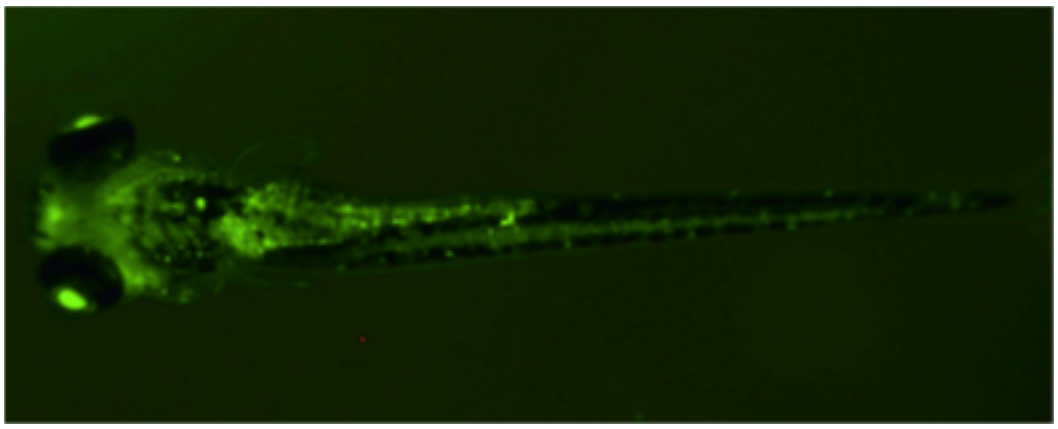
Examining the Relationship Between Type II Diabetes and Alzheimer's Disease

Using Transgenic *Danio rerio* as a Model to Study the Effects of Hyperglycemia on the Brain



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Abstract:

The Notch signaling pathway is crucial in embryonic development processes that determines cell fates, including cellular maturation of the brain. In adults, Notch is typically dormant; however, studies have found an increase in Notch signaling following traumatic brain injury. Additionally, irregular Notch signaling has been implicated in the neuropathology of Alzheimer's Disease (AD). Type II Diabetes Mellitus (T2DM) and AD share cellular and neurological deficiencies including oxidative stress, inflammation, and structural changes in the hippocampus- in addition, DM is a risk factor for AD. Our investigation will study changes in Notch signaling in the hyperglycemic brain of larval and adult zebrafish containing a Notch-GFP transgenic reporter line. To test the hypothesis that aberrant Notch signaling is involved in T2DM, we will expose zebrafish to glucose and analyze changes in Notch signaling using Western Blots, qPCR and Fluorescence Microscopy.

Proposal:

Notch is an essential protein in development and tissue renewal and helps decide the fate of developing cells-including cellular maturation in the vertebrate brain (Kopan & Ilagan, 2009 ;Selkoe & Kopan, 2003). This pathway is primarily responsible for short range communication and is classified as a single pass type I transmembrane receptor (Kopan & Ilagan, 2009). A cascade of proteins activate Notch, which then enters the nucleus to initiate transcription factors to express specific Notch-responsive genes. Apart from its role in development, evidence shows that Notch signaling is also involved in neuronal apoptosis, neurite retraction, and neurodegeneration of ischemic stroke in the brain (5). Further understanding of the Notch signaling mechanism will help to clarify how pathological events are regulated by cellular responses transduced by Notch-mediated gene regulation. Despite active studies of the developmental functions of Notch signaling, the role of Notch in adults is not well known.

