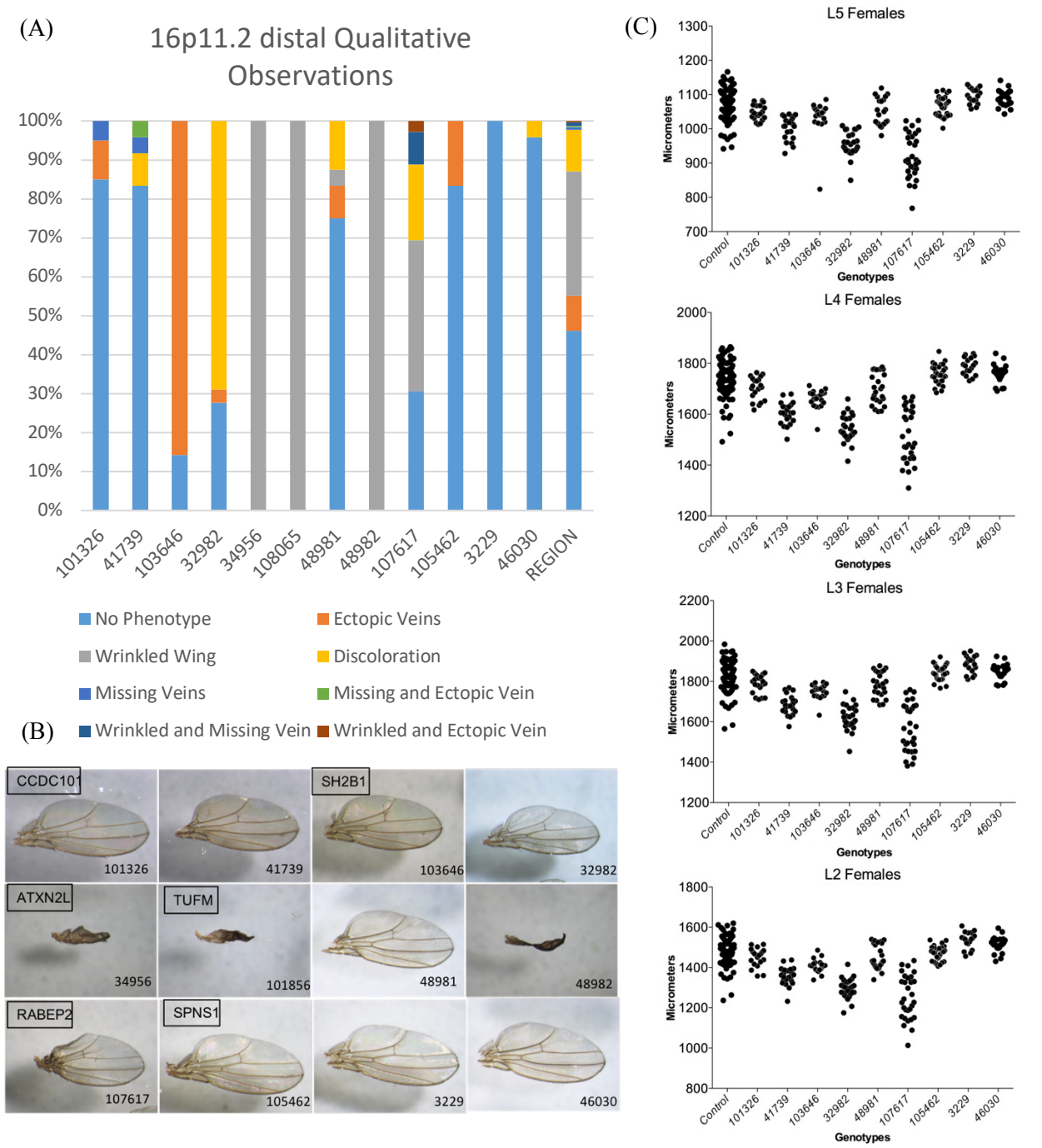

Figure 12: Qualitative and quantitative analysis of CNV region 16p11.2 distal; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

16p12.1

In the CNV region 16p12.1, four genes were analyzed: *UQCRC2, POLR3E, CDR1,* and *CG14182*.

