

THE PENNSYLVANIA STATE UNIVERSITY
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EXAMINING *DROSOPHILA MELANOGASTER* WING SIZE UPON TISSUE SPECIFIC
RNAI-INDUCED KNOCKDOWN OF CANDIDATE GENE REGIONS OF
NEURODEVELOPMENTAL DISORDERS

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ABSTRACT

In humans, deletions and duplications in the genome known as rare copy number variants (CNVs) are known to be associated with neurodevelopmental disorders, such as autism, intellectual disability, and schizophrenia. While specific genes, both in these regions and elsewhere in the genome, are known to contribute to nervous tissue development, these genes could also have implications in general (not necessarily neuronal) tissue development. To explore this possibility, this study utilized the tissue specific RNAi knockdown of homologs of these genes in wing pouch tissue of *Drosophila melanogaster* to evaluate the effects on wing area. The homologs of 79 genes, categorized in 10 CNV regions or core neurodevelopmental gene categories, were studied in a high throughput manner. The results of this study indicate that 23 genes possibly contribute to the development of non-neuronal tissue in both male and female flies; however, further testing on additional lines would increase confidence in these conclusions.

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INTRODUCTION

Neurodevelopmental disorders, such as autism, intellectual disability, and schizophrenia, have been associated with sizable regional deletions and duplications in the human genome known as rare copy number variants (CNVs).¹ While specific genes both in these regions and elsewhere in the genome are known to contribute to nervous tissue development, these genes could also have implications in general (not necessarily neuronal) tissue development. Previous work by the Girirajan group has identified 10 such rare CNV regions in individuals in the DECIPHER database² that show a variety of non-neuronal phenotypes including craniofacial, skeletal, and organ system defects.

To examine whether such genes play a role in general tissue development, the *Drosophila* homologs of 79 human genes, categorized in 10 CNV regions or core neurodevelopmental gene categories, were studied in a high throughput manner through the simulation of deletion using RNA interference in the wing pouch tissue of *Drosophila melanogaster* and measuring resulting wing area defects. *D. melanogaster* is an ideal model organism for this analysis, as many developmental genes, signaling pathways, and cellular processes are conserved between humans and fruit flies.³ Notably, approximately 77% of genes known to cause disease in humans have homologs in *Drosophila*.⁴

MATERIALS AND METHODS

Fly Stocks and Rearing Conditions

Drosophila melanogaster stocks used in this study were obtained from the GD and KK RNAi libraries from the Vienna Drosophila Stock Center (VDRC)⁵, with the exception of the MS1096 driver stock procured from the lab of Dr. Zhichun Lai. The stock used for the control in this experiment is called w[VDRC]. The complete list of stocks used, organized by the CNV region of the human genome or the molecular pathway they correspond to, is given in Table 1. Differing line numbers provided by VDRC that correspond to the same gene target differing areas of the gene. Lines for which only female flies were analyzed are marked as (F).

Table 1: Categorization of *D. melanogaster* and *H. sapiens* genes and corresponding VDRC UAS-RNAi line numbers

<i>H. sapiens</i> Gene CNV Region / Categorization	<i>H. sapiens</i> Gene	<i>D. melanogaster</i> Gene	RNAi Line Number(s)
Core genes	<i>CADPS2</i>	<i>Cadps</i>	25291, 25292
	<i>CHD8</i>	<i>Kis</i>	109414
	<i>SCN1A</i>	<i>para</i>	6131, 6132/TM3, 6132/TM6b, 104775
	<i>SHANK3</i>	<i>prosap</i>	21218/TM3, 103592
	<i>SLC6A1</i>	<i>Gat</i>	106638
	<i>SUCLG2</i>	<i>Sucb</i>	101554
	<i>TBX1</i>	<i>org-1</i>	104393
Microcephaly	<i>UBE3A</i>	<i>Ube3a</i>	45875, 45876
	<i>ASPM</i>	<i>Asp</i>	2910, 2911
	<i>CENPJ</i>	<i>Sas-4</i>	17975, 106051
	<i>CEP135</i>	<i>Cep135</i>	14194, 14195
	<i>KIF11</i>	<i>Klp61F</i>	52548, 52549
	<i>MCPH1</i>	<i>MCPH1</i>	21066, 28098
	<i>SLC25A19</i>	<i>Tpc1</i>	6005
β -catenin	<i>TUBGCP6</i>	<i>Grip163</i>	27482, 108586
	<i>CTNNB1</i>	<i>arm</i>	107344
	<i>EPHB1</i>	<i>Eph</i>	4771, 6545 (F), 27236
	<i>LGR5</i>	<i>rk</i>	904, 4735, 29931, 29932, 105360
	<i>NRXN1</i>	<i>Nrx-1</i>	4306, 36326, 36328
1q21.1	<i>PTEN</i>	<i>Pten</i>	35731, 101475
	<i>BCL9</i>	<i>lgs</i>	5694, 105874
3q29	<i>FMO5</i>	<i>Fmo-2</i>	42829, 42830, 101452
	<i>BDH1</i>	<i>CG8888</i>	30336, 30337
	<i>DLG1</i>	<i>dlg1</i>	41136, 109274
	<i>FBXO45</i>	<i>Fsn</i>	26577
	<i>MFI2</i>	<i>Tsf2</i>	5236
	<i>NCBP2</i>	<i>Cbp20</i>	107112 (F)
	<i>OSTalpha</i>	<i>CG6836</i>	108502
	<i>PAK2</i>	<i>Pak</i>	108937
	<i>PCYT1A</i>	<i>Pcyt1</i>	18628, 100575
	<i>PIGX</i>	<i>PIG-X</i>	101805
7q11.23	<i>PIGZ</i>	<i>PIG-Z</i>	106747
	<i>ZDHHC19</i>	<i>app</i>	100476, 106488
	<i>ABHD11</i>	<i>CG2059</i>	108390
	<i>BCL7B</i>	<i>BCL7-like</i>	20410, 106828
	<i>CLIP2</i>	<i>CLIP-190</i>	107176
	<i>DNAJC30</i>	<i>CG11035</i>	8478, 8479, 108566
	<i>FKBP6</i>	<i>shu</i>	105832
	<i>LIMK1</i>	<i>limk1</i>	25343, 25344
	<i>NSUN5</i>	<i>Nsun5</i>	39560, 46814, 106765
	<i>STX1A</i>	<i>syx1A</i>	33112, 33113

