Characterizing Alzheimer's in a *Drosophila* Model Carrying Synthetic Mutations of Amyloid Plaques

Kaliah Wood[1], Brett Chan[1], Aahwan Koirala[1], Olivia Nardell[1], Caroline Yu[1], Jorge Moran[1], Jordan Sitea[1], Jesus Quiroz[1]
Maria Sajimon[2], Jevgenij Raskatov[2], Jeremy Lee[1]
[1]Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz
[2]Department of Chemistry and Biochemistry, University of California, Santa Cruz

Abstract

Alzheimer's disease is characterized by reduced or impaired memory and motor function, affecting millions of mostly elderly patients every year around the world. Alzheimer's disease is associated with the cleavage products of the amyloid precursor protein, especially the amyloid beta 42 (Aβ42) product, which aggregates and disrupts neuron functions. Recent research by Jevgenij Raskatov's lab in Chemistry at UCSC has identified 2 specific missense mutations—Q15E and N27D—in Aβ42 that resulted in changes in its aggregation and reduced cytotoxic properties when tested *in vitro* on SH-SY5Y (neuroblastoma cells). Research by my research team has generated flies that express these altered forms of Aβ; we will compare the effects of these peptides to the effects of expressing wild type Aβ, which we have shown has deleterious effects on lifespan, eye neurons, behavior, and gene expression. Transgenic flies expressing Aβ Q15E and N27D—individually and together—will be compared to flies expressing wild type Aβ; specifically, we will conduct olfactory learning and climbing assays for hypothesized improvements in learning, memory, and motor function alongside lifespan assays. Scanning electron microscopy will visualize the developmental differences in the eyes with the use of a GMR-GAL4 driver, which drives expression of the transgene in eye neurons. Additionally, RNA sequencing will quantify and compare gene expression amongst the various lines—correlating expression with observations. This research aims to advance the understanding of Alzheimer's disease by elucidating the roles of specific Aβ structural components and their role in the neurodegenerative process, potentially guiding future treatments.

Background

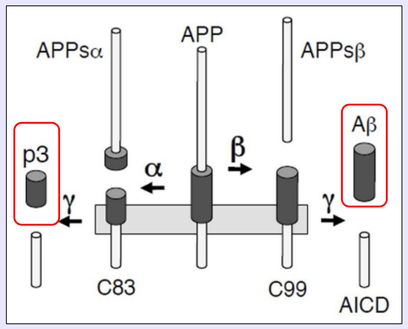

Figure 1: APP cleavage pathways producing Aα also known as P3(left) & Aβ(right)

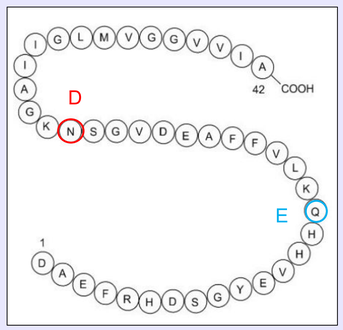

Figure 2: Amino Acid Sequence of $Aβ_{42}$ with highlighted mutations discovered by Jevgenij Raskatov

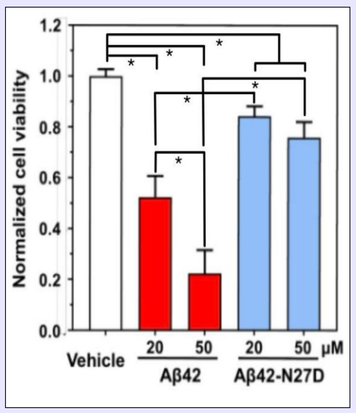

Figure 3: Neurotoxicity of WT $Aβ_{42}$ (red) vs $Aβ_{42}$-N27D (blue) against SH-SY5Y cells at 20 & 50 μM, p<0.05 (Unpublished data from the Raskatov lab at UCSC)

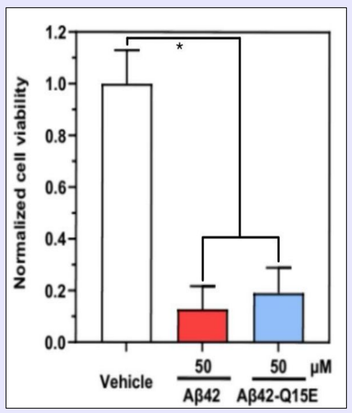

Figure 4: Neurotoxicity of WT $Aβ_{42}$ (red) vs $Aβ_{42}$-Q15E (blue) against SH-SY5Y cells at 50 μM, p<0.05 (Unpublished data from the Raskatov lab at UCSC)

Methods

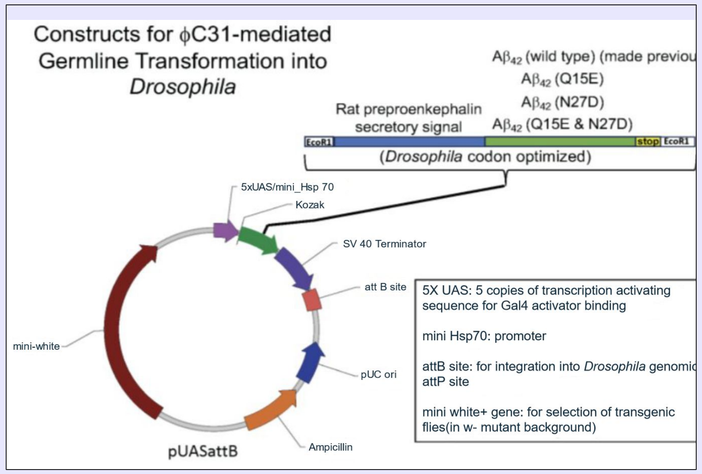

Figure 5: Aβ transgenic constructs for creating *Drosophila melanogaster* expressing synthetic mutations

