Regional Progression on Diffusion and Transport of Aβ Protein in Transgenic Mice Brain

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ABSTRACT
Accumulation of amyloid plaques is considered one of the pathological markers of Alzheimer's disease (AD). In this work, the hypothesis: "The accumulation of amyloid-beta plaque is initiated from the hippocampus and Aβ plaques diffuse to the entire cortex." is tested by designing two experiments to find out the mechanism of transport and production of amyloid-beta protein, using transgenic mice without familial Alzheimer's disease (FAD) gene expression in the hippocampus in experiment 1. The second experiment uses transgenic mice with E693Q Dutch mutation of amyloid precursor protein (APP) gene expression in the hippocampus and normal FAD gene expression in the cortical cells. CRISPR Cas-9 gene-editing technology, viral transduction, IP – immunoprecipitation, elusion and HPLC – high-performance liquid chromatography is used in this experiment. The purpose of this paper is to find the transport of amyloid-beta protein to investigate the cause of Alzheimer's Disease.

Keywords*: diffusion; APP; Aβ; hippocampus; cortex; transgenic mice.*

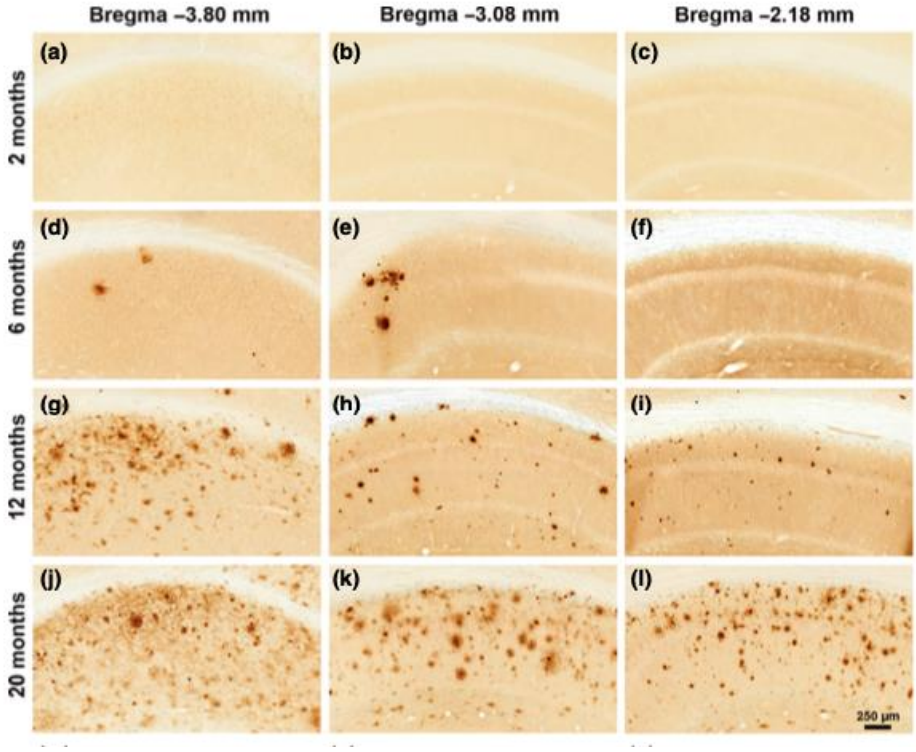
1.INTRODUCTION

Alzheimer's disease (AD) is a neurogenerative disease, which is an irreversible and incurable disease by using modern science and technology. Large numbers of amyloid-beta (Aβ) plaque surrounded by neurons containing neurofibrillary tangles [1] are considered as one of the pathological markers of Alzheimer's disease. Aβ protein molecule is a short sequence of peptides, containing 39-42 amino acids. Aβ protein monomers are cleaved from amyloid precursor protein (APP) by beta-secretase and gamma-secretase instead of alpha-secretase and beat secretase, then they can assemble to form soluble oligomers which are toxic to nerve cells. Moreover, the insoluble extracellular deposits -- Aβ plaques are formed by the construction of misfolded Aβ oligomers. The symptoms of Alzheimer's disease are associated with episodic amnesia followed by significant deficits in semantic memory and procedural memory. [2] Additional clinical manifestations such as loss of judgment, problem-solving impairment, depression, and sleep disorders are also frequently associated with the early stages of the disease. [3] (Grontvedt et al., 2018)

Based on the available experiment result and hypothesis, a regular pattern has been identified that ~80% of the 3xTg-AD mice analysed had Aβ plaques in the caudal hippocampus at 6 months age, while 100% of them have Aβ plaques in the hippocampus at 12-month age. Cortical Aβ plaques were first detected at 12 months of age, including in the entorhinal cortex. [4] A new guess was introduced that Aβ plaques seem to have a fixed starting producing point and experience simple diffusion to the rest of the brain. The hypothesis is the accumulation of Aβ protein is initiated from the hippocampus and Aβ protein diffuses to the entire cortex. The experimental animal models are normal mice and transgenic mice which overexpress human genes associated with familial AD gene (FAD).

