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A Study on Alzheimer's — Model Organism: Drosophila Melanogaster

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Abstract

Alzheimer's disease is a neurological disorder and the most common form of dementia within humans. Throughout the decades of the disease's known existence, there has yet to be a cause and subsequent cure that has been discovered ever since the disease was discovered by Alois Alzheimer.

Keywords: Neuroscience, Alzheimer's, CNT2, cGAS-STING pathways

1. Introduction

However, throughout decades of meticulous research, some recurring patterns and characteristics have started to surface after comparing the brains of humans with Alzheimer's and humans without.

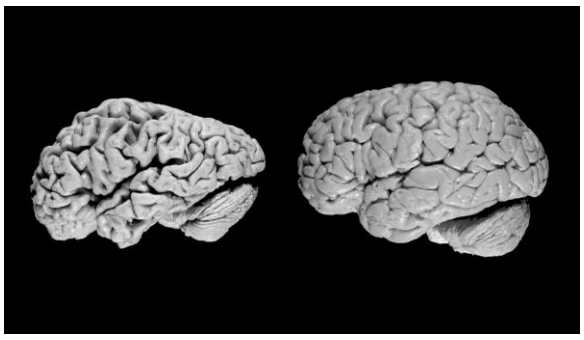

Figure 1. A side-by-side view of a brain with Alzheimer's disease (left) and a healthy brain (right) (Sindhu Ramesh et al., 2023)

One big recurring factor was the presence of a certain protein that appeared much more in affected individuals. This protein was the amyloid-precursor protein, and it has been discovered to have a near direct link with Alzheimer's disease. Examination of affected individuals' brains showed that a brain with Alzheimer's had a lot more of the amyloid-precursor protein expressed than in a healthy brain. Amyloid-precursor protein is a protein that is found in a neuron's cell membrane, with part of it outside the cell, part of it inside, and part of it in between. The amount of said protein was measured using one of its pieces that could be directly examined forming in large plaques across the brain cavity. Amyloid-precursor protein could be cleaved by beta secretase and gamma secretase to form three different smaller proteins. The external portion of the cleaved protein was called soluble amyloid-precursor protein, or sAPP, though its relevance to Alzheimer's is still debated upon. (National Institute on Aging, 2024)

The protein that's found within the cell membrane is called amyloid-beta, or Aβ. It seems to have the most profound and direct effect on Alzheimer's. The intracellular part of

the cleaved protein is the amyloid intracellular domain, or AICD.

Studies of brains with Alzheimer's have discovered large plaques of the Aβ protein present within the tissues of the brain, where it has been hypothesized that an overexpression of either the gene that creates APP or the genes that cause the cleavage of APP have contributed to the plaques of Aβ present within the brain matter, as every single examined brain has shown these same Aβ plaques. (Hampel, Harald, et al., 2021)

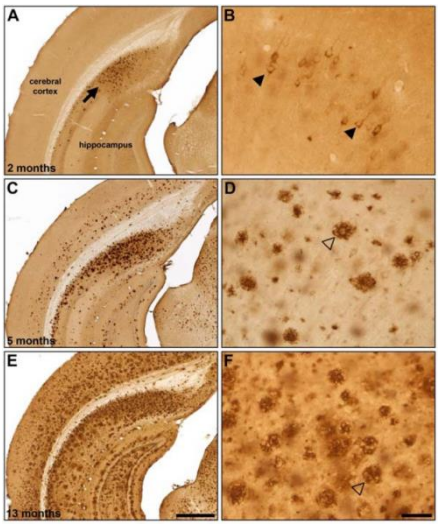

Figure 2. Microscope images of a genetically altered Mus musculus to display an overexpression of Aβ showing the eventual buildup of the plaques from 2 to 13 months of growth (Dorsey Griffith, 2019)

This research project is going to take a deeper look into the genes and gene expression present within the development of Alzheimer's and Aβ plaques. To do this, experiments have been performed using genetically altered *Drosophila melanogaster*, more commonly known as the common fruit fly. The genes of the *D. melanogaster* have been altered so that they can express the genes needed to produce the APP protein, and subsequently the Aβ plaques. The behavioural changes of the *D. melanogaster* will be monitored during their lifespan, and their gene expression will be used to measure the relative amount of each gene that is expressed in an individual with Alzheimer's, and a healthy individual. The procedure used to measure this is transcriptional profiling, which gives some information regarding how much of certain genes are expressed relative to their normal counterparts.

Over the last half decade, numerous studies have been conducted with the newfound knowledge of cGAS-STING and microglia as leading contributors to Alzheimer's disease.