For gene *UQCRC2*, three lines (100818, 26404, 26405) were analyzed, and all three showed severe phenotypes (Figure 13A). While two lines (100818, 26405) showed complete lethality, one (26404) resulted in severely wrinkled wings (Figure 13A, 13B). This indicates strongly that gene *UQCRC2* is important in wing development. Similarly, gene *POLR3E* was analyzed with three genes (51696, 52094, 108941). Two (52094, 108941) had severely wrinkled wings, and one (51696) led to complete lethality (Figure 13A, 13B). This indicates that *UQCRC2* and *POLR3E* are both important in wing development.

Gene *CG14182* was analyzed using two lines; one (5370) resulted in varied phenotypes that were ranged from moderate to severe in severity, and the other (5371) resulted in no phenotypes (Figure 13A, 13B). Further investigation into the success of RNAi knockdown will elucidate whether this gene is involved in wing development.

Lastly, gene *CDR1* was tested using one line (33444) that yielded no phenotype (Figure 13A, 13B). However, validation that this is not a false negative using qPCR would be a necessary step in confirming this result.

In conclusion, *UQCRC* and *POLR3E* seem to be involved in wing development, while *CDR1* and *CG14182* yielded inconclusive results from this study. An assay such as qPCR should be conducted to confirm these conclusions.