The designed experiment has two components. In experiment 1, the diffusion direction of Aβ protein and its original production place is tested. Group one uses normal mice. Group 2 uses transgenic mice with FAD gene expression in all brain cells. Group 3 uses transgenic mice with FAD gene expression in the cortex except for the hippocampus. The transgenic mice are treated with CRISPR Cas-9 gene-editing technology to insert the human FAD gene, and then deletes the FAD gene in hippocampus cells. The mice behaviour is observed and recorded. The cutting slices of mice brain and hippocampus are made and observed, leading to three possible results: Aβ plaque is present in Group 3 transgenic mice both at cortex and hippocampus; Aβ plaque is present in Group 3 transgenic mice only in the

cortex and Aβ plaque do not present anywhere in Group 3 transgenic mice. Experiment 2 is based on the third possible result. It shows the spreading of Aβ protein from the hippocampus to the cortex might be possible. However, a new question arises, how does Aβ protein diffuse from the hippocampus to the cortex? In other words, the mechanism of diffusion of Aβ protein needs to be investigated. The idea is about using two different forms of Aβ protein in the hippocampus and cortex. There are many mutations in amyloid precursor protein (APP) protein, E693Q Dutch mutation is chosen because this mutation happens in the place where beta-secretase and gamma-secretase are cut to produce Aβ protein. E693Q Dutch mutation has glutamine instead of glutamic acid at No.693 amino acids. Viral transduction is used to transfer the mutated gene which makes E683Q mutation APP to the Group A transgenic mice. The mutated gene is then deleted using CRISPR Cas-9 gene-editing technology for all brain cells except the hippocampus. E693Q mutated APP is only expressed in the hippocampus. Group A transgenic mice are observed and recorded activities and behaviour. Cutting slices of transgenic mice hippocampus and brain cells are made and observed under light microscope. The Aβ protein is extracted from APP using immunoprecipitation, then it is washed out by elusion. Both Aβ proteins extracted from the hippocampus and cortex need to be put in high-performance liquid chromatography (HPLC) thus E693Q mutation within glutamine can be identified. The second experiment has two possible results: 1, the Aβ protein extracted from cortex cells are also E693Q mutation, thus the hypothesis is proved; 2, the Aβ protein extracted from cortex cell is different to E693Q mutation. The guess of the second possible situation is that the hippocampus sends a signal to the cortex cell and the cortex make copies of Aβ protein by itself.



(4)

FIGURE 1 Age-dependent Aβ pathology in the hippocampus. (a–l)

2.RESULT

2.1Group 3 transgenic mice without FAD gene expression in hippocampus show a non-spreading related pathway of amyloid beta plaque production

To analyse the production pathway of Aβ in transgenic mice, sections of 2-, 6-, 9-, 12-, 18-months-old mice are immunostained with Aβ-specific antibodies. The hippocampus section selected is from the right hemibrains that were -3.80, -3.08-, and -2.18-mm posterior to bregma, representing the caudal, medial, and rostral hippocampus. [4] In this result, no extracellular Aβ plaques in 2-months old mice, it begins to appear in the caudal hippocampus of the 6-months-old Group 3 transgenic mice are expected to be observed. Six strong, healthy mice contain three males, and three females are analysed. Most of the Group transgenic mice have Aβ plaques in the caudal hippocampus, a small percent in the medial hippocampus and non in the rostral hippocampus are expected to be observed. It is expected to see that 6-months Group 3 transgenic mice have little or no symptoms of Alzheimer's diseases such as dementia. At 9-months old, Group 3 transgenic mice are assumed to

have Aβ plaques in the entire hippocampus. Later in 12 months, Aβ plaques are expected to be observed in cortex cells, first detected in lateral entorhinal cortex (I-ENT). As the mice age, the amount of Aβ is expected to increase in the medial and rostral cortex. Overall, the expected result in Group 3 transgenic mice would be. Aβ plaque first develops in the caudal hippocampus, once filled in the entire hippocampus, they start to present in the caudal cortex and fills the entire cortex. This shows the amyloid-beta plaque is not spread from hippocampus to cortex and the prediction is not correct.

