To what extent does PLCG2 expression have a positive impact on AD

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Abstract:
Late-onset Alzheimer's disease (LOAD) is a prevalent form of AD that lacks a cure. Previous research has shown that PLCG2, associated with inflammatory response, influences AD by affecting amyloid plaque density. Inflammation plays a crucial role in AD progression, as Aβ activates microglia, triggering an inflammatory response with varied consequences for neuronal survival. This study aims to explore the impact of different PLCG2 expression levels on AD using 5xFAD mouse models. Four experiments were conducted to investigate the effect of PLCG2 expression on inflammation, amyloid plaque density, and cognitive function. The GAL4-UAS system and CRISPR-Cas9 system were employed for genetic modification, while immunofluorescence staining and the Morris water navigation task were used for analysis. Results indicate a positive correlation between PLCG2 expression and immune response, with the PLCG25x mice exhibiting the highest immune response. Additionally, higher PLCG2 expression levels were associated with slower growth in amyloid plaque density. The optimal PLCG2 expression level was found to be PLCG22x, which had a positive impact on AD conditions. This research provides valuable insights into the role of PLCG2 in AD pathogenesis and highlights the potential for PLCG2-directed therapeutics as a promising avenue for developing novel treatments for LOAD.

Keywords: Late-onset Alzheimer's disease, PLCG2, inflammation, amyloid plaque density, cognitive function.

1. Introduction

Late-onset Alzheimer's disease (LOAD) is the most common form of Alzheimer's disease (AD), accounting for about 95% of cases, and typically develops in individuals over the age of 65. The symptoms of LOAD vary from person to person, but the symptoms generally include memory loss, confusion, difficulty with language and communication, changes in mood and behavior, etc. Unfortunately, there is currently no cure for LOAD. [1]

According to previous research, PLCG2 has been proven to have a huge impact on AD and is associated with inflammatory response. Increased PLCG2 expression levels are associated with increased amyloid plaque density in certain regions of the human brain. [2]

It is well understood that inflammation plays an important role in the progression of Alzheimer's disease (AD) because amyloid-β (Aβ) can activate microglia, triggering an inflammatory response that may have varied consequences for neuronal survival. On the one hand, because microglia phagocyte Aβ and release enzymes responsible for Aβ breakdown may help slow the onset of AD. Conversely, microglia become less efficient at these processes as we age, becoming over-activated in response to stimulation and triggering an overly potent reaction, which may induce neuronal damage in and of itself [3].

Thus, the impact of PLCG2 on inflammation, along with the effect on amyloid plague and macrophage with overexpression of PLCG2, needs to be further explored for the development of PLCG2-directed therapeutics.

In this research, we use four experiments to investigate the effect different PLCG2 expression levels have on AD. 5xFAD mouse models are applied in this experiment to simulate environments in the human brain. The GAL4-UAS system and the CRISPR-Cas9 system are used in this experiment for genetic modification. For statistical analysis, we use immunofluorescence staining for marking. Morris water navigation task is also applied to assess spatial learning and memory in mice.

2. Hypothesis

At first, according to a previous study, we know that amyloid plaque density has a positive correlation with PLCG2 expression [2]. Thus, the higher the PLCG2 expression level, the higher the amyloid plaque density. But inflammation also increases with the PLCG2 expression level and reduces the amyloid plaque density, so the higher the PLCG2 expression level, the slower the growth. Consequently, we predict that when the PLCG2 expression level is about twice as much as the normal expression level, it positively impacts AD.

3. Methodology

To prove this hypothesis, we designed four experiments hoping to show that certain PLCG2 expression levels

could positively impact AD. The first experiment is intended to investigate how over-expression and knock-out of PLCG2 affect inflammation. The second and third experiments aim to show how an increase in PLCG2 expression affects amyloid plaque density and inflammation, respectively. The fourth experiment uses a water maze to study mice's cognitive function and memory.

