The Direction and Mechanism of Temporal and Regional Progression of Amyloid Beta Plaques in Mice's Brains

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Keywords: Alzheimer's Disease, Transgenic Mice, Hippocampus, Amyloid Beta, APP E693Q.

Abstract: The amyloid beta (Aβ) is one of the major characteristics of Alzheimer's Disease, the neurodegenerative disease. This paper hypothesized that the progression of amyloid beta is by diffusion from the hippocampus to the cortex. To test this idea, this work is firstly designed to compare brain slices of C57BL/6J wild type mice with human FAD gene in all brain area and C57BL/6J wild type mice without human FAD gene in the hippocampus at 2, 6, 9, 12 months of age. If the result shows that there are no amyloid beta plaques present in both hippocampus and cortex after FAD gene knockout in the hippocampus, this indicates amyloid beta plaques are originally produced in the hippocampus. Then, the work is designed to compare brain slices of C57BL/6J wild type mice with wild-type FAD expression in the brain and C57BL/6J wild type mice with E693Q mutation FAD expression only in the hippocampus at 2, 6, 9, 12 months of age. If E693Q mutated FAD gene is present in the amyloid beta from the cortex, this indicates amyloid beta diffuses from the hippocampus to the cortex. This paper only provides theoretical experiment design and possible results about the direction and mechanism of temporal and regional progression of amyloid beta, which needs further research in the pathology of Alzheimer's Disease.

1 INTRODUCTION

Alzheimer's disease is considered a neurodegenerative disease (Alzheimer's Association 2016), meaning it causes the degeneration, or loss, of neurons in the brain. This leads to the symptom characteristic of dementia. Alzheimer's disease is progressive, meaning the patients will gradually suffer from memory loss and other cognitive inabilities throughout the rest of their life.

Although the causes of Alzheimer's disease remain mysterious, one of the major characteristics of it is amyloid beta (Aβ) (Billings, Oddo, Green, McGaugh, LaFerla 2005). The transmembrane protein, amyloid precursor protein, or APP, is responsible to produce amyloid beta protein.

In the Alzheimer's case, APP is cut by β and γ secretase instead of α and γ in the normal situation The peptide remained is insoluble and creates a monomer: amyloid beta (Aβ). These monomers are more chemically sticky, bond together extracellularly, and form amyloid beta plaques.

These plaques can potentially block the neurons, which inhibits neuron-to-neuron signaling. It is also thought that these plaques can start-up an immune response and cause inflammation which might damage surrounding neurons.

As shown in **Figure 1**, the amyloid beta plaques labeled by brown-dye antibodies have a progression pathway that starts from the entorhinal cortex and spread to the hippocampus, and finally spread throughout the cortex in the mice's brains as the mice grow up.

The spread of amyloid beta is possibly caused by diffusion, meaning the amyloid beta is produced in the hippocampus and diffuse to the cortex through membranes. Thus, a hypothesis of "The accumulation of amyloid beta plaque is initiated from the hippocampus and Aβ plaques diffuse to the entire cortex." is established.

2 HYPOTHESIS

The accumulation of amyloid beta plaque is initiated from the hippocampus and Aβ plaques diffuse to the entire cortex.

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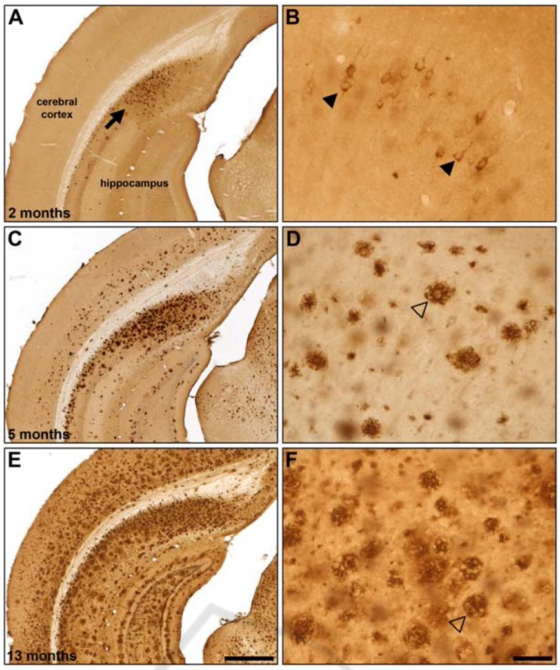

Figure 1: Representative -amyloid (A) immunohistochemistry in 2 (A, B), 5 (C, D) and 13 (E, F) month old 5XFAD mice. (Macdonald, DeBay, Reid, O'Leary, Jollymore, Mawko et al. 2014).

