Drosophila models of pathogenic copy-number variant genes show global and non-neuronal defects during development

Tanzeen Yusuff[1], Matthew Jensen[1], Sneha Yennawar[1], Lucilla Pizzo[1], Siddharth Karthikeyan[1], Dagny J. Gould[1], Avik Sarker[1], Yurika Matsui[1,2], Janani Iyer[1], Zhi-Chun Lai[1,2], and Santhosh Girirajan[1]

1) Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA;
2) Department of Biology, Pennsylvania State University, University Park, PA
3) Department of Anthropology, The Pennsylvania State University, University Park, PA.

INTRODUCTION

Rare copy-number variants (CNV), or deletions and duplications in the genome, are associated with neurodevelopmental disorders such as autism, intellectual disability (ID), and schizophrenia. While dosage alteration of CNV regions contribute predominantly to defects in nervous system development, several CNV-associated disorders also lead to early developmental features in other organ systems, including cardiac defects, kidney malformations, and skeletal abnormalities. Functional studies evaluating these regions have focused on the molecular basis of neuronal defects, implicating novel genes towards specific neuropsychiatric disorders. However, our understanding of how these genes contribute towards non-neuronal phenotypes is still limited. In particular, the *Drosophila* wing is useful to assess how homologs of human disease genes alter conserved cellular and developmental mechanisms, as key components of conserved signaling pathways were identified using fly wing models. Here, we examined fly homologs for 79 human neurodevelopmental genes, and found that they contribute towards global and tissue-specific developmental phenotypes mediated by conserved cellular processes and signaling pathways.

RESULTS

Knockdown of fly homologs of CNV genes contribute to a range of adult wing developmental defects