3.1 How over-expression and knock-out of PLCG2 affect inflammation

We set up two experimental and two control groups in the first experiment. For the experimental group, 5xFAD mouse models will be used. The PLCG2 expression level will be amplified five times in mice of the first group using the GAL4-UAS system. In the second experimental group, the PLCG2 gene will be knocked out using the CRISPR-Cas9 system.

The GAL4-UAS system is a genetic tool used to control gene expression precisely. It comprises the GAL4 transcription factor and the upstream activation sequence (UAS). GAL4, which is derived from yeast, binds specifically to the UAS. The UAS is inserted upstream of a gene of interest in the target organism's genome. A transgenic organism is engineered to express GAL4 under a specific promoter. When the promotor is activated, the GAL4 transcriptional activator gene is transcribed and translated, and then the GAL4 binds to the UAS, activating the expression of the gene of interest. (Fig. 1) [4]

In the CRISPR-Cas9 system, the sgRNA guides the Cas9 enzyme to the exact location in the gene of interest. Once Cas9 is guided to the target site, it acts as molecular scissors and cuts both DNA strands (Fig. 2). We use this technology to knock out the PLCG2 gene and act as an experimental group. [5]

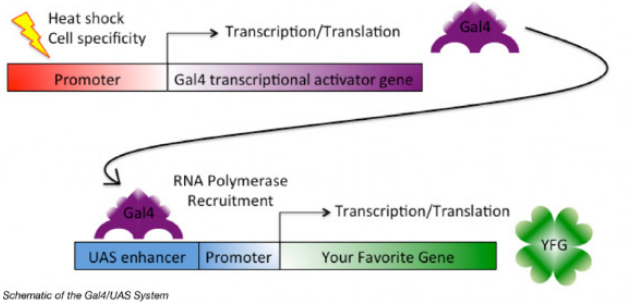

Figure 1. The GAL4-UAS system (blog. add gene, John Chow)

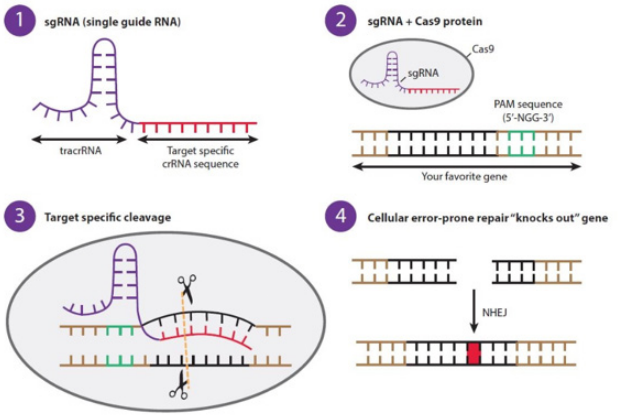

Figure 2. The CRISPR-Cas9 system (BTX, Michelle M. Ng, Ph. D)

The two control groups will be mice with normal PLCG2 expression and wild type, respectively. Each experimental group and control group contains ten mice.

Then, we will use immunofluorescence to quantify the immune response in three groups and use statistical analysis to determine the optimal level of PLCG2 expression.

Immunofluorescence staining allows us to visualize the presence and distribution of specific proteins or cellular structures within a tissue or cell sample. In this technique, fluorescently labeled antibodies target and bind to the specific protein or structure of interest. These antibodies are designed to recognize and attach to a specific epitope on the target molecule. Once bound, the antibody-antigen complex can be visualized under a fluorescence microscope. In this experiment, we will use EGFP and mCherry (Fig. 3).

