Selective vulnerability of Dopaminergic neurons revealed by Genome-wide analysis

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Abstract

However, little is known about why DA neurons are selectively vulnerable to PD [2]. To identify genes that are associated with DA neuron loss, we screened through over 200 wild-caught populations of *Drosophila melanogaster* as part of the Drosophila Genetic Reference Panel (DGRP) [5]. Here we identify the top associated genes containing SNPs that render DA neurons vulnerable. We tested these genes further by using mutant analysis and tissue-specific knockdown for functional validation. We found that this loss of DA neurons caused progressive locomotor dysfunction in mutants and gene knockdown analysis as well. We plan to investigate *Sestrin* and *CG42339,* two of the most significant candidate genes from our screen [1,3,4]. Further analysis of these genes should help to identify the factors that render DA neurons selectively vulnerable to PD.

Methods

Drosophila melanogaster brains were dissected and stained to assess DA neuron number.
Immunohistochemistry was performed by using Tyrosine hydroxylase antibody, which stains the DA neurons. Neuronal clusters were counted.
Locomotor behavior was depicted when using a climbing assay. Percentage of flies able to climb up to an 8cm mark on a glass vial in 20 seconds in groups of about 10 were recorded as climbing index.

Results

Genome wide screen to identify mutations that render DA neurons vulnerable

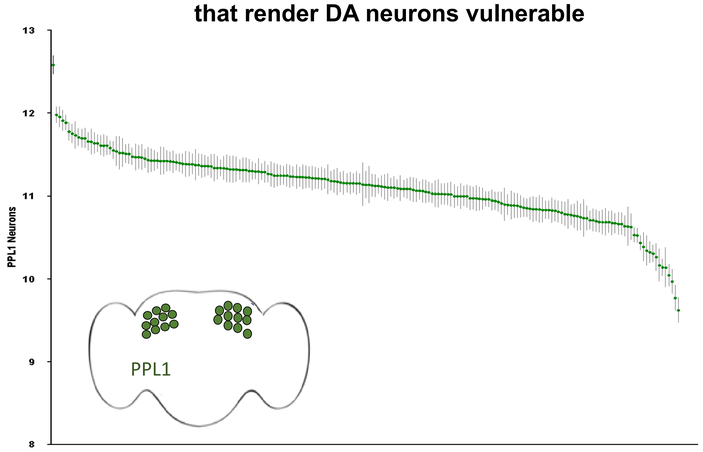


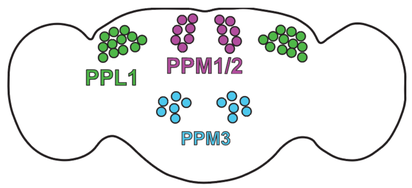
Figure 1. Vulnerability of DA neurons various across genetic backgrounds. Green dots measure the average number of PPL1 per cluster in each of the 201 DGRP fly stocks at Day 21. Genes harboring SNPs most significantly associated with fewer DA neurons are listed in Table 1.

Table 1. Top Candidate Genes

Rank	Candidate Gene	SNP[a]	P-Value[a]	Human Ortholog	OMIM
1	*tow*	3L:6937595	2.56E-07	C1orf21	---------
2	*CG42339*	X:10933694	4.32E-07	SBSPON	---------
3	*megalin*	X:9318757	8.05E-07	LRP2	600073
4	*plexus*	2R:18423826	1.06E-06	none	---------
5	*Trf2*	X:8325561	1.78E-06	TBPL1	605521
6	*kirre*	X:2800634	2.77E-06	KIRREL	607428
7	*tweek*	2L:16900031	3.24E-06	KIAA1109	611565
8	*sestrin*	2R:19614642	3.40E-06	SESN3	607768
9	*Lim3*	2L:19086842	9.20E-06	LHX3	600577

[a] SNP with the most statistically significant association for this candidate gene.

Functional validation of DGRP candidate genes leads to progressive loss of DA neurons



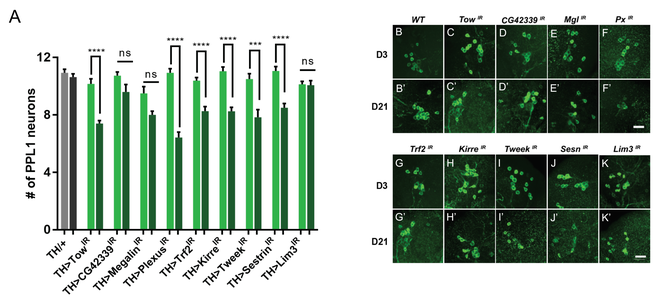


Figure 2. Loss of DA neurons in the PPL1 cluster is significant upon knockdown of candidate genes (A-J) shows the cluster containing PPL1 neurons in the order of Wildtype, *Tow, CG42339, Megalin, Plexus, Trf2, Tweek, Sestrin*, and *Lim3* at Day 3 and Day 21. ***, p < 0.001 ****, p < 0.0001.