<i>H. sapiens</i> Gene CNV Region / Categorization	<i>H. sapiens</i> Gene	<i>D. melanogaster</i> Gene	RNAi Line Number(s)
15q11.2	<i>CYFIP1</i>	<i>Sra-1</i>	34907, 34908
	<i>NIPA2</i>	<i>spict</i>	110180
	<i>TUBGCP5</i>	<i>Grip128</i>	29073, 29074
15q13.3	<i>CHRNA7</i>	<i>nAChRalpha5</i>	10328, 10330, 39411
	<i>MTMR10</i>	<i>CG14411</i>	17576, 17579
	<i>TRPM1</i>	<i>Trpm</i>	30609, 30610, 33669
16p11.2	<i>ALDOA</i>	<i>Ald</i>	27541 (F), 101339
	<i>PAGR1</i>	<i>pal</i>	45978, 101952 (F)
	<i>CDIPT</i>	<i>pis</i>	106842
	<i>CORO1A</i>	<i>coro</i>	44672
	<i>DOC2A</i>	<i>rph</i>	52438 (F)
	<i>FAM57B</i>	<i>CG17841</i>	103772
	<i>KCTD13</i>	<i>CG10465</i>	107131
	<i>MAPK3</i>	<i>rl</i>	109108
	<i>PPP4C</i>	<i>pp4-19C</i>	25317
	<i>TBX6</i>	<i>Doc2</i>	103431
16p11.2 distal	<i>YPEL3</i>	<i>CG15309</i>	101370
	<i>ATXN2L</i>	<i>Atx2</i>	34956
	<i>CCDC101</i>	<i>Sgf29</i>	41739 (F), 101326
	<i>SH2B1</i>	<i>Lnk</i>	32892, 103646
	<i>SPNS1</i>	<i>Spin</i>	105462
	<i>TUFM</i>	<i>EfTuM</i>	48982, 108065
16p12.1	<i>CDR2</i>	<i>Cen</i>	33444 (F)
	<i>MOSMO</i>	<i>CG14182</i>	5370, 5371 (F)
	<i>POLR3E</i>	<i>Sin</i>	51696, 108941
	<i>UQCRC2</i>	<i>UQCR-C2</i>	26404, 26405, 100818
16p13.11	<i>ABCC6</i>	<i>MRP</i>	105419
	<i>KIAA0430</i>	<i>CG17018</i>	32810, 106964
	<i>NDE1</i>	<i>nudE</i>	29788, 100713
17q12	<i>AATF</i>	<i>Aatf</i>	18182, 106977
	<i>ACACA</i>	<i>acc</i>	8105
	<i>DDX52</i>	<i>CG5589</i>	44322, 108642
	<i>DHRS11</i>	<i>CG9150</i>	16877
	<i>DUSP14</i>	<i>CG15528</i>	14865, 105484
	<i>LHX1</i>	<i>Lim1</i>	13996, 104468
	<i>PIGW</i>	<i>PIG-Wb</i>	4092
	<i>TADA2L</i>	<i>Ada2a</i>	23308
	<i>ZNHIT3</i>	<i>CG8204</i>	100755

In order to reduce expression of these genes in the *Drosophila* wing pouch, the *UAS-GAL4* system and RNAi interference was employed.⁶ This system combines two fly lines containing two different constructs that when combined, leads to an RNA interference-mediated reduction of

expression of the genes of interest. The first of these lines are the 136 upstream activator sequence (*UAS*) RNAi lines in Table 1. The second line is the driver stock, which contains a wing pouch specific gene (*MS1096*) coexpressed with a yeast derived transcriptional activator protein (GAL4) specific to the *UAS* of the other line. When these two lines are individually crossed, the GAL4 protein prompts the *UAS* to promote the transcription of a double stranded hairpin RNA, which prompts the RNA interference system to target and degrade the mRNA of the gene of interest in the wing pouch due to the coexpression of *MS1096*. This results in a functional knockdown of the gene of interest in a wing tissue specific manner. The 136 *UAS* RNAi lines were included as a result of previous qPCR work indicating knockdown success; as such, for some lines, males or females were not included as the knockdown could not be confirmed.

The crosses were incubated at 25°C. Adult flies were isolated on day 0-1, but remained at 25°C until day 2-5, at which point they were frozen at -80°C. Wings were subsequently imaged and analyzed. Samples in the freezer for longer than one month were moved to -20°C.

Imaging

Female and male *drosophila* wings were captured using a Zeiss Discovery V20 stereoscope (Zeiss, Thornwood, NY, USA), with a ProgRes Speed XT Core 3 camera and CapturePro v.2.8.8 software (Jenoptik AG, Jena, Germany) at 40X magnification. The slides were prepared after the flies were frozen by plucking the wings and sealing the cover slips over the wings using clear nail polish.

Quantitative and Statistical Analysis

For the non-lethal lines, fly wing area was measured in Fiji ImageJ software using the Measure Area tool.⁷ The image scale was set to 0.785 pixels to 1 μm , thus area measurements were recorded in square micrometers (μm^2). The area measurement was taken by following the perimeter of the wing, excluding the jugal region and flesh from the proximal side of the wing if present (Figure 1).



Figure 1: Example Wing Area Measurement. Area in red (excluding jugal region) was recorded.

The wing area measurements were compared to corresponding male and female w[VDRC] controls using two-tailed Mann-Whitney tests in R v.3.6.1.⁸ Boxplots of wing areas for each region was created in GraphPad Prism 8.0.0.⁹ Wing areas that were found to statistically significantly differ ($p < 0.05$) from the respective male and female control lines are marked with an asterisk; non-significant results are marked as such (ns). For significant values, comparison to the median wing area values for the respective controls (shown with a red dotted line) gives directionality to the effect on wing size.

RESULTS

Core Genes

Eight genes in this study were categorized as “Core Genes” due to their known involvement in human neurodevelopmental disorder. By knocking down the *drosophila* analogues of these genes in the wing, the phenotypic effect of these genes can be observed in tissue outside of the nervous system. The genes in this category include *CADPS2*, *CHD8*, *SCN1A*, *SHANK3*, *SLC6A1*, *SUCLG2*, *TBX1*, and *UBE3A*.

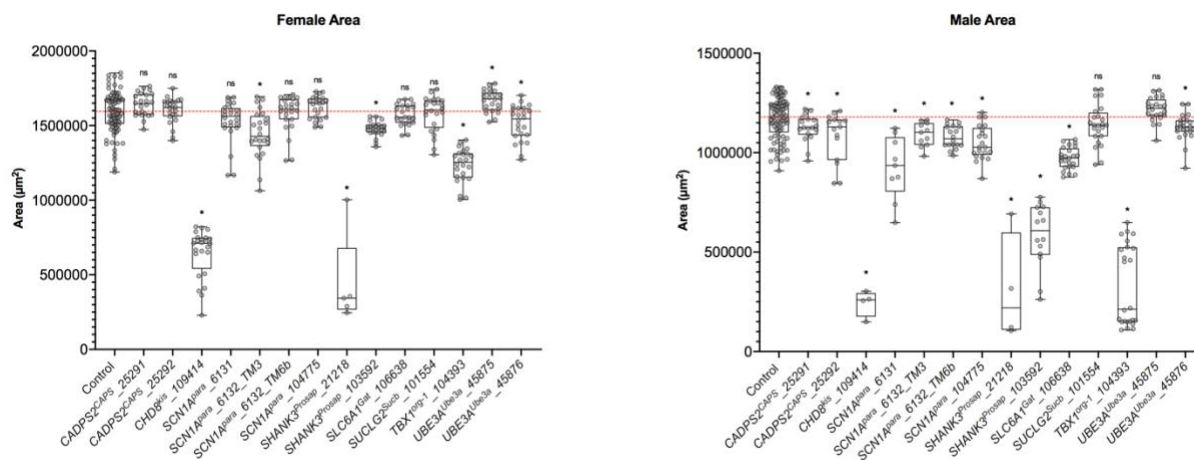


Figure 2: Core Genes Wing Area Boxplots.

In females, the areas of the wings significantly differed in 7 knockdown lines corresponding to 5 genes, while in males, the areas of the wings differed in 12 knockdown lines corresponding to 7 genes. However, discordant results were observed within females for the genes *SCN1A* and *UBE3A*—in the former, only one line showed a significant decrease in area; in the

latter, the lines showed significant differences in both directions. In males, all results except the lines for *UBE3A* were concordant.