A lab at Harrison College had recently conducted a research study about cGAS-STING and its relation to Alzheimer's in May of 2023. While brief, it utilizes the findings and research in the previous sections in order to try and find ways and works around cGAS-STING and Alzheimer's, using *M. musculus* as their model organism. The results are largely successful, with a lot of the research and tests indicative of some sort of decline from the tests utilized through varying levels of gene expression; however, the area of piqued interest was how there was little mention of the genes that code for parts of the pathway such as the transmitters and transmembrane transporters, when the research paper focused more on the genes and proteins that make up the main parts of the pathway itself, such as cGAS. cyclic-di-GMP was also not explicitly mentioned within the article itself. (Govindarajulu, Manoj, et al., 2023)

Another study looked similarly at the relationships between cGAS-STING and neurological disorders as its own literary analysis in November 2023; written by a group of students in Weill Cornell Medicine school in New York. Its specific look into Alzheimer's had found multiple specific genes and roles of cGAS-STING and microglia in the development of Alzheimer's in the field of molecular genetics, such as mentioning various different genes, types of nucleic acids, and the general timeline for the development of Alzheimer's disease. However, the study did not explicitly mention the different procedures used by the experimenters to discover the findings. (Huang, Yige, et al., 2023)

Ongoing research at the Alzheimer's Drug Discovery Foundation has also helped to work out the links between cGAS-STING and Alzheimer's disease, with the latest studies and research being implemented in September 2020. It had looked into the specifics of cGAS-STING and whether the inhibition of the pathway could ultimately prove to be beneficial or detrimental to treating Alzheimer's disease. Using lab cultured *M. musculus*, it was found out that 5-month old lab mice actually had a protective result against neurodegenerative cytokines;

furthermore, a different study was used where people who are obese and/or smokers were given reduced cGAS-STING activation, and their results came back with a reduced chance of dementia, this research helped develop the idea that the inhibition of cGAS-STING would have a beneficial effect on vascular dementia. Despite all this evidence, Alzheimer's is such a vast neurological disease with large amounts of heterogeneity that the treatment cannot be useful for every type of Alzheimer's disease, meaning that treatment of cGAS-STING may have varying results. (Alzheimer's Drug Discovery Foundation, 2020)

One other study that was conducted as of May 2023 saw the expansion of ideas addressed in other studies, which helped further solidify the usage of different genes, mitochondrial DNA, and neurofibrillary tangles in the development of neurological inflammation. The study utilized genetically modified *M. musculus* containing faulty codes in the genetic sequence that would allow for rapid neuroinflammation, however –as pointed out by the study itself– the usage of *M. musculus* has one big downside in that the transferring of gene therapy or drugs will most likely not be a fit for both species, and thus specific types of gene therapy must be engineered using the *H. sapiens* genome. (Sindhu Ramesh, et al., 2023)

2. Research Question

What is the role of the CNT2 gene and its effects and impacts on the cGAS-STING pathways and microglia cells, and how may it be used to gain understanding about Alzheimer's Disease?

3. Background

Much of the research stems around the autoimmune aspect of Alzheimer's disease, where immune cells called microglia were found to have likely taken part in the destruction of the brain matter found in Alzheimer's infected individuals. Immune genes also simply seemed to be pretty heavily linked with Alzheimer's in terms of their expression rate and their function.

Research has indicated that the microglia's actions in destroying and enveloping foreign and excess brain material has also had an adverse side effect of the microglia actually also enveloping parts of the neurons that make up the brain itself. Thus, as the microglia work to contain all the various Aβ plaques, they are also inadvertently eating up various bits of neurons and synapses in the brain itself.

The big part of the immunodeficient aspect of Alzheimer's also lies within a signaling pathway known as the cGAS-STING pathway. The aforementioned pathway is an innate contingency that ensures the death of cells that may otherwise be damaged or virally infected by sensing DNA in the cell's cytoplasm by way of nucleic acid sensors present within the cells. Should DNA exist in the cytoplasm either by a broken nuclear membrane or a virus injecting its DNA into the cell, the pathway would kill said cell to stop it from reproducing.