2.2Group 3 transgenic mice without FAD gene expression in hippocampus show a gene related production on amyloid beta plaque

Group 3 mice are completely the same as previously described in 2.1. In the second possible result, Aβ plaque is expected to be firstly detected in the caudal cortex of 12-months old Group 3 transgenic mice and as mice age, they are expected to appear in the medial and caudal cortex in 18-months-old mice. No Aβ plaque would be detected inside the hippocampus. Therefore, this means the production of Aβ plaque is related to the FAD gene inserted as Group 3 transgenic mice only have the gene in their cortex, not in the hippocampus. It is also expected to see Group 2 transgenic mice have Aβ plaque in the whole brain and developed some symptoms of Alzheimer's disease, for example, dementia and loss of memory.

2.3Group 3 transgenic mice without FAD gene expression in hippocampus show a spreading system of amyloid beta plaque production

Group 3 mice are completely the same as previously described in 2.1. This third possible result expects none of Aβ plaque appears in neither hippocampus nor cortex in Group 3 transgenic mice at any age. Also, all Group 3 transgenic mice should remain healthy and normal without any symptoms related to Alzheimer's disease. However, Group 2 transgenic mice are expected to have Aβ plaque firstly in the hippocampus at 2 months old, then in the cortex at 6 to 9 months old whereas Group 1 normal mice work as control with no changes in the brain. Based on the expected observed result, it reveals the production of Aβ is not controlled by the FAD gene. The production should begin at the hippocampus and spread to the rest of the brain which matches with the hypothesis and lead to the second section of the designed experiment.

2.4Group A transgenic mice with E693Q Dutch mutation gene expression in hippocampus show a diffusion model of production on amyloid beta plaque

The transgenic mice used in the second section of the experiment had been inserted with the FAD gene into whole-brain cells by CRISPR Cas-9 gene editing technology, then the FAD gene in Group A transgenic mice has been taken out and inserted new gene – E693Q mutation FAD gene expression only on the hippocampus. To identify the diffusion pathway and mechanism of diffusion of Aβ plaque, sections of 12-, and 18-months-old mice are immunostained with Aβ-specific antibodies. The hippocampus section selected is the caudal hippocampus from the right hemibrains. [4] Slides are made from six strong, healthy mice, including three male and three female. Moreover, the cortical sections were selected from the caudal cortex on the same mouse. Amyloid-beta plaque samples from two chosen areas are extracted by immunoprecipitation. Slides are being testified using high-performance liquid chromatography (HPLC). They are compared between the hippocampus and cortex of the same mouse. The amyloid-beta plaque produced from the hippocampus and cortex are the same type of Aβ -- E693Q Dutch mutation is expected. Finally, it proves the hypothesis -- the accumulation of amyloid beta plaque is initiated from the hippocampus and Aβ plaques diffuse to the entire cortex.

2.5Group A transgenic mice with E693Q Dutch mutation gene expression in hippocampus show a signalling pathway of Aβ plaque production

The mice in experiment 2 are the same as previously described in 2.4. In the second possible result of experiment 2, the result expected is the Aβ plaque in the hippocampus and cortex is not the same. Only Aβ plaque in the hippocampus shows E693Q Dutch mutation whereas Aβ in the cortex is normal. The guess for this situation is maybe the hippocampus can send a signal to the cortical cell which starts to produce Aβ once received the signal. The signal might be a special electrical impulse transported through a special passageway and being detected by the GCR receptor, therefore activates the expression of a gene and start production.