Microcephaly

Genes categorized as Microcephaly genes are known to be associated with smaller craniofacial features, but they were investigated here to assess whether there are possible effects outside of the nervous system. This category includes the genes *ASPM*, *CENPJ*, *CEP135*, *KIF11*, *MCPH1*, *SLC25A19*, and *TUBGCP6*.

In females, the areas of wings in 8 RNAi lines corresponding to 6 genes were significantly different than the control, while in males, 7 lines differed in 5 genes, with one line exhibiting lethality. *KIF11* exhibited lethality in males, while females exhibited the largest effect size of all the microcephaly lines. *SLC25A1* showed a significant difference from the respective controls in males, but not in females.

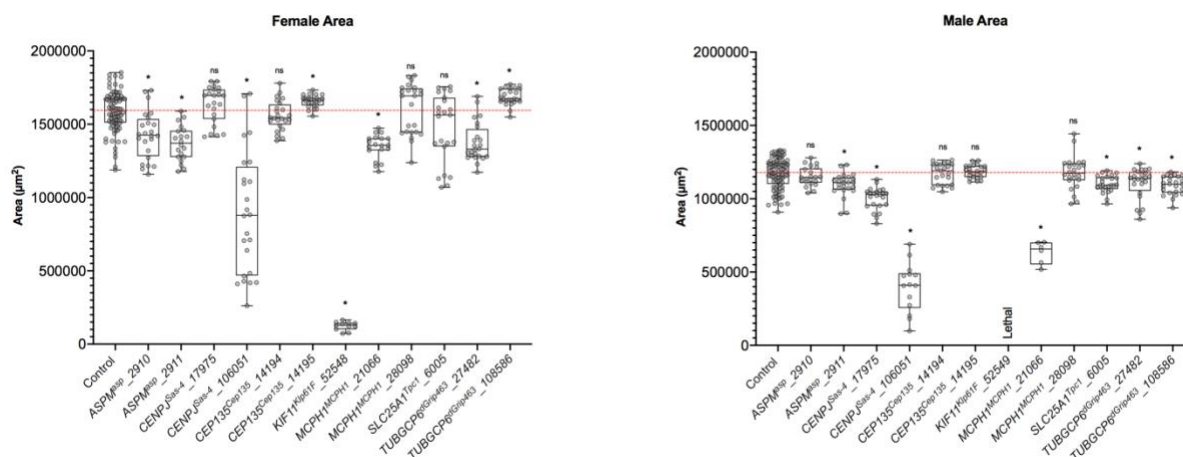


Figure 3: Microcephaly Wing Area Boxplots.

β -Catenin

Proteins grouped in the Beta-Catenin category are known to be involved with the signaling functions of the Beta-Catenin protein, which are numerous. Beta catenin has been implicated in the Wnt signaling pathway, cell to cell adhesion, and general development. The genes of interest are known to be involved in neural development, including *CTNNB1*, *EPHB1*, *LGR5*, *NRXN1*, and *PTEN*.

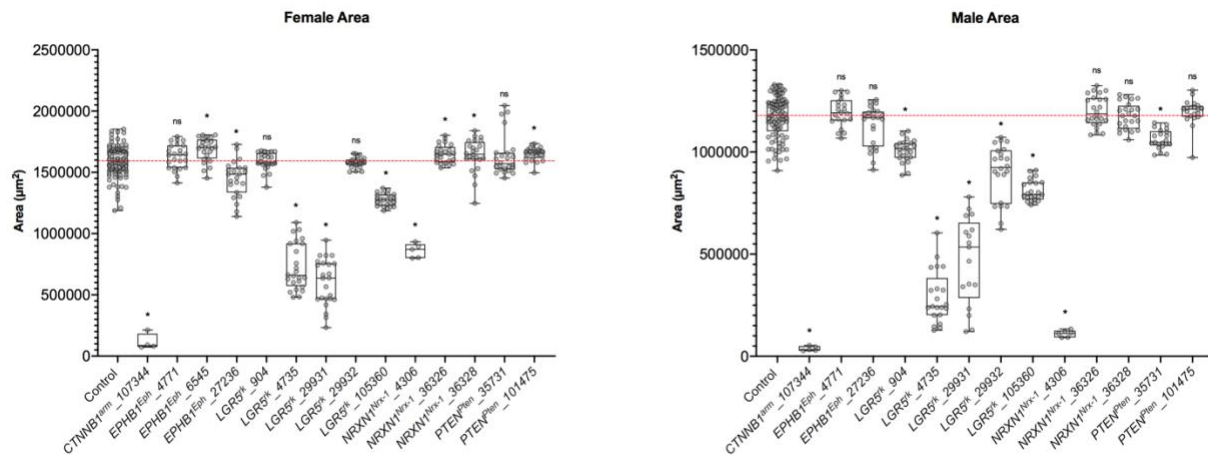


Figure 4: β -Catenin Wing Area Boxplots.

In females, the areas of 11 RNAi lines corresponding to 8 genes showed a significant difference compared to the control, while in males, 8 RNAi lines corresponding to 4 genes showed such differences. No lines in either sex exhibited lethality. Statistically significant differences were seen in the 3 RNAi lines for *NRXN1* in females; however, 1 line showed a significant decrease in area, while 2 showed significant increases. Concordance was seen across all 5 lines corresponding to *LGR5* – in males, all 5 lines showed significant decreases in area, while in females, 3 of 4 lines

showed such decreases in area. As such, the role of *LGR5* outside of the central nervous system should be further investigated.

1q21.1

CNVs of the 1q21.1 region are associated with intellectual disability, schizophrenia, microcephaly, macrocephaly, and congenital heart defects.¹⁰ This region contains the genes *BCL9* and *FMO5*.

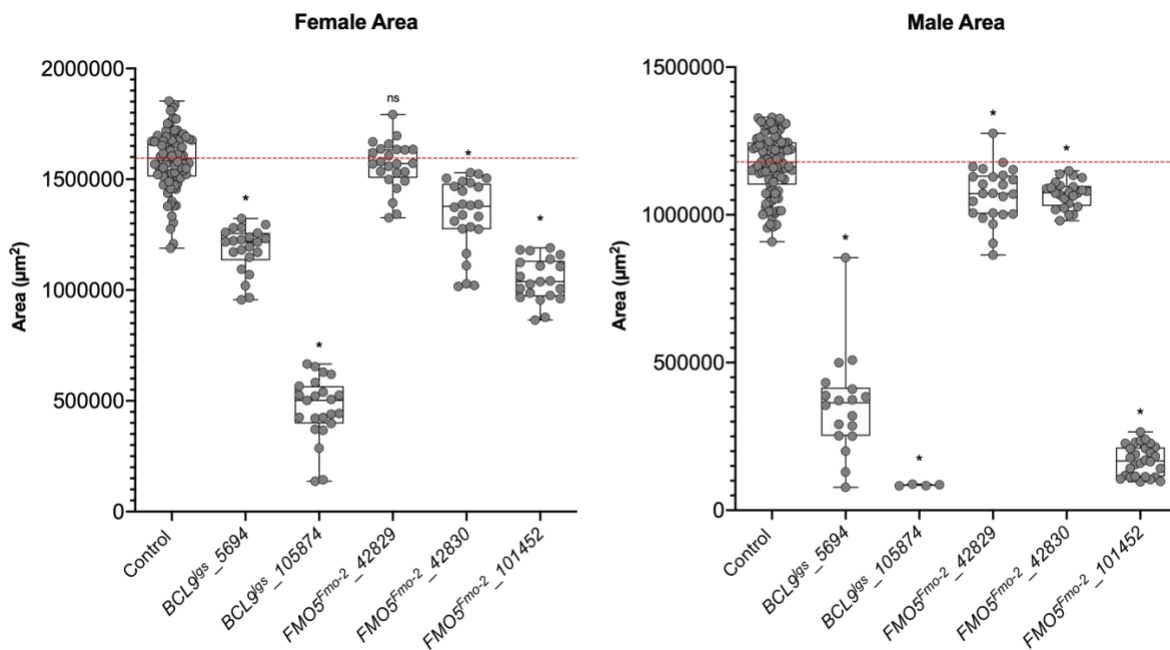


Figure 5: 1q21.1 Wing Area Boxplots.