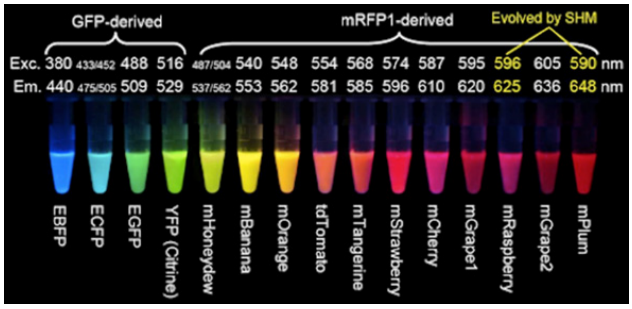

Figure 3. Immunofluorescence staining color samples

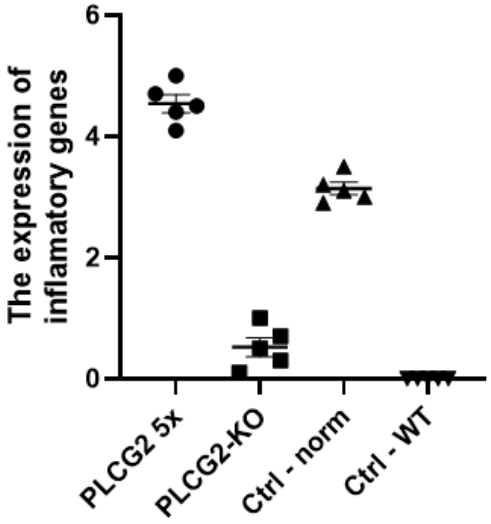

Figure 4. Predicted result 1

For the expected results, we predict that Group 1, the PLCG2^{5x} mice, has the highest immune response since more microglia are activated with the increase in PLCG2 expression, followed by the PLCG2^{norm} mice and the knock-out group (Fig. 4).

3.2 How does an increase in PLCG2 expression affect amyloid plague density?

Experiment 2 investigates how an increase in PLCG2 expression would affect the number of amyloid plaques. The experiment involves four groups: wild-type (WT) mice as a negative control, 5xFAD mice with normal PLCG2 expression, 5xFAD mice with twice the expression of PLCG2, and 5xFAD mice with five times the expression of PLCG2. The GAL4-UAS system will induce PLCG2 overexpression in the 5xFAD mice. Immunofluorescence staining will be employed to examine and compare the changes in amyloid plaque formation in the different groups at four-time points: one month, four months, eight months, and twelve months. Each group contains ten mice.

At first, the higher the PLC G2 expression level, the higher the Amyloid plaque density. But inflammation also increases with the PLCG2 expression level and reduces the amyloid plaque density, so we predict that the higher the PLCG2 expression level, the slower the growth. At last, the PLCG2 normal group will have the most amyloid plaque density, followed by the two times PLCG2 group, and then the five times PLCG2 group (Fig. 5).

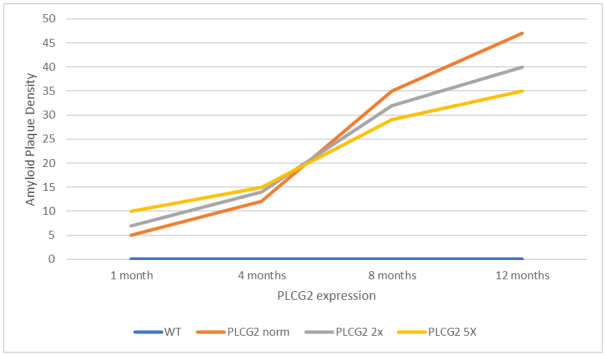

Figure 5. Predicted result 2

3.3 How does an increase in PLCG2 expression affect inflammation

Experiment 3 will look into how an increase in PLCG2 expression affects inflammation. The investigation includes four groups: wild-type (WT) mice as a negative control, 5xFAD mice with normal PLCG2 expression, 5xFAD mice with twice the PLCG2 expression, and 5xFAD mice with five times the PLCG2 expression. The GAL4-UAS technology will be used to increase PLCG2 expression in 5xFAD mice. Immunofluorescence staining will be used to detect the longitudinal morphological change of macrophage density in the different groups at four-time points: one month, four months, eight months, and twelve months. Each group has ten mice.

At first, the higher the PLC G2 expression level, the higher the Amyloid plaque density. But inflammation also increases with the PLCG2 expression level and reduces the amyloid plaque density, so we predict that the higher the PLCG2 expression level, the slower the growth. At last, the PLCG2 normal group will have the most amyloid plaque density, followed by the two times PLCG2 group, and then the five times PLCG2 group (Fig. 6).