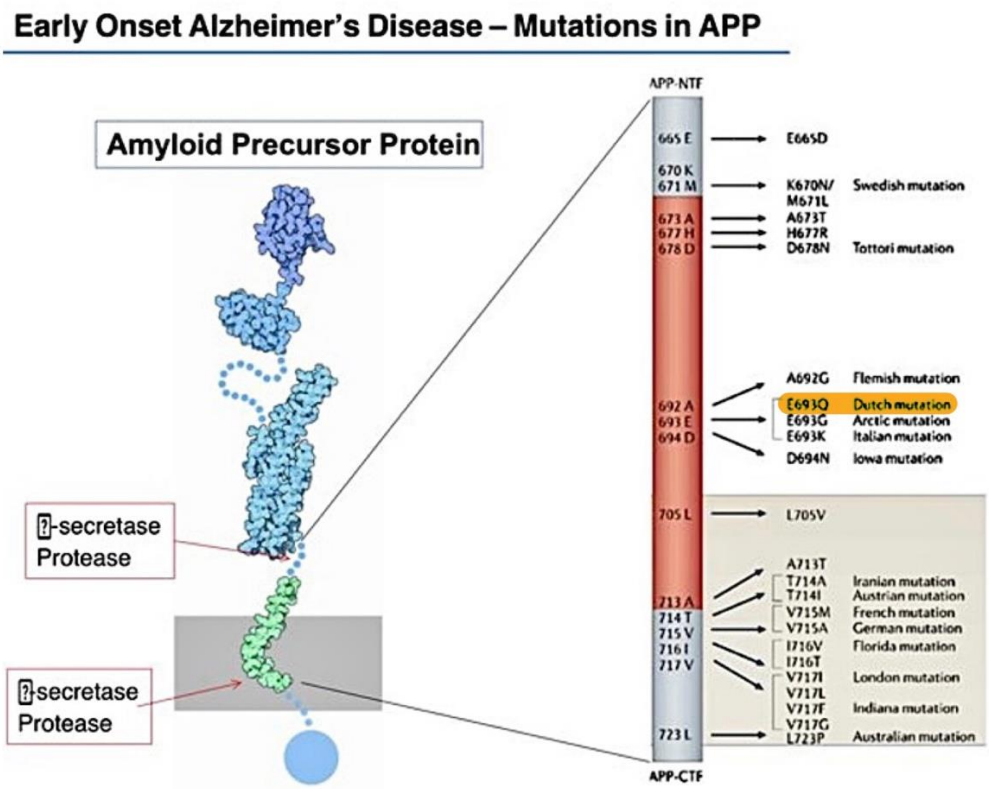


FIGURE 2 The position of E693Q Dutch mutation in Amyloid Precursor Protein (APP)

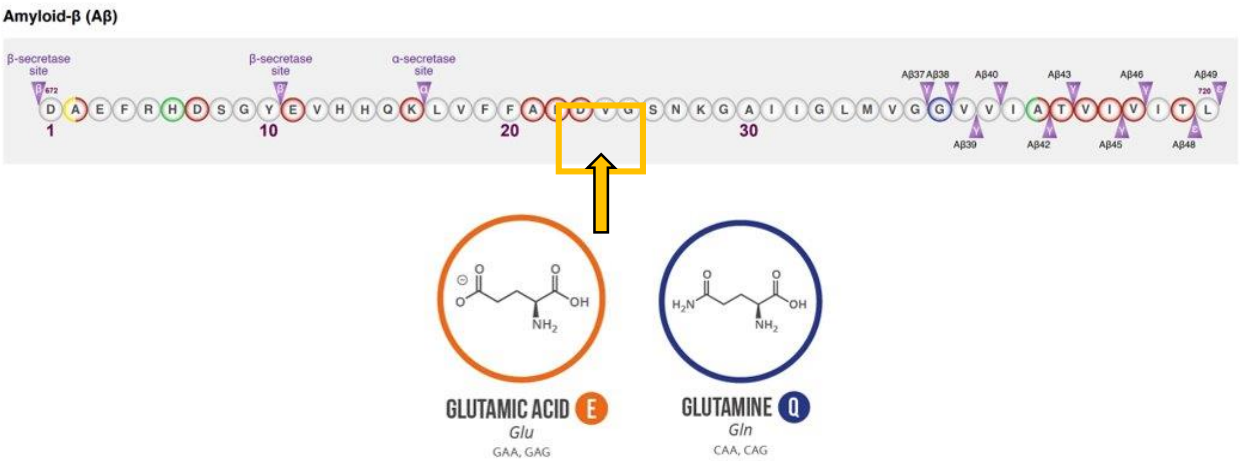


FIGURE 3 Difference of molecular structure between normal APP (left) and E693Q Dutch mutation APP (right)

3.DISCUSSION

The hypothesis of amyloid-beta diffusion is a completely new idea; thus, its validity is uncertain and no related research papers are found. However, this makes it more significant to prove this new hypothesis as it might open a new direction of Alzheimer's disease. In this experiment, the feasibility of using Dutch mutation to examine the present type of Aβ needs to be testified, if Dutch mutation is hard to be identified, a new method is required to distinguish amyloid beta. Also, this experiment leaves much to be improved such as the better technology can be used to insert the FAD gene more quickly. The suggestion of future direction would be if the Aβ plaque originates in the hippocampus, how to control the amount of protein produced? How to clear these proteins to cure Alzheimer's disease?

4.EXPERIMENTAL PROCEDURE

4.1Animal

The experimental mice use in this experiment are colonies, it is maintained by breeding to each other. Mice were housed 4–5 per cage, kept on a 12-hr light/dark

cycle, and were given ad libitum access to food and water. [4]

4.2HPLC high-performance liquid chromatography

Sample 1 made of E693Q Dutch mutation amyloid beta protein is injected into the HPLC column and recorded the retention time. Sample 2 made of normal amyloid beta protein is injected into the HPLC column and recorded the retention time. Then the sample amyloid beta protein extracted from the cortical cell is injected into HPLC column, compared its retention time with the previous recorded two data.

5.CONCLUSION

Although in this paper both experiments are only designed, no actual operation had been done to confirm the results. However, the multiple possibilities that this paper leads to can be deserved further in future studies and discussions. The focus of this paper is to discuss the production and transport of Aβ proteins which could be used as a base to hypothesize the cause of Alzheimer's disease, so as carry out treatment and cure research in the future.

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