In this region, 5 RNAi lines among 2 genes were analyzed for both sexes. In females, 4 lines corresponding to the 2 genes showed significant decreases in area, with only 1 line for *FMO5* not showing such a decrease. In males, all 5 lines showed significant decreases in area. Such

concordance among decreases in area among females in males suggests an important role for these genes in development for the fly wing.

3q29

3q29 microdeletion syndrome is associated with autism spectrum disorder, schizophrenia, anxiety, bipolar disorder, developmental and speech disorders, with varied exhibition of symptoms. Associated congenital defects include microcephaly, jaundice (yellowing of eyes and skin), and heart defects.¹⁰ The genes studied as part of this CNV region include *BDHI*, *DLG1*, *FBXO45*, *MFI2*, *NCBP2*, *OSTalpha*, *PAK2*, *PCYT1A*, *PIGX*, *PIGZ*, and *ZDHHC19*.

In this region, 15 RNAi lines amongst 11 genes were analyzed in both males and females. In females, 10 of the 15 RNAi lines showed statistically significant differences in size from the controls, with 1 line exhibiting lethality. In males, 13 of the 15 RNAi lines showed such differences, with 3 lines exhibiting lethality. Across both sexes, line 41136 for the gene *DLG1* exhibited lethality. However, the other RNAi line for *DLG1*, 109774, showed significant size differences in opposite directions across the two sexes, with females exhibiting an increase in size and males displaying a decrease in size. Such a discordance in size change can also be seen with the 106488 line of *ZDHHC19*, with females showing an increase in area and males showing a decrease. *OSTalpha* also showed such a discordance. However, other non-lethal lines across both sexes exhibited concordance. Both of the lines for *BDHI* showed significant decreases in area in both sexes, as did the two lines for *PCYT1A*. Only one line for *PAK2* and *PIGX* were analyzed, but each of these lines showed decreases in area in both sexes.

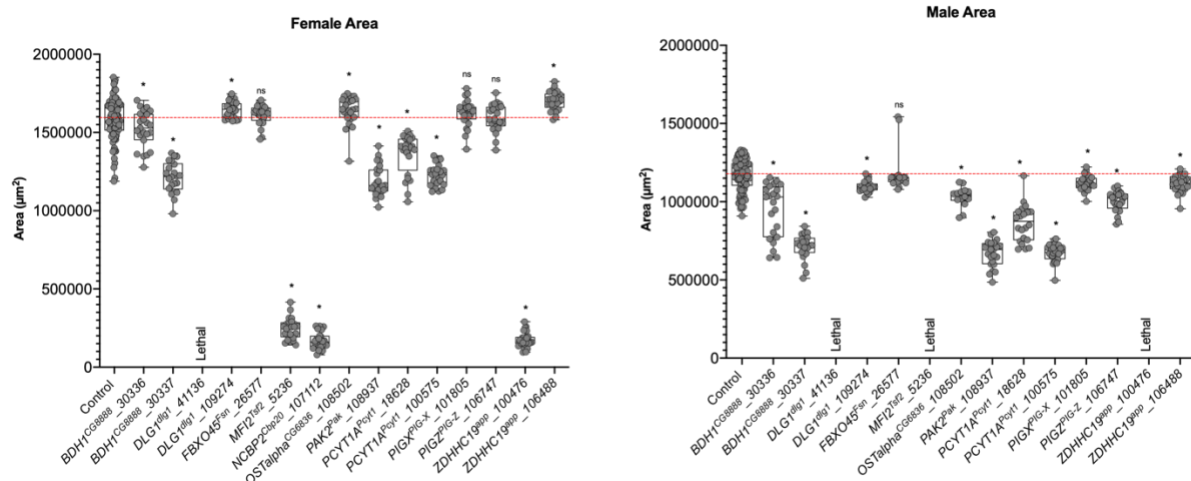


Figure 6: 3q29 Wing Area Boxplots.

7q11.23

The 7q11.23 microdeletion is associated with Williams syndrome, which manifests in patients as intellectual disability along with craniofacial features.¹¹ The genes studied in this CNV region include *ABHD11*, *BCL7B*, *CLIP2*, *DNAJC30*, *FKBP6*, *LIMK1*, *NSUN5*, and *STX1A*.

The areas of wings in 15 RNAi lines across these 8 genes were analyzed. In females, 7 non-lethal lines showed significant differences from the controls, while in males, 8 non-lethal lines showed such differences. Across both sexes, RNAi line 33112 for the gene *STX1A* exhibited lethality, while the other RNAi line 33113 showed no significant changes in neither males nor females. This is a mixed result, requiring verification using other methods such as qPCR to see if a knockdown occurred in the latter line. The one line for *CLIP2* in both sexes showed a significant decrease in wing area; however, with only one line, this result will require confirmation through another knockdown. Both lines for *LIMK1* in both sexes showed significant decreases in wing area, which indicates this gene may contribute to general tissue development.

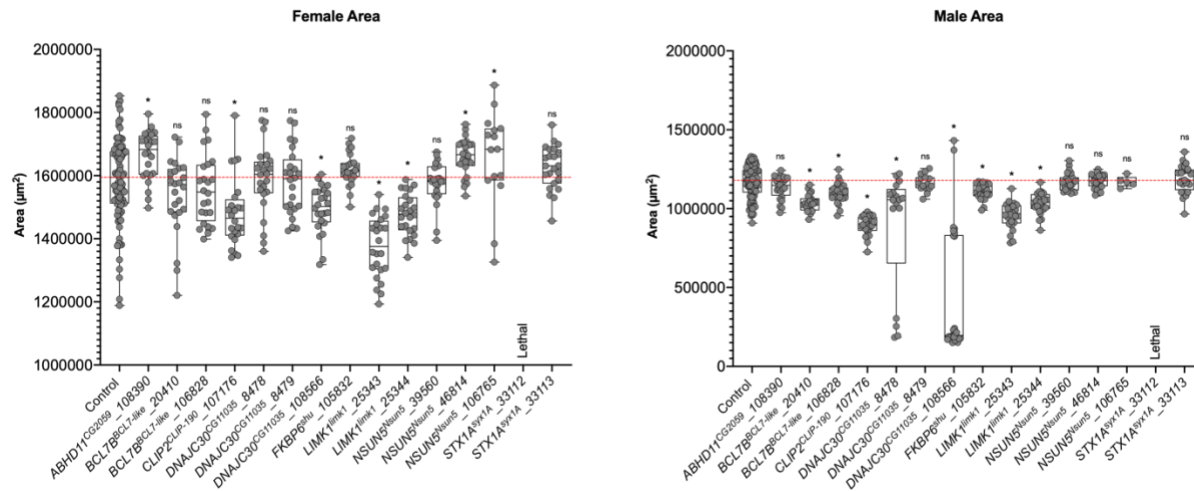


Figure 7: 7q11.23 Wing Area Boxplots.