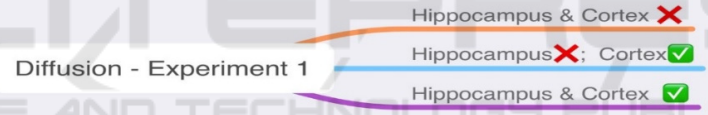

Figure 2: First experiment design for the hypothesis.

3 METHODS AND MATERIALS

3.1 Amyloid Beta Progression Pathway

To testify this hypothesis, the following experiments are designed. There are two subsets in this investigation.

Firstly, to determine whether the amyloid beta is originated from the hippocampus, the amyloid production in the hippocampus is designed to be suppressed and the presence of amyloid beta plaques is detected in the cortex, as shown in **Figure 2**.

3.1.1 Animals

60 mice wild-type (C57BL/6J), 30 female and 30 male. (Manocha et al. 2019)

3.1.2 Transgenic Material

Human FAD (Familial Alzheimer's disease) gene

3.1.3 Transgenic Method

Virus-mediated gene delivery: Import the FAD gene into the mice's brains at the embryo stage.

3.1.4 Cre-loxP System

The Cre-loxP recombination is a special type of site-specific recombination, which can remove a certain gene in a certain area. To stop amyloid beta production in the hippocampus, the FAD gene in the hippocampus is removed immediately after transgene. The transgenic line in which Cre recombinase expression is restricted in the

hippocampus is used, so the specific promoter can activate loxP sites in the same direction in the hippocampus and the FAD gene is deleted, as shown in **Figure 3**.

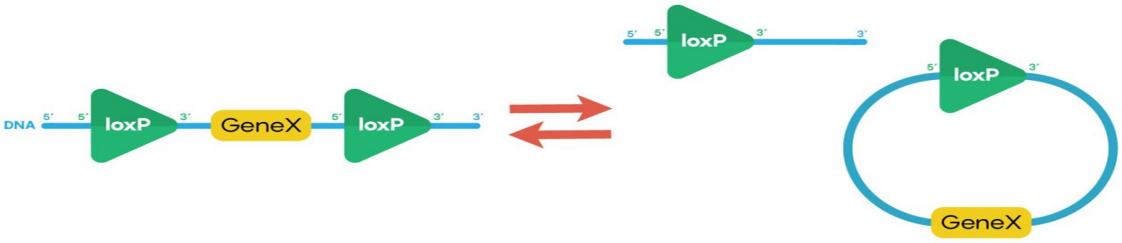

Figure 3: Excision cis placement of loxP sites in same directional orientation causes a gene deletion (Ju 2020) .

3.1.5 Procedure

Experimental group

Transgenic mice with FAD gene expression in all brain cells

Transgenic mice without FAD gene expression in the hippocampus

Firstly, the FAD gene is imported using virus-mediated gene delivery into all 60 mice's brains at the embryo stage. After one day, the FAD gene is removed in the hippocampus region of 30 mice (15 female and 15 male) using the Cre-loxP system.

By 2, 6, 9, 12 months of age, six strong, healthy mice (three males and three females) were taken from each group. The brain of each mouse is taken and cutting slices of each mouse's hippocampus and cortex are made. Brown-dye antibody is added to the cutting slices and observes under the microscope.