However, should this pathway actually be overexpressed, it can become one of the most defining parts and characteristics for the cause of Alzheimer's disease. The overexpression of the cGAS-STING pathway causes both neuroinflammation as well as causing abnormal microglia behaviour, which also contributes to Aβ induced neurotoxicity. The symptoms and side effects of these toxicities are markedly similar to the common symptoms associated with Alzheimer's, including the plaques and neurofibrillary tangles commonly associated with said disease. (Chen, Qi, et al., 2016)

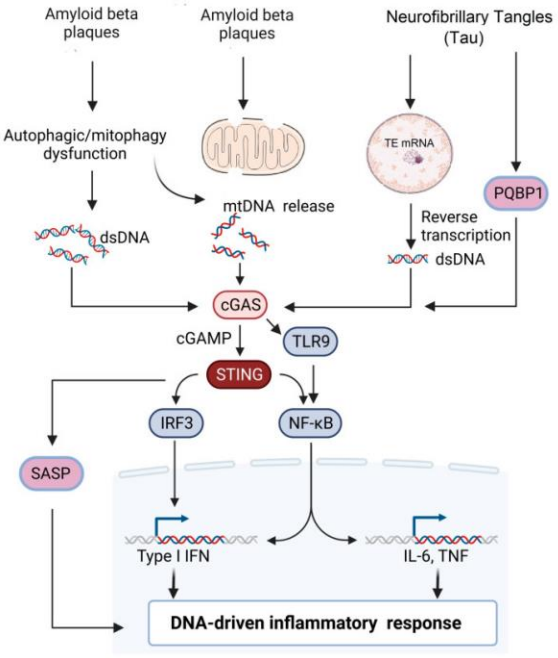

Figure 3. The pathway that shows the relationship Between cGAS-STING and AD (Govindarajulu, Manoj, et al., 2023)

One specific gene that has sparked some interest is the CNT2 gene, present in *D. melanogaster*. In most adult individuals, the gene is often present in the head of the adults, presumably in their neural tissue. CNT2 has also been demonstrated

to be orthologous to various human genes that contribute to the same function, including SLC28A1. It is linked directly with the cGAS-STING pathways and the information stored within this gene. The CNT2 gene specifically acts as an on-switch for cyclic-di-GMP transmembrane transporters. It is one of the DNA sensors that is present within the cGAS-STING pathway, and is able to pass signals throughout cells on nucleotide mediated signaling. It often sees the most usage with mitochondrial damage causing leaking mitochondrial DNA into the cytoplasm. The leaky mitochondrial DNA as well as viruses would usually be where the cGAS-STING pathway and CNT2 gene are often used the most, in regular *D. melanogaster* individuals. (Govindarajulu, Manoj, et al., 2023)

Alzheimer's infected individuals seem to have very different responses and stimulations for the pathway and the gene to become activated. Recent research done at Harvard University expressing various genes of the model organism of *D. melanogaster* have shown that affected individuals have roughly 10.5 times more CNT2 gene expression than in regular organisms. This unprecedented spike in gene expression is almost undoubtedly linked to the formation of Alzheimer's disease, though the cause of the unnatural spike and the overexpression of the cGAS-STING pathway has been researched for years. However, it stands to reason that the extraordinarily high gene expression rate will almost certainly be paralleled in humans and their corresponding orthologous genes.

D. melanogaster has been chosen as the model organism for this experiment for a number of reasons. For starters, it is one of a group of animals on the planet that scientists possess the entire known genome for (alongside *H. sapiens*, *M. musculus*, and many others). The entire genome of *D. melanogaster* is also not as long as some other model organisms that could be used for this experiment like *M. musculus*, only possessing four chromosomes. This means that it is easier to show and compare the entire genome as compared to other model organisms.

The lifespan of *D. melanogaster* is also quite short, only living for a couple of months. This means that data can be gathered in a much quicker fashion than the likes of an animal like *M. musculus*, which has a lifespan of up to 30 months. (Facts, n.d.; ScienceDaily, 2023)

Using a method known as transcriptional profiling, the entire genetic sequence of *D. melanogaster* across its four chromosomes can be laid out for display. Each gene has its own name and function. By genetically modifying certain genes in *D. melanogaster,* it can be manipulated into overexpressing certain genes related to the production and cleavage of APP into Aβ. Every single gene from both a genetically modified individual and a natural individual is then expressed, where the difference in the amount of genes expressed in a genetically modified individual is recorded in the format of logarithm base two as well as its statistical relevance (the R^2 value).