15q11.2

This microdeletion region is associated with Burnside-Butler syndrome. The genes studied as a part of this region include *CYFIP1*, *NIPA2*, and *TUBGCP5*.

In this region, 5 RNAi lines for the 3 genes were analyzed among male and female flies. No lines exhibited lethality. Three lines in females (34907 and 39408 for *CYFIP*, 29074 for *TUBGCP5*) and 4 lines in males (34907 and 39408 for *CYFIP*, 29703 and 29074 for *TUBGCP5*) showed significant decreases in wing area. *NIPA2* showed no significant changes in wing area amongst males and females. Both lines for *CYFIP1*, for both sexes, showed significant decreases in wing area; thus, this gene may be involved in wing disc cellular development.

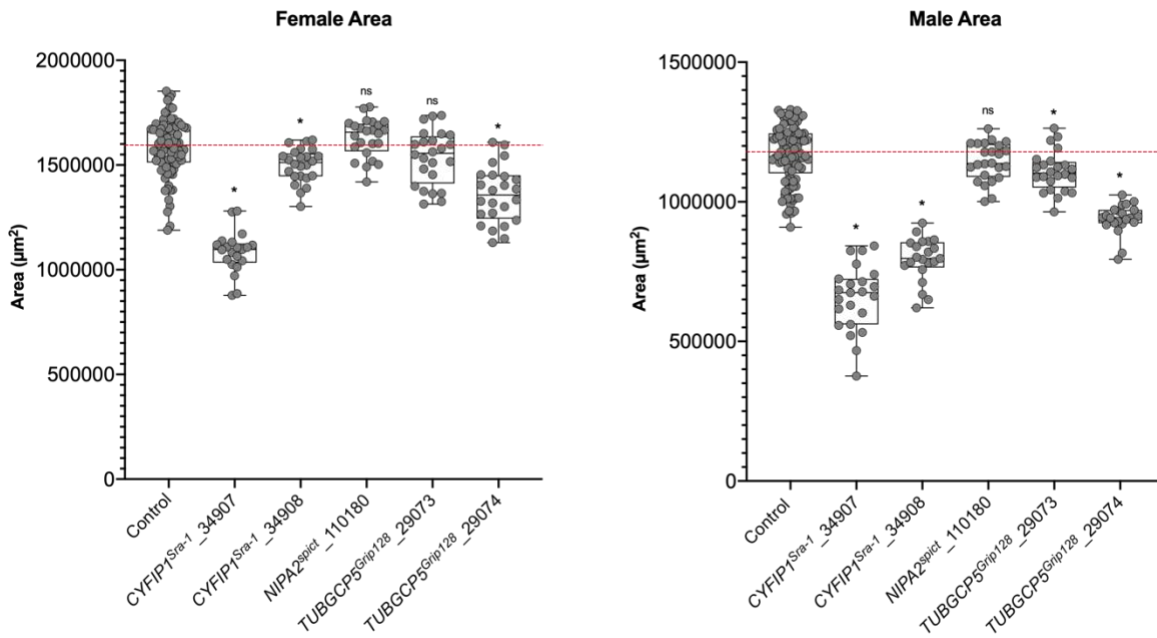


Figure 8: 15q11.2 Wing Area Boxplots.

15q13.3

In this CNV region, 3 genes (*CHRNA7*, *MTMR10*, and *TRPM1*) across 8 RNAi lines in both sexes were examined. In females, 6 lines showed significant differences in wing areas compared to the controls, while in males, 7 lines showed such differences. Among both sexes and in all 3 RNAi lines for *CHRNA7*, there was a significant decrease in wing area.

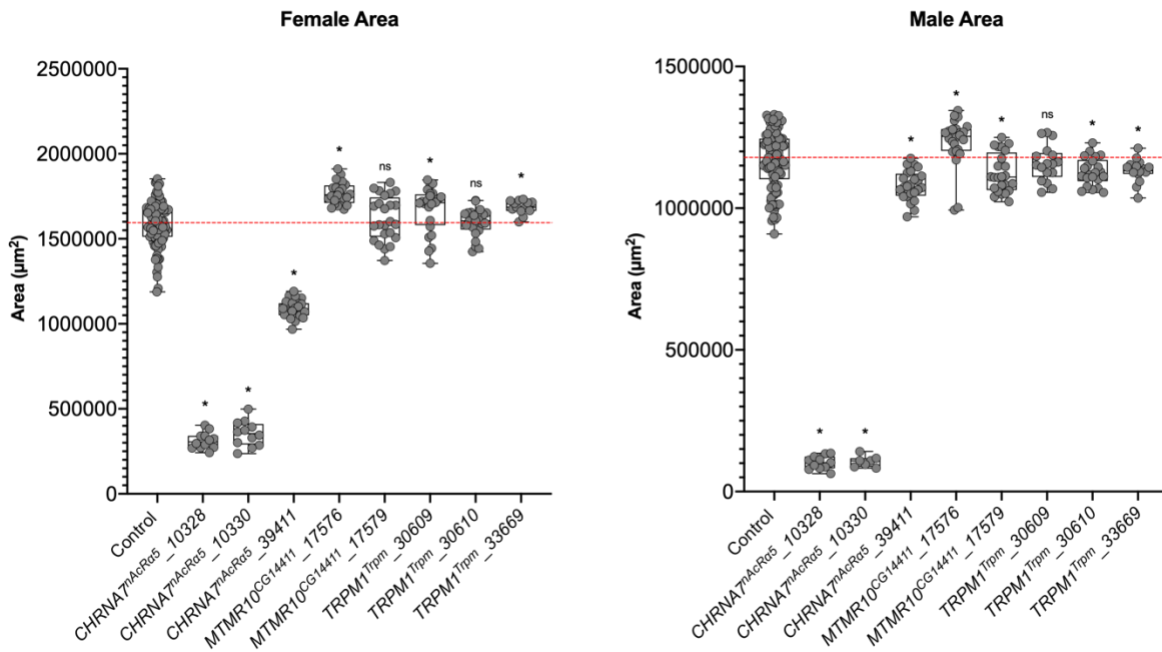


Figure 9: 15q13.3 Wing Area Boxplots.

16p11.2

In this region, 10 RNAi lines were analyzed corresponding to 10 genes (*ALDOA*, *PAGR1*, *CDIPT*, *CORO1A*, *DOC2A*, *FAM57B*, *KCTD13*, *MAPK3*, *PPP4C*, *TBX6*, and *YPEL3*) in both males and females. Among both males and females, all but 1 RNAi line (44672) showed significant differences in wing area or were lethal. The line 101339 for the gene *ALDOA* showed a statistically significant decrease in wing area among both males and females.