3.2 Mechanism of Amyloid Beta Progression

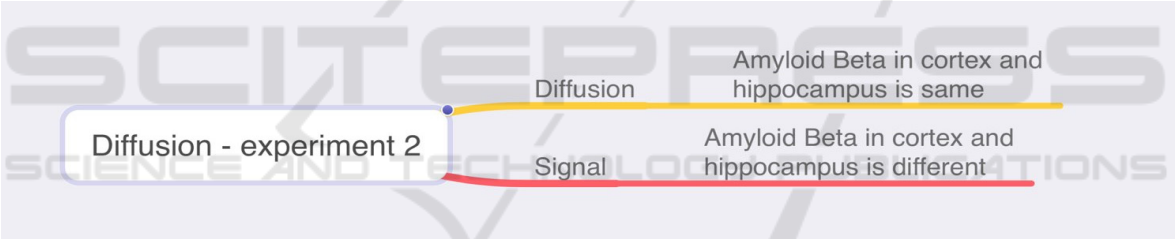

Figure 4: Second experiment design for the hypothesis.

Once the pathway of progression is confirmed that it is from the hippocampus to the cortex, the mechanism of amyloid beta spreading requires a second experiment to testify, as shown in **Figure 4**.

3.2.1 Animals

60 mice wild-type (C57BL/6J), 30 female and 30 male. (Manocha et al. 2019)

3.2.2 Transgenic Material

Human FAD (Familial Alzheimer's disease) gene:

Familial AD, which represents a minority of AD cases, is due to mutations in one of three genes, presenilin (PS) 1 and 2 and the amyloid precursor protein. (Van Cauwenberghe, Van Broeckhoven, Sleegers 2015)

E693Q mutated FAD gene:

To determine whether the amyloid beta plaques present in the cortex are produced in the hippocampus and diffuse out, a method to distinguish the amyloid beta in the hippocampus and the cortex is required. In this experiment, the Dutch mutation, E693Q on the amyloid precursor protein is used.

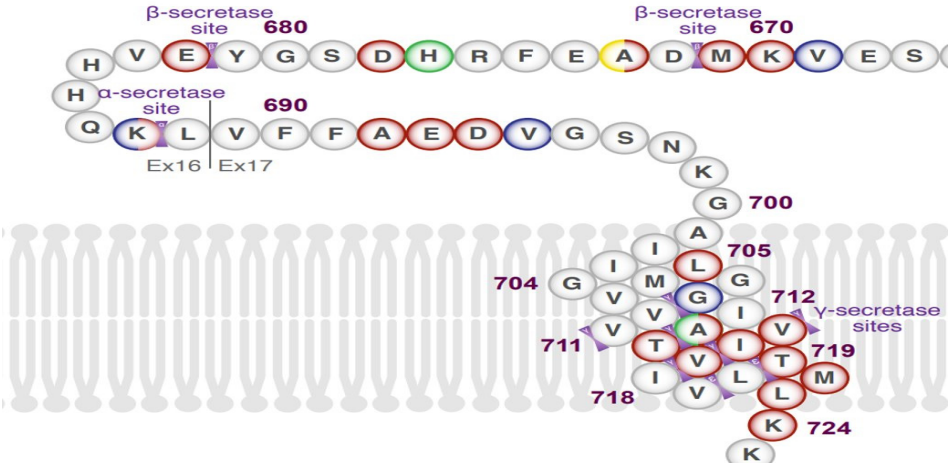

Figure 5: Partial amino acid sequence of APP containing multiple secretases' cutting sites (APP E693Q (Dutch) 2021).

As shown in **Figure 5**, the amyloid beta which forms plaques and causes neurodegeneration in Alzheimer's disease is cut by β and γ secretase at specific cutting sites.