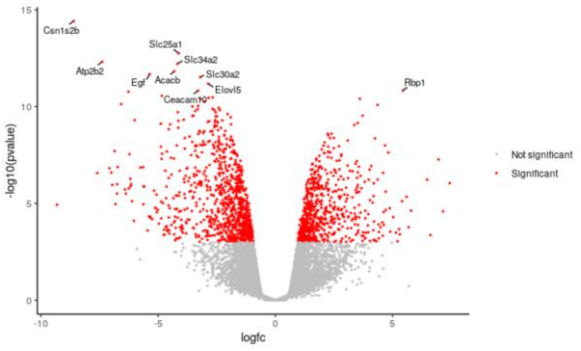

Figure 4. A typical volcano plot of a certain organism (Bonnin & Sarah, 2020)

This collection of data can be used and compared to the functions of the different genes in the bodies of *D. melanogaster*, which can in turn be used to determine any sort of relevance between the gene functions and the amount of which they are expressed in a *D. melanogaster* with Alzheimer's.

The data will then be plotted using a volcano plot to give a comprehensive look at the different genes that have a different magnitude of change and their corresponding statistical relevance.

This volcano graph as well as other types of data collection (yet to be determined) will hopefully help build a more comprehensive look at the gene expression and ways of detecting changes in gene expression that could help in mitigating Alzheimer's disease, or at the very least give insight into which genes could have an effect in causing Alzheimer's disease. (Bonnin & Sarah, 2020)

This type of experiment and data collection has been performed many times within a similar

moniker all with the intention of deciphering the relevance and correlation between APP, Aβ and Alzheimer's disease. Research between *D. melanogaster* and *H. sapiens* in terms of Alzheimer's and the nervous system in general has been done many times in the past. Alongside *M. musculus*, *D. melanogaster* is one of the most used model organisms in the study of Alzheimer's disease.

My personal relation to this research project in Alzheimer's disease comes from one of my family members having been affected by Alzheimer's disease. By researching this topic, I wish to help others who are either affected or have someone close to them affected by this disease, in the legacy of my family member who I looked up to dearly.

4. Variables

The manipulated variable used to collect the data in this experiment is the genetic alteration of certain individuals from the species *D. melanogaster*. This is done by using CRISPR-Cas 9, a pair of molecular scissors that can be used to both cut, and also turn on and off specific genes. The specific genes of *D. melanogaster* that relate to the production of APP and Aβ will be overexpressed and injected into a recently fertilized *D. melanogaster* embryo, where the gene will integrate in the budding cells of the embryo and produce a genetically modified *D. melanogaster*. This method in genetically modifying *D. melanogaster* is by far the most efficient, cheap, and effective way of genetically modifying the cells in order to gain conclusive results.

The responding variable for the data will be the amount of change in fold between affected individuals and regular individuals, as it is directly influenced by the genetic altering of the sequence of *D. melanogaster,* where the difference in fold between genes of both individuals are measured using log base two, which gives a specific value. These values, when plotted using a volcano plot, should theoretically provide conclusive enough results about the research question.

5. The Next Step

While the research that has been done on Alzheimer's up to this point has remained steadfast, and many new intriguing discoveries have been made in its causes and effects, much still remains a mystery in the disease and the study for future medicines and therapies to treat Alzheimer's remain paramount. There still exist a few major gaps that separates the scientific world's current understanding of Alzheimer's to its eventual cure. Despite the extensive research that has been done on amyloid plaques, cGAS-STING, and other contributing factors to AD (or simply dementia in general), scientists have yet to discover what is actually fundamentally wrong with Alzheimer's. All the contributing factors listed have only been to show subtle changes in an individual's mental decline, and aren't the main basis on the development of AD. This will most likely be the biggest part of research that scientists still need to tackle. If they can figure out what is fundamentally wrong with the human brain in AD, that area can be expanded upon and thoroughly researched until the problem is discovered.

The first steps in accomplishing this task will most likely be expanded upon via use of model organisms as well as autopsies of deceased Alzheimer's infected patients, neither of which will give the most comprehensive or completely accurate results. Model organisms cannot simulate the human brain, and since there's a lack of knowledge of what fundamentally causes AD in humans, replicating it in model organisms can be a challenge. Autopsies of brains infected with AD would most likely be already so deteriorated and worn down by AD that it can be hard to get a fix on the specifics of the causes of AD, as well as the heterogeneity of AD meaning that there must be many different comprehensive autopsies with extensive enough data to draw a reliable conclusion. Given the extraordinarily little chance of accomplishing this feat, it alone will likely take decades more study.