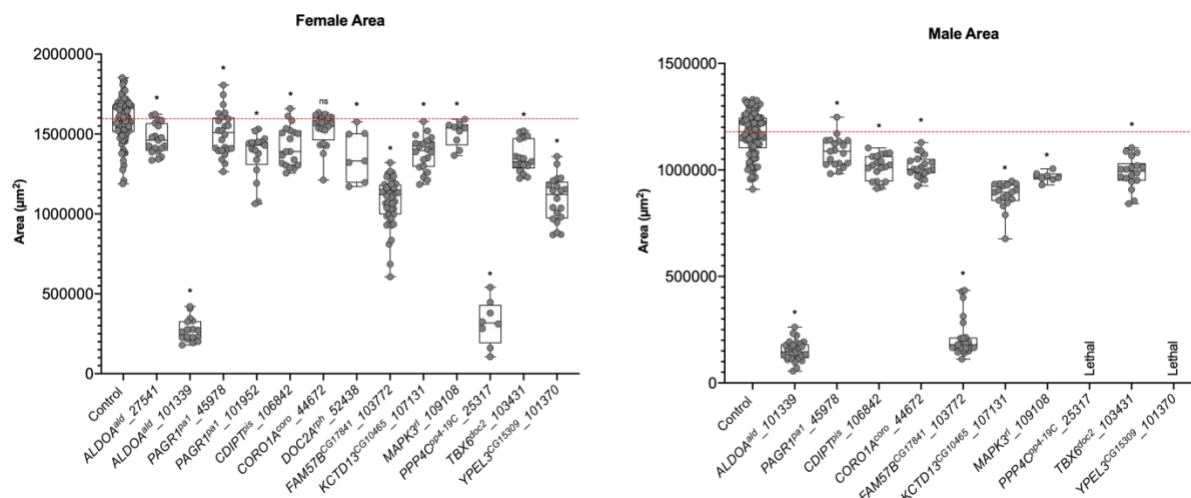


Figure 10: 16p11.2 Wing Area Boxplots.

16p11.2 Distal

In this region, 7 RNAi lines for 5 genes (*ATXN2L*, *CCDC101*, *SH2B1*, *SPNS1*, and *TUFM*) were analyzed for both males and females. All RNAi lines, except for 105462 for *SPNS1*, showed significant differences in area or exhibited lethality. Significant wing area decreases among both lines for *SH2B1* and *TUFM* among both sexes suggests involvement with wing pouch development. *ATXN2L* and *CCDC101* also showed concordance in this manner, with the one line for each gene exhibiting lethality or a significant decrease in wing area. However, with only 1 RNAi line for each gene, more confirmation is required to validate this result.

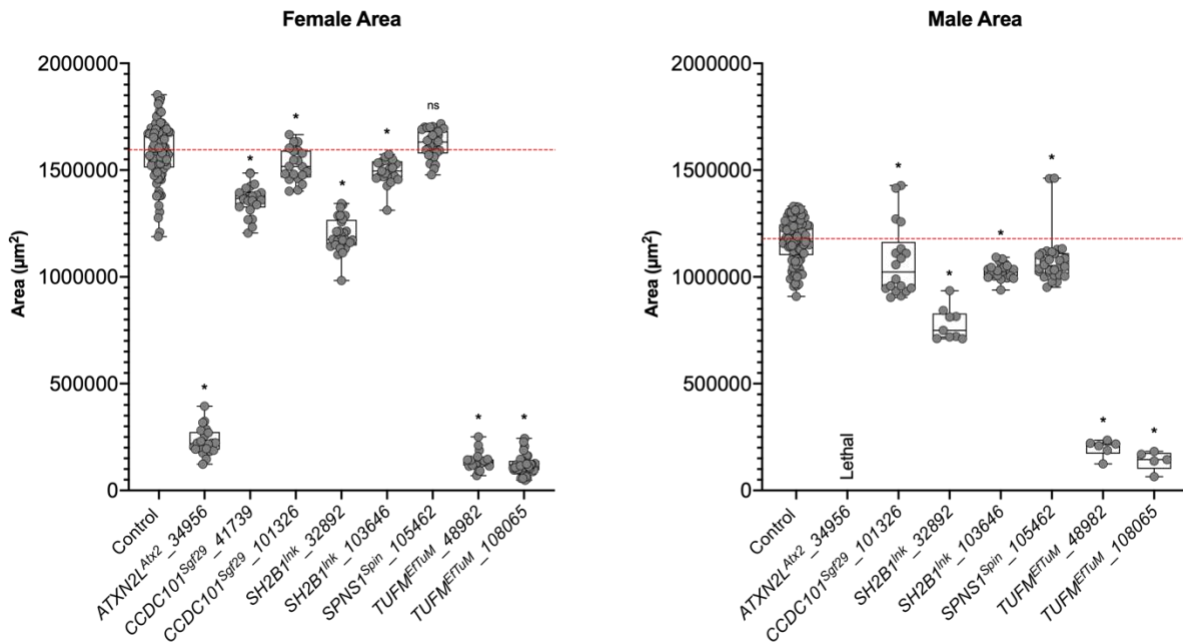


Figure 11: 16p11.2 Distal Wing Area Boxplots.

16p12.1

In this region, 5 RNAi lines were analyzed for 4 genes (*CDR2*, *MOSMO*, *POLR3E*, and *UQCRC2*) in male and female flies. In both males and females, all lines exhibited lethality or showed significant decreases in wing area. *MOSMO* showed significant decreases in area in line 5370, suggesting an involvement with development but requires confirmation with another line. *POLR3E* showed lethality in 1 line in both males and females, but also requires further confirmation. Notably, *UQCRC2* exhibited lethality in 2 of 3 lines in females and all 3 lines in males, with the sole line in females (26404) showing highly significant decreases in wing area. The concordant results among male and female flies suggest that *UQCRC2* may have an impact on wing pouch development.

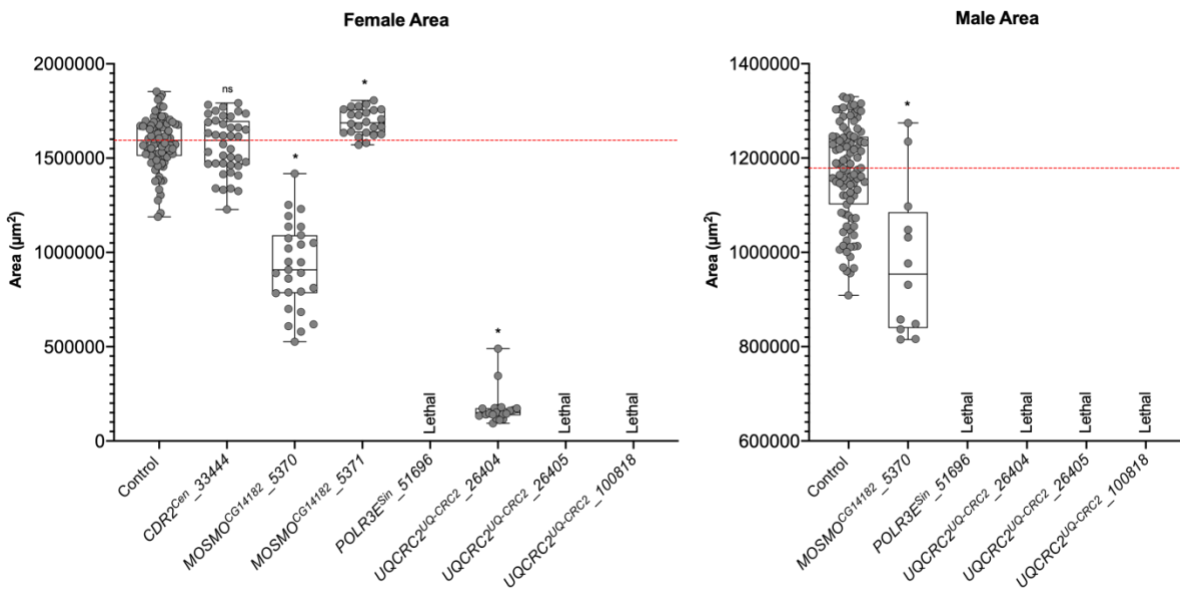


Figure 12: 16p12.1 Wing Area Boxplots.