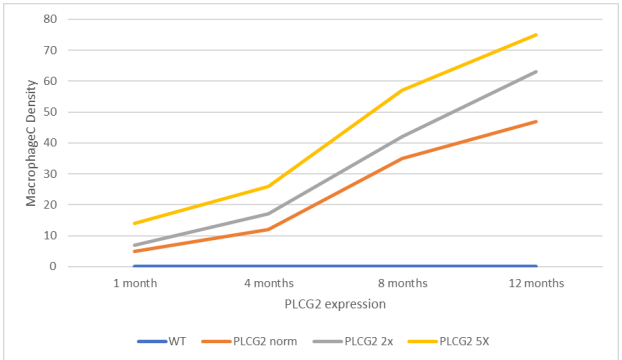

Figure 6. Predicted result 3

3.4 Morris water navigation task

The Morris water navigation task is a behavioral test to assess spatial learning and memory in mice. It is often used in studies related to Alzheimer's disease and other

neurological disorders.

It consists of a large pool of water with an invisible escaping platform hidden just beneath the water's surface. The mice are placed in the pool and must learn to find the hidden platform based on this visual landmark. Over several trials, the mice could gradually improve their performance by learning the platform's location. The Morris water navigation task is used to study cognitive function and memory. It tests the animals' ability to navigate and remember spatial information and helps understand the brain's cognitive processes. [6]

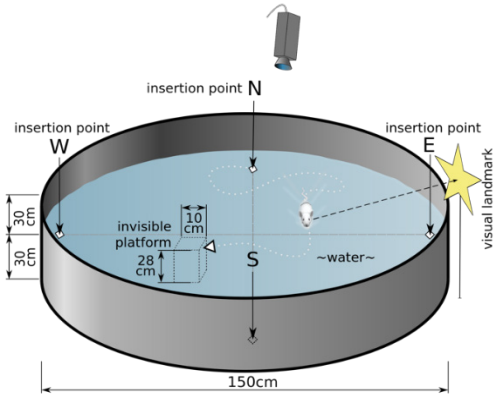

Figure 7. Morris water navigation task (Samueljohn.de, 2010)

4. Conclusion

To summarize, four experiments were conducted in the research. The first experiment explores the relationship between inflammation and the presence of the PLCG2 gene, and we predicted a positive correlation between the two factors. The second experiment investigates the relationship between PLCG2 expression level and amyloid plaque density. With the influence of inflammation, we conclude that the PLCG2 normal group will have the most amyloid plaque density. Then, the third experiment shows the effect of increasing the expression level of PLCG2 on inflammation. Finally, the Morris water navigation task is carried out to test the functionality of the mice's brains under different PLCG2 expression conditions.

Since PLCG2 has a positive relationship with inflammation, on the other hand, PLCG2 decreases the rate of increase of amyloid plaque density. Through the four experiments, we conclude that the optimal expression level of PLCG2 is $PLCG2^{2x}$, which positively affects AD conditions. $PLCG2^{2x}$ has the best functionality based on the cognitive test result.

However, the experiments have many limitations. To begin with, the number of mice on whom we test this technique must expand; a large number of them is required to obtain a more exact result, and the ten mice in each group from our design are insufficient to do that. Since the difference between experimental groups is significant, we also need to do more experiments on different expression levels to improve our results' accuracy; by adding $PLCG2^{3x}$ and $PLCG2^{4x}$, we can generate a more accurate plot. Furthermore, the anatomical differences between mice and humans are significant. Also, even though this approach worked successfully in mice models, we cannot use it directly on humans because genetic manipulation in humans is prohibited, which raises ethical concerns. However, it is possible to use techniques such as gene therapy to introduce a functional gene copy. The vector carrying the PLCG2 gene could be introduced into the patient's body to control the PLCG2 gene expression.

References

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