Notch signaling is of great interest to researchers because of its previous demonstrated role in neurodegenerative diseases such as AD and traumatic brain injury events. Irregular Notch signaling is involved in cleavage of the amyloid precursor protein (APP) which generates a neurotoxic amyloid beta-peptide (Ab) that is thought to be associated with the neurodegeneration observed in AD (5). Previous research has also found that Notch expression is elevated two fold in AD patients compared with match-controlled individuals (Woo et. al, 2009). However, the biological significance of increases or decreases in Notch signaling in patients who experience neurodegenerative diseases such as AD can be be further elucidated (Woo et. al, 2009). In addition to a role in AD, Notch signaling increases following traumatic brain injuries in zebrafish (Kishimoto et. al, 2012). Telencephalon injury prompted the proliferation of Notch-expressing neuronal precursor cells (NPCs) in the ventricular zone (VZ) of the injured hemisphere. The number of NPCs reaching the injury site significantly decreased when the fish were treated with an inhibitor of γ-secretase, a component of the Notch signaling pathway (Kishimoto et. al, 2012).

This study showed, that the adult zebrafish brain possesses a remarkable capacity for neuronal regeneration (Kishimoto et. al, 2012). Furthermore, this suggests that injury-induced neurogenesis mechanisms are at least partly conserved between fish and mammals (Kishimoto et. al, 2012).

Type II Diabetes Mellitus (T2DM) is characterized by hyperglycemia, decreased production of insulin or its availability, and insulin resistance (Nazareth et. al, 2017). Type 2 Diabetes (T2DM) and AD share cellular and neurological deficiencies including oxidative stress, inflammation, and structural changes in the hippocampus - in addition, DM is a risk factor for AD. It has been shown that the the metabolic changes that occur in T2DM can increase the presence of Beta Amyloid (BA) plaques, hyperphosphorylated tau proteins and loss of neurons, which is consistent with the progression of AD (Nazareth et. al, 2017). Although no studies have directly assessed the role of Notch signaling in T2DM, a previous study found that Notch inhibition promotes neovascularization in diabetic mice (Bonegio & Susztak, 2012). Additionally, Notch may induce apoptosis in these cells which would lead to neuronal death (one of the pathological events of AD). As such, we hypothesize Notch signaling may be altered in fish exposed to hyperglycemic conditions that model T2DM (Figure 1).

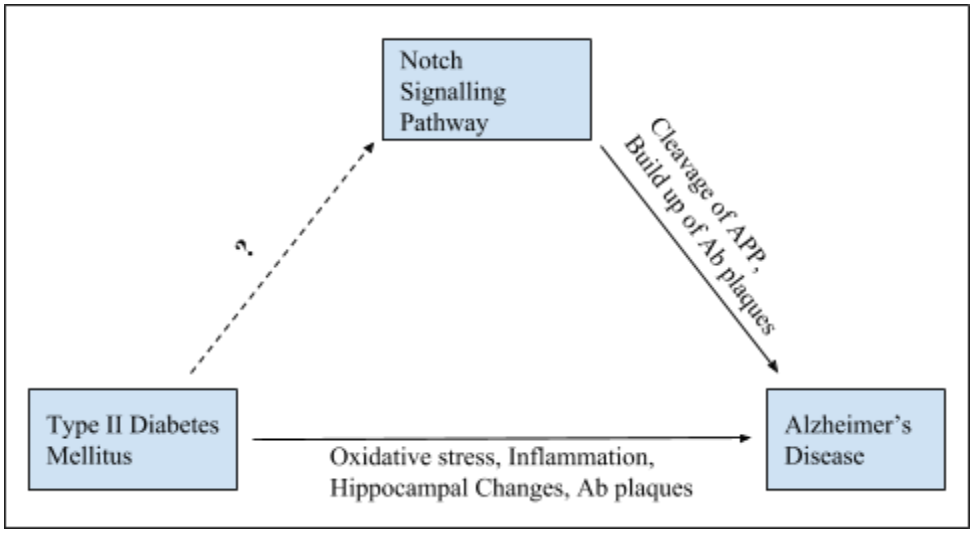

Figure 1. The arrows indicate the connections between the Notch signaling pathway, Alzheimer's Disease, and Type II Diabetes Mellitus. The dotted arrow indicates the connection this investigation is examining.