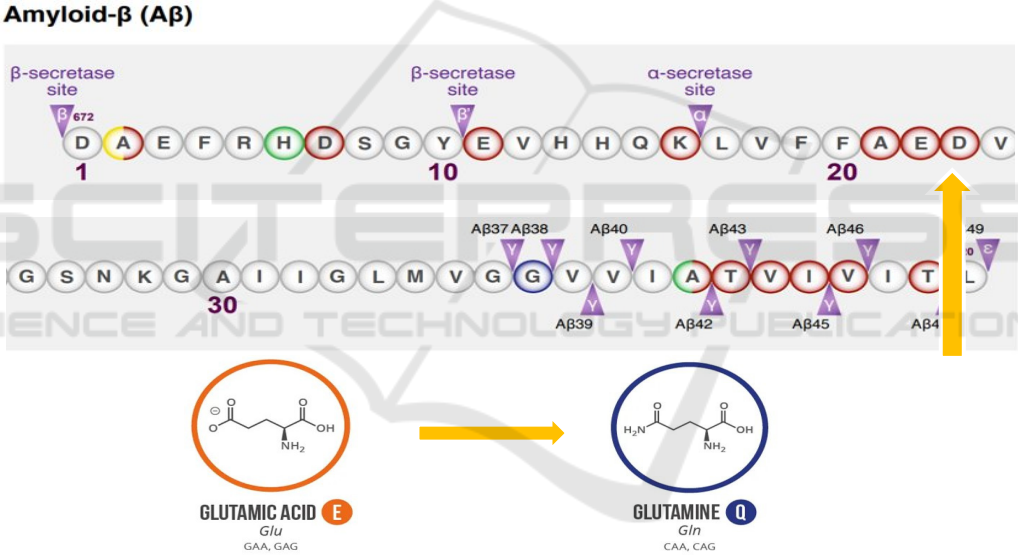

Figure 6: Amino acid sequence of amyloid beta and Dutch mutation (APP E693Q (Dutch) 2021).

The Dutch mutation is on the 693rd amino acid of the APP, which is the 22nd amino acid on the amyloid beta, as shown in **Figure 6**. The Dutch mutation changes the glutamic acid into glutamine. Since "the mutated gene may also undergo accelerated aggregation and accumulation" (Knight et al. 2014), so the function of E693Q is similar to the wild-type FAD gene.

3.2.3 Transgenic Method

Virus mediated gene delivery: Import the strand containing the FAD gene into the mice's brains at the embryo stage, as shown in **Figure 7**.

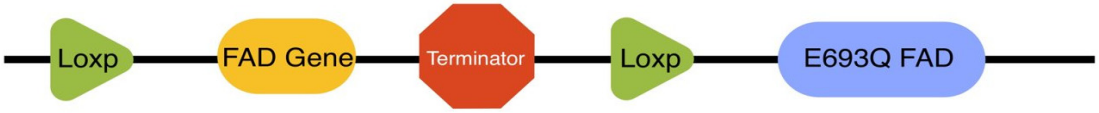

Figure 7: Simplified structure of imported gene strand.

3.2.4 Cre-loxP System

The Cre-loxP recombination is a special type of site-specific recombination, which can remove a certain gene in a certain area, as shown in **Figure 3**. In the imported gene strand, as shown in **Figure 7**, a wild-type FAD gene and a terminator are added between two loxP sites in the same direction and an E693Q mutated FAD gene after the loxP sites. After virus-mediated gene delivery, the gene can be expressed throughout the brain. During transcription, the only gene before the terminator, stop codon, can be expressed successfully, which is the wild-type FAD gene. After that, the transgenic line in which Cre recombinase expression is restricted to the hippocampus is used, so the specific promotor activates two loxP sites in the same direction in the hippocampus. The gene between the two loxP sites is deleted, including the terminator, snd only the E693Q mutated FAD gene is remained and is expressed in the hippocampus.

3.2.5 IP – immunoprecipitation

To testify the presence of the E693Q mutated FAD gene in the cortex, firstly, the amyloid beta plaques in the cortex of mice's brains should be extracted. IP, immunoprecipitation, is a technique of precipitating a protein antigen out of solution using an antibody that specifically binds to the amyloid beta. In this experiment, the brown-dye antibody is used. IP can be used to isolate and concentrate the amyloid beta from a sample of cortex mixture, as shown in **Figure 8**.