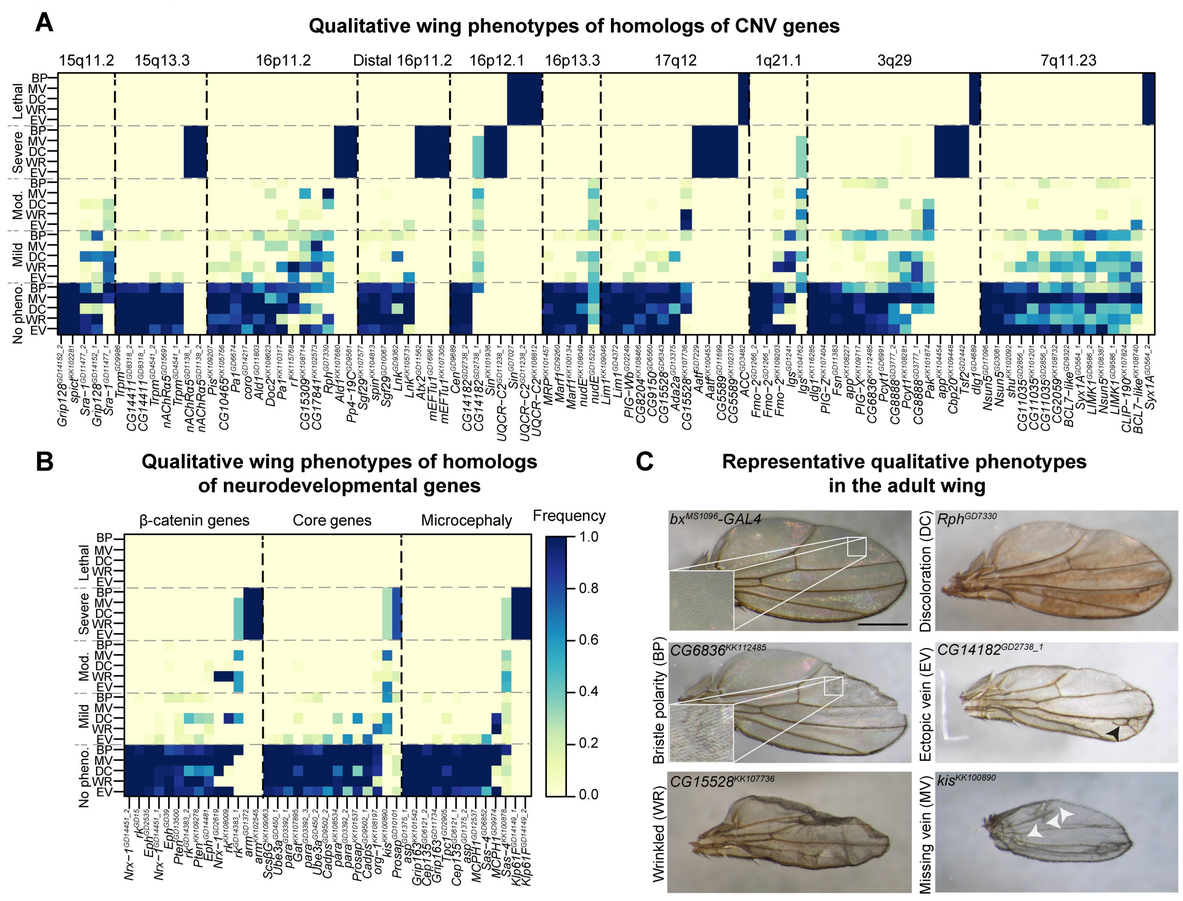

Figure 1. Heatmaps representing five qualitative adult wing phenotypes (wrinkled wings, WR; ectopic veins, EV; missing veins, MV; discoloration, DC; bristle planar polarity, BP) for 136 tested RNAi lines, with **(A)** 59 tested homologs for 10 CNV regions and **(B)** 20 homologs for neurodevelopmental-associated genes (β-catenin, core neurodevelopmental genes, and microcephaly genes), are shown. The color of each cell represents the frequency of each qualitative phenotype by severity, ranging from no phenotype to lethality. **(C)** Representative brightfield images of adult fly wings (scale bar = 500μm) with wing-specific knockdown of homologs of CNV and neurodevelopmental genes show the five assessed qualitative phenotypes, including discoloration, wrinkled wings, bristle polarity, ectopic veins, and missing veins.

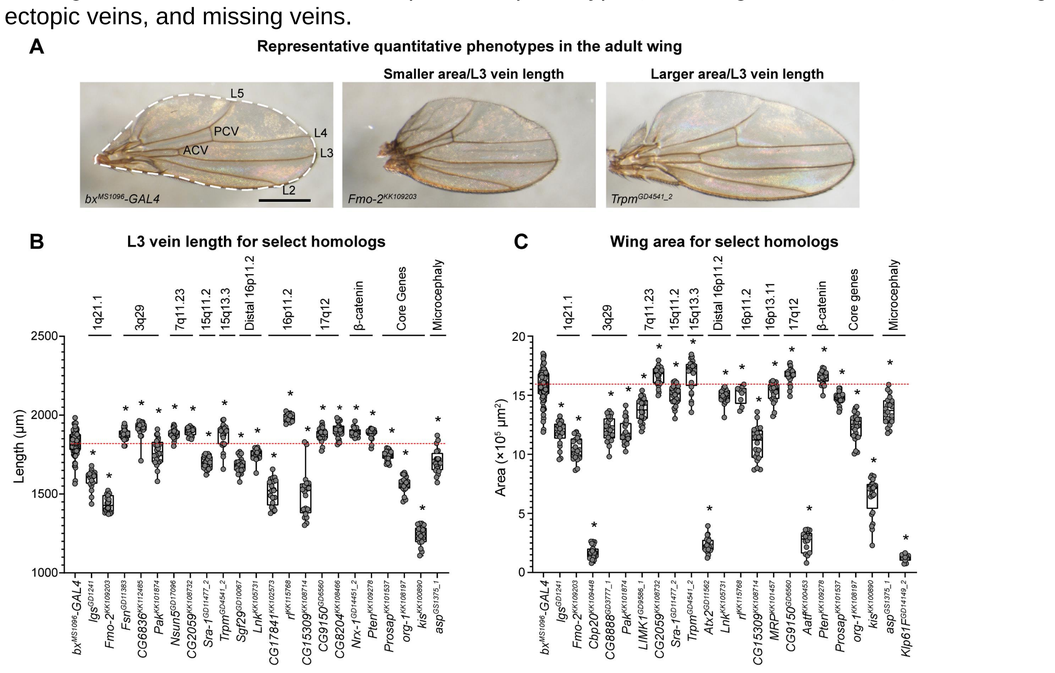

Figure 2. (A) Representative brightfield images show adult fly wings (scale bar = 500μm) with wing-specific knockdown of homologs of CNV and neurodevelopmental genes with size defects. **(B)** Boxplot of L3 vein lengths for knockdown of select homologs in adult fly wings (n = 9-91, *p < 0.05, two-tailed Mann–Whitney test with Benjamini-Hochberg correction). **(C)** Boxplot of wing areas for knockdown of select homologs in adult fly wings (n = 9-91, *p < 0.05, two-tailed Mann–Whitney test with Benjamini-Hochberg correction). Boxplots indicate median (center line), 25th and 75th percentiles (bounds of box), and minimum and maximum (whiskers), with red dotted lines representing the control median.