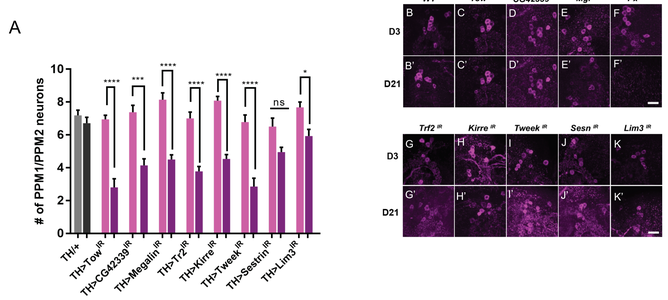


Figure 3. Loss of PPM1/2 DA neurons is significant upon knockdown of candidate genes. (A-J) shows the cluster containing PPM1/ 2 neurons in the order of Wildtype, *Tow, CG42339, Megalin, Plexus, Trf2, Tweek, Sestrin*, and *Lim3* Day 3 and Day 21. *, p <0.05 ****, p < 0.0001.

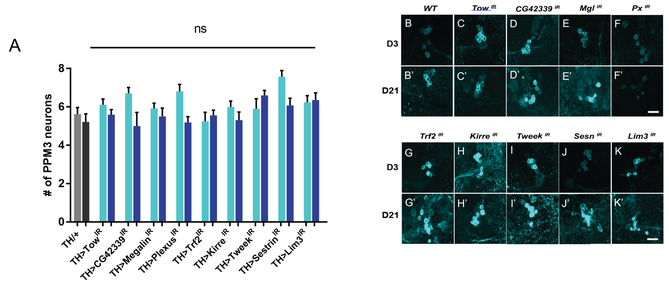


Figure 4. No significant loss of DA neurons in the PPM3 cluster upon knockdown of candidate genes. (A-J) shows the control with Wildtype, *Tow, CG42339, Megalin, Plexus, Trf2, Tweek, Sestrin*, and *Lim3* at Day 3 and Day 21.

Progressive neurodegeneration in *Sestrin* and *CG42339* mutants

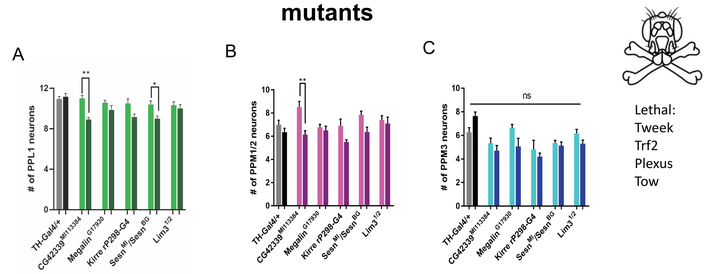


Figure 5. Significant loss of DA neurons for select genes in clusters PPM1/2 and PPL1. (A) Significant loss of DA neurons in the PPL1 cluster for *CG42339* and *Sesn* double mutants. (B) shows a loss of PPM1/2 neurons for *CG42339.* (C) There is no significant loss of neurons in the PPM3 cluster. Day 3 and Day 21 were compared for all mutants. *, p <0.05 **, p <0.01.

Locomotor dysfunction found in mutant and knockdown in the DA neurons

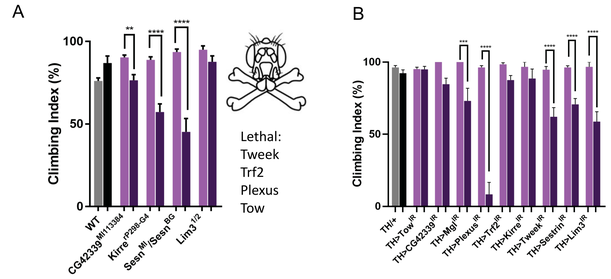


Figure 6. Progressive locomotor dysfunction found when candidate genes are not maintained. (A) Locomotor dysfunction observed in *CG42339^{MI113384}*, *Kirre*, *Sesn* double mutants at D21 (B) Dysfunction found in *Megalin, Plexus, Tweek, Sesn,* and *Lim3* knockdown in DA neurons.***, p <0.001 ****, p <0.0001.

Conclusions and Future Directions

200 lines were screened from the Drosophila Genome Reference Panel and 9 candidate genes were identified that caused the loss of DA neurons.
In the future we plan to further characterize our genes of interest based on these data collected.
Sestrin and *CG42339* were two genes we are further characterizing based on the mutant, knockdown and climbing data that has been collected.

Acknowledgements

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References

Araque, A., & Navarrete, M. (2010). Glial cells in neuronal network function. *Philosophical Transactions of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rstb.2009.0313
Bayersdorfer, F., Voigt, A., Schneuwly, S., & Botella, J. A. (2010). Dopamine-dependent neurodegeneration in Drosophila models of familial and sporadic Parkinson's disease. *Neurobiology of Disease*. https://doi.org/10.1016/j.nbd.2010.02.012
Kraneveld, A. D., de Theije, C. G. M., van Heesch, F., Borre, Y., de Kivit, S., Olivier, B., … Garssen, J. (2014). The neuro-immune axis: Prospect for novel treatments for mental disorders. *Basic and Clinical Pharmacology and Toxicology*. https://doi.org/10.1111/bcpt.12154
Lee, J. H., Budanov, A. V., Park, E. J., Birse, R., Kim, T. E., Perkins, G. A., … Karin, M. (2010). Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. *Science*. https://doi.org/10.1126/science.1182228
MacKay, T. F. C., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., Zhu, D., … Gibbs, R. A. (2012). The Drosophila melanogaster Genetic Reference Panel. *Nature*. https://doi.org/10.1038/nature10811