Our investigation will study Notch expression in the hyperglycemic brain of larval and adult zebrafish containing a Notch-GFP transgenic reporter line. To test the hypothesis that Notch signaling changes in hyperglycemic conditions, we will expose zebrafish to glucose and analyze changes in Notch signaling using Western Blots, qPCR and Fluorescence Microscopy. To assess the expression of Notch, we will use a transgenic zebrafish model. A transgenic line is

a genetically modified zebrafish containing the Green Fluorescent Protein (GFP) under the control of Notch signaling (Parsons et. al, 2009). The use of this line will allow us to evaluate Notch signaling temporally and spatially. Once Notch signaling is active, GFP will be expressed and observed as fluorescence under the microscope (see figure on cover sheet).

We plan to induce hyperglycemia via glucose exposures to both larval and adult zebrafish.

Both the larval and adult fish will be exposed to three different conditions: (1) Glucose, (2) Mannitol (to control for osmolarity), and (3) Water. Larval animals will be exposed for 72 hours during the first week of development, when Notch is known to be active. Adult fish (more than 3 months old) will be exposed for 8 weeks to induce systemic hyperglycemia.

We hypothesize that Notch signaling will increase in response to the hyperglycemic conditions and the interruption of homeostasis in the neuronal cells in both larval and adult trials. As stated, Notch functions differently during the early stages of development versus in adults. In addition, we can conduct cognitive assessments on the treated and control adult fish (Lucon-Xiccato & Dadda, 2014). By testing two different age groups, we can learn the differences in Notch signaling pathways in larvae and adult zebrafish. Altogether the data we propose to collect will allow us to determine if there is a bridge between Notch expression and T2DM. After we determine what happens in regards to Notch in the hyperglycemic brain, we can perform long term behavioral and cognitive assessments to correlate induction of hyperglycemia with clinical presentation of AD (Lucon-Xiccato & Dadda, 2014).

Timeline

Time	Experiments	Goals	Other
Spring 2018	Stabilize Transgenic Line Trial Exposures on Transgenic larvae fish.	Gather Preliminary Data	Attend NIH Alzheimer's Conference Present preliminary findings at Mathias Conference
May 2018	Continue larvae exposures Begin first adult exposures	Have adult fish ready for exposures	
June 2018	Collect data from larvae exposures and run quantitative tests	Analyze data to observe trends	
July 2018	2nd exposure to adults	To have at least 2 exposures on adults	
August 2018	Compile all data and analyze Cognitive assessments	Create a final project presentation and poster	Write Final Paper

References

Bonegio, R., & Susztak, K. (2012). Notch signaling in diabetic nephropathy. *Experimental cell research*, *318*(9), 986-992.

Kishimoto, N., Shimizu, K., & Sawamoto, K. (2012). Neuronal regeneration in a zebrafish model of adult brain injury. *Disease models & mechanisms*, *5*(2), 200-209.

Kopan, R., & Ilagan, M. X. G. (2009). The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*, *137*(2), 216-233.

Lucon-Xiccato, T., & Dadda, M. (2014). Assessing memory in zebrafish using the one-trial test. *Behavioural processes*, *106*, 1-4.

Nazareth, A. M. D. (2017). Type 2 diabetes mellitus in the pathophysiology of Alzheimer's disease. *Dementia & Neuropsychologia*, *11*(2), 105-113.

Parsons, M. J., Pisharath, H., Yusuff, S., Moore, J. C., Siekmann, A. F., Lawson, N., & Leach, S. D. (2009). Notch-responsive cells initiate the secondary transition in larval zebrafish pancreas. *Mechanisms of development*, *126*(10), 898-912.

Selkoe, D., & Kopan, R. (2003). Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. *Annual review of neuroscience*, *26*(1), 565-597.

Woo, H. N., Park, J. S., Gwon, A. R., Arumugam, T. V., & Jo, D. G. (2009). Alzheimer’s disease and Notch signaling. *Biochemical and biophysical research communications*, *390*(4), 1093-1097.