16p13.11

In this region, 5 RNAi lines corresponding to 3 genes (*ABCC6*, *KIAA0430*, and *NDE1*) were analyzed in male and female flies. The only line that showed concordance among both males and females was line 29788 for *NDE1*, which exhibited a statistically significant large decrease in wing area compared to the controls. While some of the remaining lines showed small, statistically significant, decreases in area, none showed such large decreases in both sexes. Thus, in this region it can only be concluded that *NDE1* may impact wing pouch development.

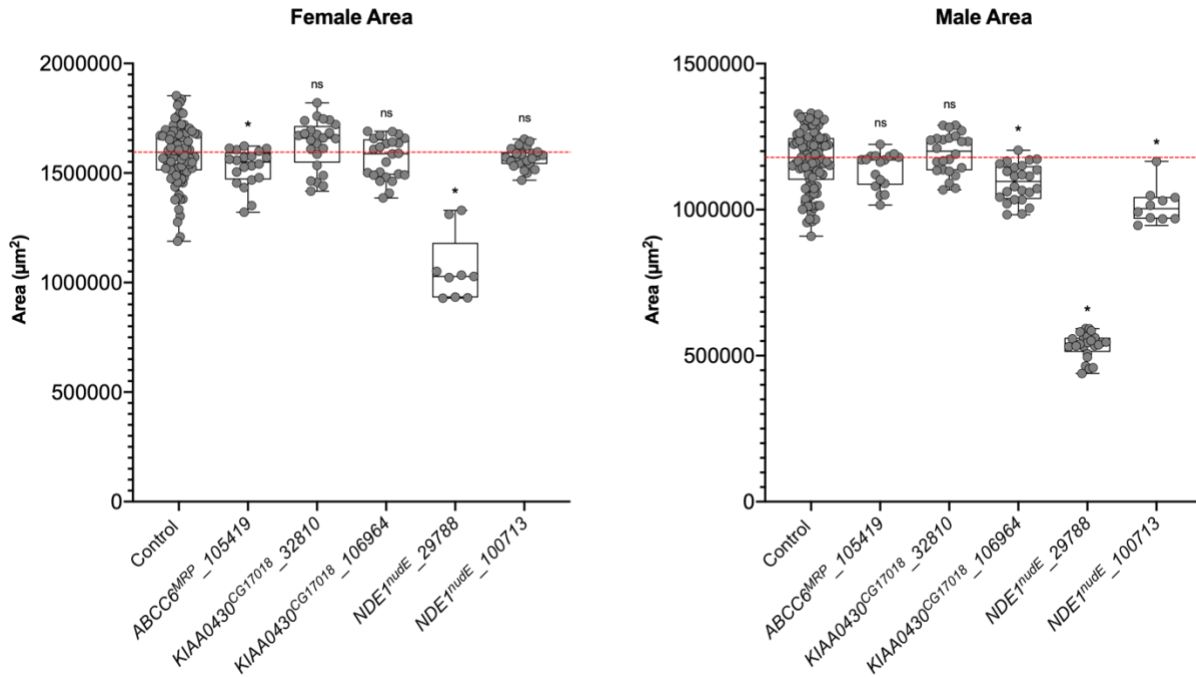


Figure 13: 16p13.11 Wing Area Boxplots.

17q12

In this region, 12 RNAi lines for 9 genes (*AATF*, *ACACA*, *DDX52*, *DHRS11*, *DUSP14*, *LHX1*, *PIGW*, *TADA2L*, and *ZNHIT3*) were analyzed for both males and females. One line in females (8105 for *ACACA*) and 5 lines in males (106977 for *AATF*, 8105 for *ACACA*, 44322 and 108642 for *DDX52*, 14865 for *DUSP*) exhibited lethality. All lines for *AATF*, *ACACA*, and *DDX52* in males exhibited lethality. Taken together, this is suggestive that these genes may be important in the development of the wing pouch. Additionally of note, 1 of 2 female lines and both male lines for *LHX1* resulted in a significant increase in wing area, suggesting this gene potentially affects the proliferation of cells in the development of the wing pouch.

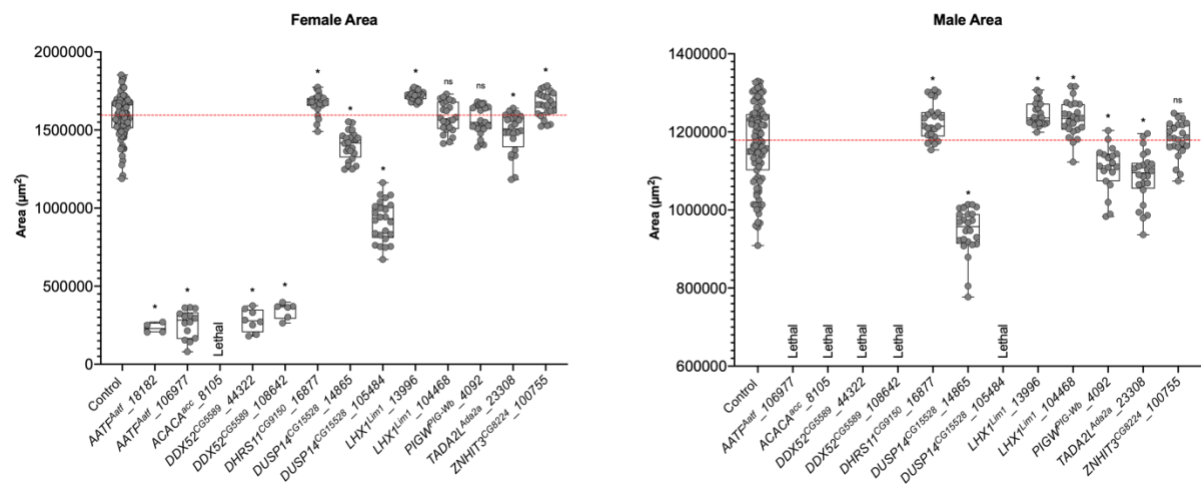


Figure 14: 17q12 Wing Area Boxplots.

DISCUSSION

This project sought to identify homologs of human CNV genes in *Drosophila* that are potentially involved with the development of the wing. Previous work by the Girirajan group has analyzed the role of these genes in the development of the neurons, particularly the eye¹²; however, these genes may also be of importance in the development of other non-neuronal tissue such as the wing. The results of this analysis indicate that the RNAi-induced knockdown of many of these genes was associated with changes in the wing area across a multitude of lines in one or both sexes, as summarized in Table 2. Twenty-nine genes among the 10 CNV regions and 3 NDD categories studied showed a majority of RNAi lines with concordant results, suggesting that these genes are possible candidates for involvement in fly wing pouch development, and potentially elucidate understanding of the development of human congenital defects associated with the neurodevelopmental disorders of the homologous genes in these regions.

Future experiments would include a robust categorization of other phenotypes exhibited, such as vein defects and abnormal bristle growth. The latter would require higher quality images to be captured of the wings to examine bristle polarity defects, for which there are tools available such as FijiWings¹³ to assess such features. Additionally, further confirmation with additional stock lines for the genes tested would be able to confirm discordant results and for genes for which concordant results were observed with only 1 RNAi line available. The stocks used in this experiment from the Vienna *Drosophila* Resource Center include stocks from the GD and KK libraries; however, part of a newer library of stocks called short hairpin RNA (shRNA) is available

with additional lines under development and should be explored if there are lines corresponding to genes of interest.⁵

Table 2: Summary of Significant Findings. Genes highlighted in yellow indicate a majority of lines showed consistency in area effects and/or lethality. Discordant lines are marked, reflecting the presence of both larger and smaller area effects.