RESULTS

Homologs of CNV genes show tissue-specific developmental effects

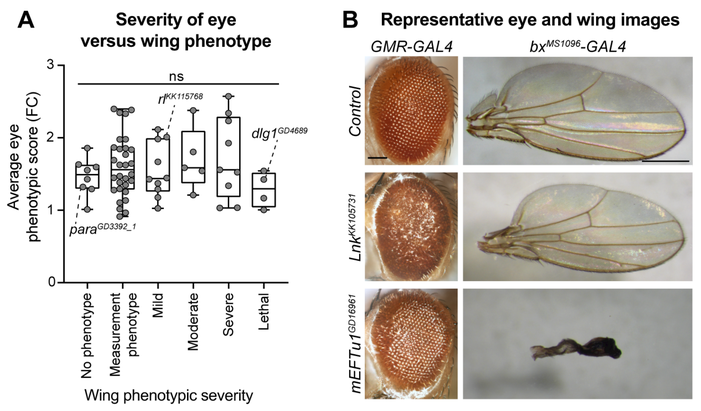

Figure 3. (A) Boxplot showing average eye phenotypic scores for 66 RNAi lines of select fly homologs, normalized as fold-change (FC) to control values, according to wing phenotypic severity (n=4–30 RNAi lines per group). No significant changes in eye phenotype severity were found across the five wing phenotypic categories (Kruskal-Wallis test, p=0.567, df = 5, $\chi 2 = 3.881$). Examples of average eye phenotypic scores for RNAi lines with no phenotype, mild, and lethal wing phenotypes are highlighted in the plot. **(B)** Representative brightfield adult eye (scale bar = 100 μm) and wing (scale bar = 500μm) images show tissue-specific effects due to knockdown of CNV homologs.

Knockdown of homologs of CNV genes disrupt conserved cellular processes and signaling pathways