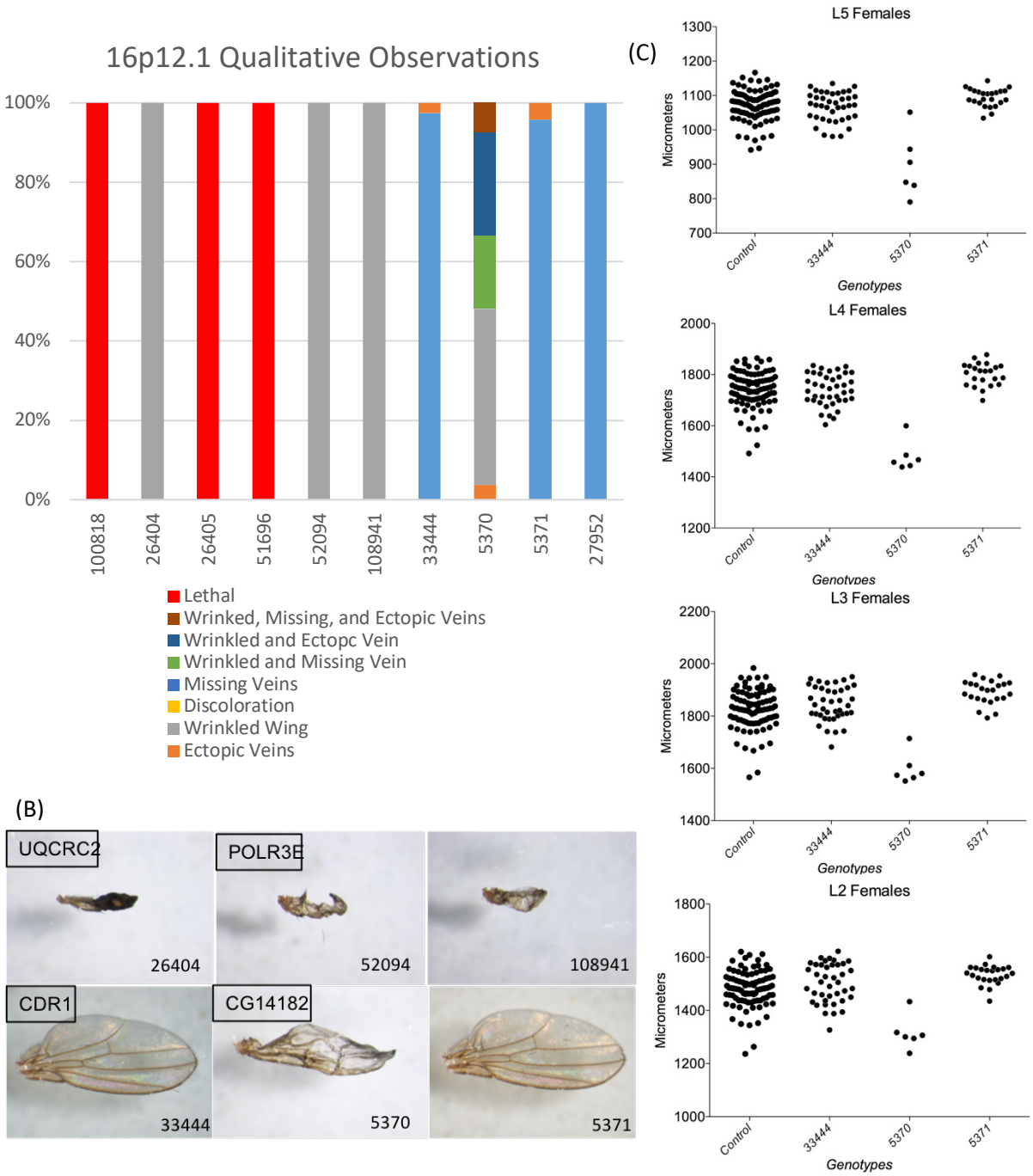

Figure 13: Qualitative and quantitative analysis of CNV region 16p12.1; female adult Drosophila melanogaster wing (A) Each wing was qualitatively categorized by type according to the schematic laid out in Figure 1, but not according to severity. (B) Representative images from each line. (C) Wings were measured and quantitative data points of the four veins were graphed. Wings that were too wrinkled to distinguish veins were not measured

Chapter 4

Discussion and Future Experiments

The experiments detailed here give an important insight into the potential involvement of genes in the development of wings. Previous work in this lab has begun to analyze the role of these genes in the development of neurons, as is important in the context of neurodevelopmental disorders, but involvement in wing tissue indicates that these genes could be more central to general tissue development – not just for neurons. The following table summarizes the positive results from each region/category of genes, based on these experiments (Table 11). Several other genes showed discordant results, and still others should no phenotype.

Table 11: Summary table of genes important for wing development

Region	Gene
Core Region	*CHRNA7*
	CHD8
	TBX1
Microcephaly Genes	*KIF11*
	RRBP1
ß-catenin	*CTNNB1*
1q29	*BCL9*
15q11.2	*CYFIP1*
3q29	*PAK2*
	MELTF
16p11.2 distal	*SH2B1*
	TUFM
	RABEP2
	ATXN2L
16p12.1	*UQCRC2*
	POLR3E

Future experiments would ultimately need to include qPCR on each line in order to assess RNAi knockdown success. In addition, more lines for some of the genes where there were few lines tested or discordance within those tested would help to increase confidence in conclusions

made. Future plans for this experiment involve conducting these qPCR experiments, in order to understand the discrepancies with our results as compared to potential published results where the genes being tested have been proven to be involved in the wing function or development. In addition, more morphological tests of the wings can be run, such as expanding upon the categories of qualitative observations to include bristle growth on the wing Lastly, immunohistochemical staining experiments are part of the future of this experiment in order to gain a molecular understanding of what is leading to the visible phenotype

Bibliography

Pandey UB, Nichols CD. Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery. Barker EL, ed. *Pharmacological Reviews*. 2011;63(2):411-436. doi:10.1124/pr.110.003293.

Scanga SE, Ruel L, Binari RC, Snow B, Stambolic V, Bouchard D, Peters M, Calvieri B, Mak TW, Woodgett JR, Manoukian AS. The conserved PI3'K/PTEN/Akt signaling pathway regulates both cell size and survival in *Drosophila*. *Nature Oncogene*. 2000; 19: 3971-3977.

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IN REVIEW: Pervasive epistasis modulates neurodevelopmental defects of the autism associated 16p11.2 deletion

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