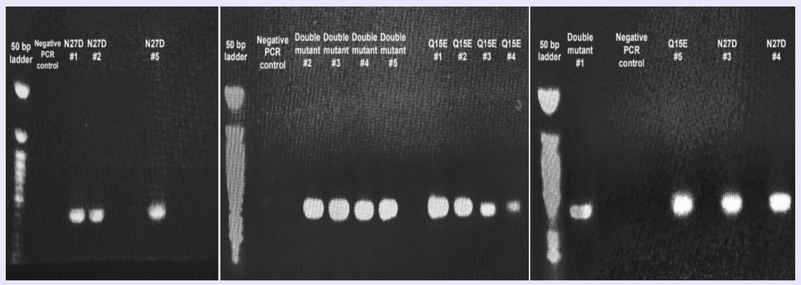

Figure 6: PCR gel for the 5 UAS transgenic fly lines (15 lines) confirming insertion of transgenes

Previous Results

Figure 7a-e: Scanning Electron Microscopy (S.E.M) performed on fly eyes 3 days after eclosion at 4000x to visualize ommatidia development

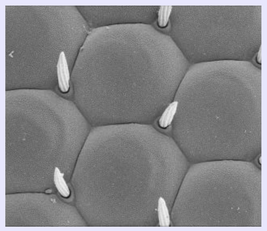

Figure 7a: +/+ control

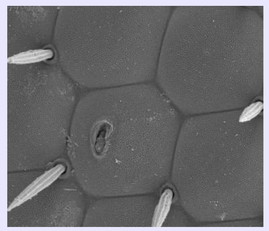

Figure 7b: Aβ/+

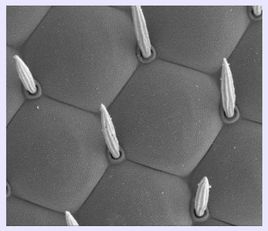

Figure 7c: Aβ-N27D/+

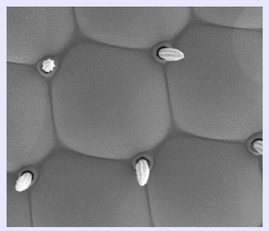

Figure 7d: Aβ-Q15E/+

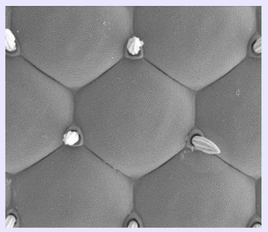

Figure 7e: Aβ-Q15E,N27D

These results were not conclusive, and the scanning electron microscopy will be redone at 20 days after eclosion.

Next Steps

Our team knows that Aβ causes negative outcomes or effects in locomotor abilities, lifespan, and eye development in *Drosophila*. Olfaction effects are not clear.

Fly lines are as follows: (1) +/+ (2) Aβ/+ (3) Aβ-N27D/+ (4) Aβ-Q15E/+ (5) Aβ-Q15E,N27D. These five lines will undergo a series of assays and analyses to compare and further characterize the effects of Aβ mutations to wildtype (Aβ/+) and nonexpressing (+/+) lines.

Assays and Analyses:

Olfactory assay: Will test how well different lines respond to odors to characterize their memory and learning.

Climbing assay: Instinctively, flies climb upwards, this assay tests that instinct, their locomotor abilities, and behavior which will characterize hypothesized improvement in transgenic flies.

Longevity assay: Aβ decreases lifespan, transgenic flies' lifespans will be monitored to test the hypothesized improvement in lifespan due to the mutations.

Scanning Electron Microscopy (S.E.M): Previously done on the 5 lines at 3 days after eclosion. Results were not conclusive, so now this analysis will visualize the ommatidia at 20 days after eclosion to give genes more time to express/regulate over the flies' lives to draw a stronger correlation between improvements in eye development and mutations.

RNA-Seq: This will confirm gene expression and relatively quantify their expression levels.

References

Kuhn, A. J., Abrams, B. S., Knowlton, S., & Raskatov, J. A. (2020). Alzheimer's Disease "*Non-amyloidogenic*" p3 Peptide Revisited: A Case for Amyloid-α. ACS Chemical *Neuroscience*, 11(11), 1539–1544. https://doi.org/10.1021/acschemneuro.0c00160

Lichtenthaler, SF. "Alpha-Secretase Cleavage of the Amyloid Precursor Protein: Proteolysis Regulated by Signaling Pathways and Protein Trafficking." *Alpha-Secretase Cleavage of the Amyloid Precursor Protein: Proteolysis Regulated by Signaling Pathways and Protein Trafficking - Ludwig-Maximilians-Universität, Chair of Metabolic Biochemistry - LMU Munich*, 23 May 2011,www.biochemie.abi.med.uni-muenchen.de/news/publications/20110523/index.html.

"Amyloid β-Peptide (1-42) (Human)." APExBIO, 2013, www.apexbt.com/amyloid-peptide-1-42-human.html.

Acknowledgements

Dr. Tom Yuzvinsky for assistance with sample preparation and electron microscopy and the W.M. Keck Center for Nanoscale Optofluidics for use of the FEI Quanta 3D Dualbeam microscope.

STEM Diversity Research Program's PBSci Dean's Diversity Fund, Jesus Quiroz

Undergraduate Research in Science and Technology, Kaliah Wood

Cowell Student Project Fund, Olivia Nardell