

Limiting lipid droplets accumulation is a way for Alzheimer's disease treatment

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Abstract. Background: In recent years, Alzheimer's disease is a serious problem in the chemical world that haven't been solved for many years. We have set up a research about Alzheimer's disease these days because the number of patients who had disease could grow to 13.8 million by 2060 if there's development of medical treatment to prevent, slow or cure AD.[7] Method: By using control variables, immunohistochemistry, western blotting and fluoresces images to investigate our experiments. Predicted results: Protective APOE variants may work as an antagonist to LDAM transition by limiting lipid droplets accumulation.

Keywords: Alzheimer's disease, lipid droplets, APOE, LDAM transition.

1. Introduction

As the disease advances, patients' nervous systems can include problems with language, disorientation(including easily getting lost), easily getting mad, loss of motivation, self-neglect, and behavioral issues.^[1] A lot of patients were affected by Alzheimer's disease and by the time, researchers also focused on this disease to invent more efficient chemicals to help patients relieve the pain. The cause of Alzheimer's disease haven't been discovered yet and many researchers did experiments to find out a way for Alzheimer's disease treatment.^[2] To investigate the effect of Alzheimer's disease, we search for some paper. At first, we focused on an article called: APOE4/4 is linked to damaging lipid droplets in Alzheimer's disease microglia. Alzheimer's disease contains several risk factors which are related to lipid metabolism, and many of these lipid genes are highly expressed in glial cells. ^[3] However, how does lipid metabolism work in glia and Alzheimer's disease pathology need more discoveries and researches.^[3] In this article, they used amount of experiments to investigate the function of microglia and lipid droplets separately. The article showed that ACSL1+ microglia appeared more significant in APOE4 brain tissues, and lipid droplets accumulation is greater in APOE4 microglia. In summary, M.S.Haney *et al* discovered that the APOE4 genes determined the microglia transition process to an evolutionarily conserved and damaging LDAM state which responses to innate immune triggers including A β .^[3] From this paper, we concluded that patients with APOE4 gene may have higher possibility to get Alzheimer's disease. When lipid droplets accumulate, proteins like A β in microglia were over accumulation, so that the function of clearance of microglia is exhausted. This article states the link between damaging lipid droplets, but it also has some aspects that they didn't mention. Future studies have to focus more on determining whether protective APOE variants like APOE2 genotype may prevent microglial transition(such as LDAM) by limiting LD accumulation.^[3] We thought that this

hypothesis was too complicated, so we divided this question into two parts and did experiments separately. The two parts are: does protective APOE limit lipid droplets accumulation, and will the limitation of LD accumulation reduce LDAM transition. Finally, we hypothesized that APOE-2 works as an antagonist to LDAM transition by limiting lipid droplets accumulation. Comparing to APOE3, APOE2 genes will decrease the possibility of getting AD and APOE2 against and protect through both A β independent and dependent mechanisms.^[4] So we thought APOE-2 as protective variant. As soon as we get this result, then we predicted that limitation of LD may improve microglia function to clear up A β to delay the time getting Alzheimer's disease. For this purpose, we start our experiment design.

2. Experiments

According to the introduction, we began to design our two experiments. Firstly, we focused on does APOE-2 limit lipid droplets accumulation? As a result, we design the following experiments to prove that protective APOE variants, which is known as APOE2, could limit lipid accumulation. To investigate this question, we mainly used mice as our experiment subjects and used western-blotting as our method. We use both female and male mice, as well as fed them regular food in the same room temperature. In the demyelination study, we decided to use 2-month-old mice in control and CPZ-treated groups are fed with regular food or food supplemented with 0.2% CPZ for four weeks.^[5] To find out how different APOE affect microglia function in the condition of demyelination we induced CPZ treatment. That's because the CPZ treatment can fully activate microglia and elicits reactive gliosis without causing blood-brain barrier (BBB) damage.^[5]

We decided to fed mice regular first and than replaced food with CPZ to induce demyelination. After a period of time, we may probably see a reduction of demyelination sensitivity with CPZ treatment compared to the control group. To access microglial responses by facing challenge, we could examine the levels of a microglial marker, ionized calcium-binding adapter molecule1, by using immunohistochemical (IHC) analysis and Western blotting.^[5] After this, we may observe that microglia in APOE-2 which induced the CPZ treatment increased. Comparing to other brain tissues with APOE-4 and 3, microglia in APOE-2 shows a significant increase in the number. This result shows that APOE2 microglia are mostly related to acute demyelination, however APOE4 microglia fail to respond efficiently to neuronal injury.^[5] As a result, we could infer from this observation that microglia is more functional and efficient in the clearance of lipid droplets. However, microglial in APOE-4 are less active to the clearance of LD, so this also shows that APOE-4 gene may be a key impact of getting Alzheimer's disease.

Secondly, we planned to do another experiment which is motivated by the previous study^[3]. The question we decided to investigate is the limitation of lipid droplets reduce the LDAM transition. We were inspired from the paper we read last week called APOE4/4 has a relationship to harmful lipid droplets in Alzheimer's disease microglia. In this article, they investigated an experiment which use human frontal cortex which is near to the tissue used in snRNA-seq experiment by immunofluorescence images.^[3] This paper also mentioned that the ACSL1 was more significant in APOE4. In this paper, it said that LD-related genes in the ACSL1+ microglia cluster with ACSL1+ cells are the same as LDAM.^[3] According to this, we had an idea about our experiment by leveraging immunofluorescence images. So we designed our paper with the two same fresh-frozen brain cortex tissues with APOE-2 gene. We kept the age, sex and other factors the same. Then, we added different amount of lipid droplets into the samples. By using the immunofluorescence images, we could probably see the difference between the brain tissues. If our experiment went through correctly, we predicted that red ACSL1 cells were less significant in the brain tissue with less lipid droplets, so this indicates that limiting lipid droplets can reduce ACSL1 cells accumulation. As a result, we predicted that the microglial LDAM transition may be reduced by limiting LD accumulation.

3. Summary

To sum up, during the experiment, we followed the logic of the question and designed two experiments separately. So we made a hypothesis that APOE-2 gene can act as an antagonist to reduce LDAM

transition by limiting lipid droplets. Our first experiment indicates that APOE-2 is a kind of protective variant and has ability to limit lipid droplets. It is the first step. The next experiment shows that the limitation of LD can reduce ACSL1 cells accumulation which means reducing LDAM transition. As we combine two process together, the final result turns out. If we have correctly done the experiments and get proper data, this shows that our hypothesis is correct. When we have designed all the things, we think about a question about Alzheimer's disease treatment and precautions. As we know that patients with APOE-4 gene have higher risks of getting Alzheimer's disease and lipid droplets accumulation may decrease the function of microglia to clear up proteins. The article hypothesized that compounds interacting with both the lipid droplets and the protein LC3 may lead to degradation of LDs.^[6] They designed and synthesized such compounds by connecting LC3-binding molecules to LD-binding probes via a linker. These compounds are useful to clear up LDs almost completely and efficiently.^[6] As a result, we suppose that chimeric autophagy-tethering compounds are promising in the treatment of Alzheimer's disease by limiting lipid accumulation.

The problem for our experiments design is the feasibility of success because we separate the original question and the relationship between two experiments need clearer research. So we hope that future study can focus more on APOE genes related to lipid droplets. This would help Alzheimer's disease treatment by preventing disease from initial stage.

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