CNV Region / Categorization	<i>H. sapiens</i> Gene	<i>D. mel.</i> Gene	# RNAi Lines (M/F total)	Larger Area	Smaller Area	Lethal	Discordant
Core genes	<i>CADPS2</i>	<i>Cadps</i>	4		2 M		
	<i>CHD8</i>	<i>Kis</i>	2		1 F, 1 M		
	<i>SCN1A</i>	<i>para</i>	8		1 F, 4 M		
	<i>SHANK3</i>	<i>prosap</i>	4		2 F, 2 M		
	<i>SLC6A1</i>	<i>Gat</i>	2		1 M		
	<i>SUCLG2</i>	<i>Sucb</i>	2				
	<i>TBX1</i>	<i>org-1</i>	2		1 F, 1 M		
	<i>UBE3A</i>	<i>Ube3a</i>	4	1 F	1 F, 1 M		x
Microcephaly	<i>ASPM</i>	<i>Asp</i>	4		2 F, 1 M		
	<i>CENPJ</i>	<i>Sas-4</i>	4		1 F, 2 M		
	<i>CEP135</i>	<i>Cep135</i>	4	1 F			
	<i>KIF11</i>	<i>Klp61F</i>	2		1 F	1 M	
	<i>MCPH1</i>	<i>MCPH1</i>	4		1 F, 1 M		
	<i>SLC25A19</i>	<i>Tpc1</i>	2		1 M		
	<i>TUBGCP6</i>	<i>Grip163</i>	4	1 F	1 F, 2 M		x
β -catenin	<i>CTNNB1</i>	<i>arm</i>	2		1 F, 1 M		
	<i>EPHB1</i>	<i>Eph</i>	5	1 F	1 M		x
	<i>LGR5</i>	<i>rk</i>	10		3 F, 5 M		
	<i>NRXN1</i>	<i>Nrx-1</i>	6	2 F	1 F, 1 M		x
	<i>PTEN</i>	<i>Pten</i>	4	1 F	1 M		x
1q21.1	<i>BCL9</i>	<i>lgs</i>	4		2 F, 2 M		
	<i>FMO5</i>	<i>Fmo-2</i>	6		2 F, 3 M		
3q29	<i>BDH1</i>	<i>CG8888</i>	4		2 F, 2 M		
	<i>DLG1</i>	<i>dlg1</i>	4	1 F	1 M	1 F, 1 M	x
	<i>FBXO45</i>	<i>Fsn</i>	2				
	<i>MF12</i>	<i>Tsf2</i>	2		1 F	1 M	
	<i>NCBP2</i>	<i>Cbp20</i>	1		1 F		
	<i>OSTalpha</i>	<i>CG6836</i>	2	1 F	1 M		x
	<i>PAK2</i>	<i>Pak</i>	2		1 F, 1 M		
	<i>PCYT1A</i>	<i>Pcyt1</i>	4		2 F, 2 M		
	<i>PIGX</i>	<i>PIG-X</i>	2		1 M		
	<i>PIGZ</i>	<i>PIG-Z</i>	2		1 M		
	<i>ZDHHC19</i>	<i>app</i>	4	1 F	1 F, 1 M	1 M	x
7q11.23	<i>ABHD11</i>	<i>CG2059</i>	2	1 F			
	<i>BCL7B</i>	<i>BCL7-like</i>	4		2 M		
	<i>CLIP2</i>	<i>CLIP-190</i>	2		1 F, 1 M		
	<i>DNAJC30</i>	<i>CG11035</i>	6		1 F, 2 M		
	<i>FKBP6</i>	<i>shu</i>	2		1 M		
	<i>LIMK1</i>	<i>limk1</i>	4		2 F, 2 M		
	<i>NSUN5</i>	<i>Nsun5</i>	6	2 F			
	<i>STX1A</i>	<i>syx1A</i>	4			1 F, 1 M	

CNV Region / Categorization	<i>H. sapiens</i> Gene	<i>D. mel.</i> Gene	# RNAi Lines (M/F total)	Larger Area	Smaller Area	Lethal	Discordant
15q11.2	<i>CYFIP1</i>	<i>Sra-1</i>	4		2 F, 2 M		
	<i>NIPA2</i>	<i>spict</i>	2				
	<i>TUBGCP5</i>	<i>Grip128</i>	4		1 F, 2 M		
15q13.3	<i>CHRNA7</i>	<i>nAChRalpha5</i>	6		3 F, 3 M		
	<i>MTMR10</i>	<i>CG14411</i>	4	1 F, 1 M	1 M		x
	<i>TRPM1</i>	<i>Trpm</i>	6	2 F	2 M		x
16p11.2	<i>ALDOA</i>	<i>Ald</i>	3		2 F, 1 M		
	<i>PAGR1</i>	<i>pal</i>	3		2 F, 1 M		
	<i>CDIPT</i>	<i>pis</i>	2		1 F, 1 M		
	<i>CORO1A</i>	<i>coro</i>	2		1 M		
	<i>DOC2A</i>	<i>rph</i>	1		1 F		
	<i>FAM57B</i>	<i>CG17841</i>	2		1 F, 1 M		
	<i>KCTD13</i>	<i>CG10465</i>	2		1 F, 1 M		
	<i>MAPK3</i>	<i>rl</i>	2		1 F, 1 M		
	<i>PPP4C</i>	<i>pp4-19C</i>	2		1 F	1 M	
16p11.2 distal	<i>TBX6</i>	<i>Doc2</i>	2		1 F, 1 M		
	<i>YPEL3</i>	<i>CG15309</i>	2		1 F	1 M	
	<i>ATXN2L</i>	<i>Atx2</i>	2		1 F	1 M	
	<i>CCDC101</i>	<i>Sgf29</i>	3		2 F, 1 M		
	<i>SH2B1</i>	<i>Lnk</i>	4		2 F, 2 M		
16p12.1	<i>SPNS1</i>	<i>Spin</i>	2		1 M		
	<i>TUFM</i>	<i>EfTuM</i>	4		2 F, 2 M		
	<i>CDR2</i>	<i>Cen</i>	1				
	<i>MOSMO</i>	<i>CG14182</i>	3	1 F	1 F, 1 M		x
16p13.11	<i>POLR3E</i>	<i>Sin</i>	1			1 F, 1 M	
	<i>UQCRC2</i>	<i>UQCR-C2</i>	6		1 F	2 F, 3 M	
	<i>ABCC6</i>	<i>MRP</i>	2		1 F		
17q12	<i>KIAA0430</i>	<i>CG17018</i>	4		1 M		
	<i>NDE1</i>	<i>nudE</i>	4		1 F, 2 M		
17q12	<i>AATF</i>	<i>Aatf</i>	3		2 F	1 M	
	<i>ACACA</i>	<i>acc</i>	2			1 F, 1 M	
	<i>DDX52</i>	<i>CG5589</i>	4		2 F	2 M	
	<i>DHRS11</i>	<i>CG9150</i>	2	1 F, 1 M			
	<i>DUSP14</i>	<i>CG15528</i>	4		2 F, 1 M	1 M	
	<i>LHX1</i>	<i>Lim1</i>	4	1 F, 2 M			
	<i>PIGW</i>	<i>PIG-Wb</i>	2		1 M		
	<i>TADA2L</i>	<i>Ada2a</i>	2		1 F, 1 M		
	<i>ZNHIT3</i>	<i>CG8204</i>	2	1 F			

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