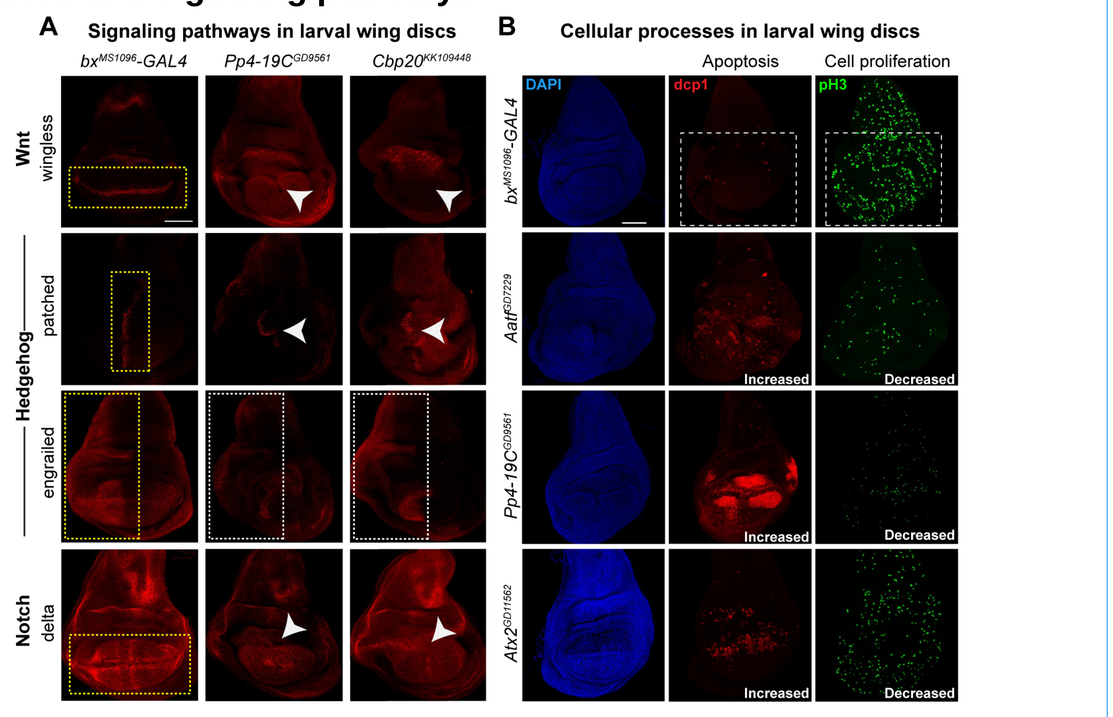

Figure 4. (A) Larval imaginal wing discs (scale bar = 50 μm) stained with wingless, patched, engrailed, and delta illustrate disrupted expression patterns for proteins located within the Wnt (wingless), Hedgehog (patched and engrailed), and Notch (delta) signaling pathways due to wing-specific knockdown of select fly homologs. White arrowheads and dotted white boxes highlight disruptions in expression patterns. **(B)** Larval imaginal wing discs (scale bar = 50 μm) stained with nuclear marker DAPI, apoptosis marker dcp1, and cell proliferation marker pH3 illustrate altered levels of apoptosis and cell proliferation due to wing-specific knockdown of select fly homologs of CNV genes.

Connectivity of human CNV genes with conserved signaling pathway genes vary across human tissue-specific networks

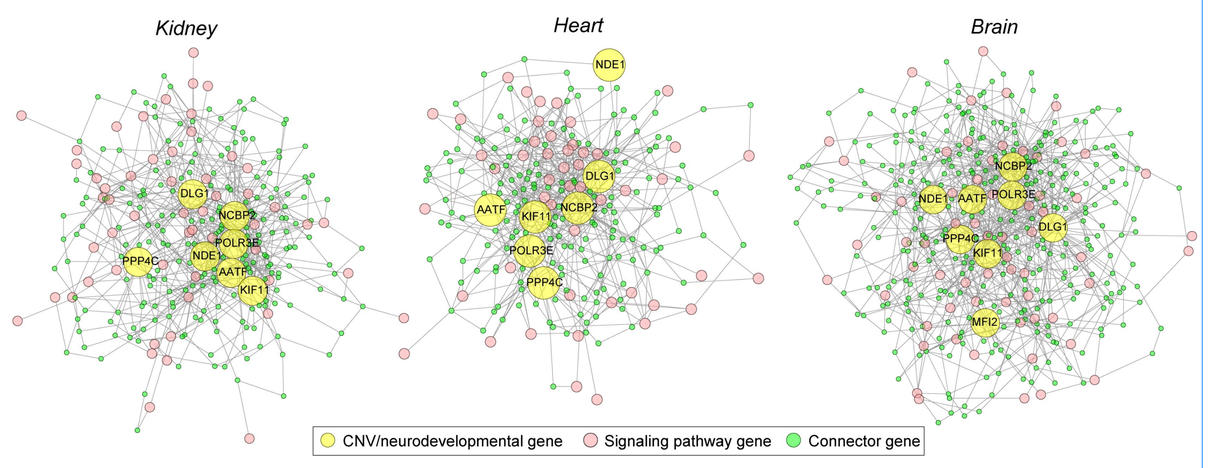

Figure 5. Diagrams representing the connectivity of eight human CNV and neurodevelopmental genes whose fly homologs disrupt the Notch signaling pathway to 57 human Notch signaling genes within kidney, heart, and brain-specific gene interaction networks are shown. Yellow nodes represent CNV and neurodevelopmental genes, pink nodes represent Notch signaling pathway genes, and green nodes represent connector genes located within the shortest paths between CNV and Notch pathway genes.

SUMMARY

- Wing-specific knockdown of 72/79 homologs CNV and neurodevelopmental genes showed qualitative and quantitative phenotypes in the *Drosophila* adult wing, including 21 lines with severe wing defects and six lines with lethality.
- Comparisons between eye and wing-specific knockdown of 37/45 homologs showed both neuronal and non-neuronal defects, but with no correlation in the severity of defects.
- Disruptions in cell proliferation and apoptosis in larval wing discs for 23/27 homologs, and altered Wnt, Hedgehog and Notch signaling for 9/14 homologs, including *AATF/Aatf*, *PPP4C/Pp4-19C*, and *KIF11/Klp61F*. These finding are further supported by tissue-specific differences in network connectivity of CNV genes to signaling pathway genes in the brain, heart and kidney.

Acknowledgements

This work was supported by NIH R01-GM121907 and resources from the Huck Institutes of the Life Sciences to S.G., and NIH T32-GM102057 to M.J. This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the DECIPHER project was provided by the Wellcome Trust.