Should the fundamental problem that causes AD in the brain be discovered, the next logical step would be to use various different model organisms in order to map out the genome and find ways of using gene therapy to treat AD in the organisms' nervous systems. Common lab animals such as *D. melanogaster* and *M. musculus* will likely be used given their already extensive usage as model organisms for the study of AD as well as the mapping of the complete genome. Humans, while the easiest organism to find direct evidence and analysis in the genome, take far too long to gain conclusive results (given that the average human lifespan is around 85 years, and it takes 50-60 years just for the first signs of

Alzheimer's to develop), and ethics would also have to be consulted. Thus *D. melanogaster* and *M. musculus* would make for the most logical choices when it comes to model organisms. Other model organisms may also be used given that they are similar enough to the human brain and results can be collected relatively quickly.

Following this development and the experimentation of genetics on the model organisms, the next step would be to isolate the specific genes, types of gene therapy and drugs that can be potentially used in order to cure AD, which can likely be generated in a few years given how advanced biotechnology has become. The types of gene therapy can be isolated in the specific organism and be proven as an effective way of curing AD in that specific organism. However, one big hurdle that still remains between the ultimate goal is the translation between the gene therapies and cures in the model organisms to the respective cures in humans. This step still proves to be one of the biggest steps between the study and curing of AD as a result. (Hurt & Avery, 2023)

References

Alzheimer's Drug Discovery Foundation. (2020, August 30). cGAS-STING Inhibitors Evidence Summary. www.alzdiscovery.org/uploads/cognitive_vitality_media/cGAS-STING.pdf.

Bonnin, Sarah. (2020, March 9). 19.11 Volcano Plots | Introduction To R. biocorecrg.github.io/CRG_RIntroduction/volcano-plots.html.

Chen, Qi, et al. (2016 September). "Regulation and Function of the cGAS–STING Pathway of Cytosolic DNA Sensing." *Nature Immunology*, *17*(10), pp. 1142–49. https://doi.org/10.1038/ni.3558.

Dorsey Griffith. (2019, March 12). "Dementia Looks Different in Brains of Latinos." University of California. www.universityofcalifornia.edu/news/dementia-looks-different-brains-latinos.

Facts. (n.d.). Fruit Flies | Max-Planck-Gesellschaft. https://www.mpg.de/10973625/why-do-scientists-investigate-fruit-flies

Govindarajulu, Manoj, et al. (2023 May). "Role of cGAS–Sting Signaling in Alzheimer's Disease." *International Journal of Molecular Sciences*, *24*(9), p. 8151. https://doi.org/10.3390/ijms24098151.

Hampel, Harald, et al. (2021 August). "The Amyloid-β Pathway in Alzheimer's Disease." *Molecular Psychiatry*, *26*(10), pp. 5481–503. https://doi.org/10.1038/s41380-021-01249-0.

Huang, Yige, et al. (2023 November). "Mechanism and Therapeutic Potential of Targeting cGAS-STING Signaling in Neurological Disorders." *Molecular Neurodegeneration*, *18*(1), https://doi.org/10.1186/s13024-023-00672-x.

Hurt, Avery. (2023, February 6). "Why Don't We Have a Cure for Alzheimer's?" WebMD, www.webmd.com/alzheimers/features/alzheimers-amyloid-hypothesis-rut.

Macdonald, Ian & Debay, Drew & Reid, Andrew & O'Leary, Timothy & C.T, Jollymore & G, Mawko & Burrell, Steven & E, Martin & Bowen, Chris & Brown, Richard & Darvesh, Sultan. (2014). Early Detection of Cerebral Glucose Uptake Changes in the 5XFAD Mouse. *Current Alzheimer research*. 11. 10.2174/1567205011666140505111354.

Moloney, A. M., Sattelle, D. B., Lomas, D. A., & Crowther, D. C. (2010). Alzheimer's disease: insights from Drosophila melanogaster models. *Trends in Biochemical Sciences*, *35*(4), 228–235. https://doi.org/10.1016/j.tibs.2009.11.004

National Institute on Aging. (2024, January 19). "What Happens to the Brain in Alzheimer's Disease?" www.nia.nih.gov/health/alzheimers-causes-and-risk-factors/what-happens-brain-alzheimers-disease.

Prüßing, K., Voigt, A., & Schulz, J. B. (2013). Drosophila melanogaster as a model organism for Alzheimer's disease. *Molecular Neurodegeneration*, *8*(1). https://doi.org/10.1186/1750-1326-8-35

ScienceDaily. (2023, January 23). Fruit flies help researchers decode genetic link to Alzheimer's disease. https://www.sciencedaily.com/releases/2023/01/230109145202.htm

Sindhu Ramesh, et al. (2023, May 10). cGAS–Sting Signaling in Alzheimer's Disease. encyclopedia.pub/entry/44070.