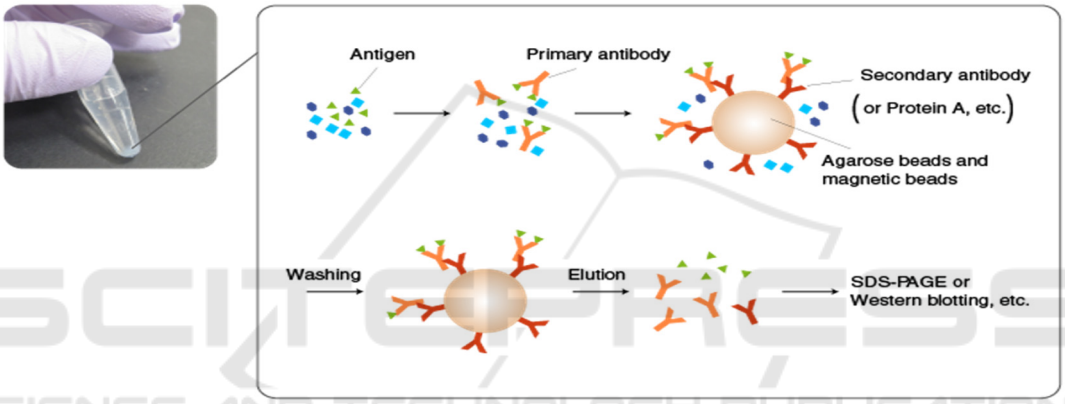

Figure 8: Process of IP (MBL Life Sience -ASIA-. Mblbio.com. Retrieved 21 September 2021).

3.2.6 Elution

To extract out amyloid beta from the antibody-amyloid eta complex, a process called elution is used. As shown in **Figure 9**, by washing the extraction with a solvent, as in the washing of loaded ion-exchange resins to remove captured ions, the pure amyloid beta molecule can be extracted.

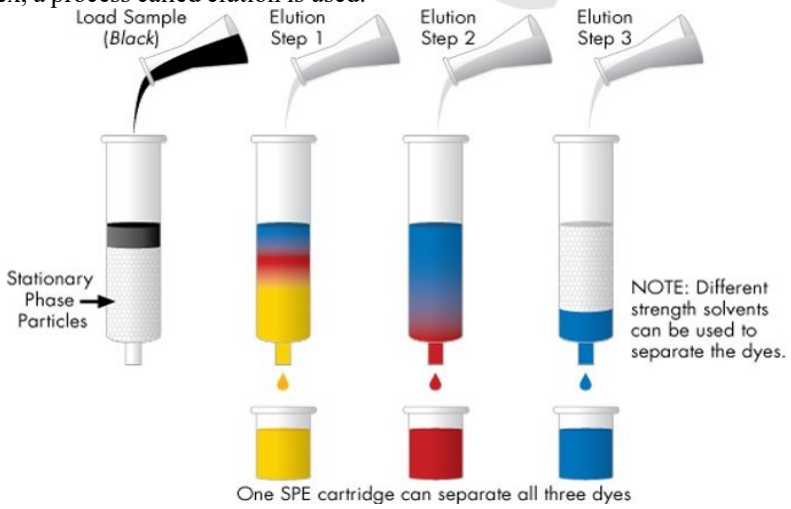

Figure 9: Process of elution (Solid Phase Extraction/SPE Guide | Waters. Waters.com. Retrieved 21 September 2021).

3.2.7 E693Q Mutated FAD Gene Testing

HPLC-High performance liquid chromatography

After extraction of the pure amyloid beta molecule, HPLC is used to testify the presence of the E693Q mutated FAD gene on amyloid beta. As shown in **Figure 6**, the E693Q mutated amyloid beta molecule has one glutamine instead of glutamic acid compared to the wild-type amyloid beta. As shown in **Figure 10**, the solvent is forced through a metal tube under high pressure. The particle size of the stationary phase is much smaller, which leads to better separation of the components. The two amyloid beta samples (wild-type and E693Q mutated type) are injected into the column. Finally, the components are detected after passing the column, by their polarity. Then the retention times of two different amyloid beta forms are compared. Firstly, samples of HPLC on both E693Q mutated type and wild-type amyloid beta are made, and results are recorded. When testing the amyloid beta form from the cortex of the transgenic mice's brains, its result can be compared with the two recorded results to see which result matches with it, thus determine the type of amyloid beta.

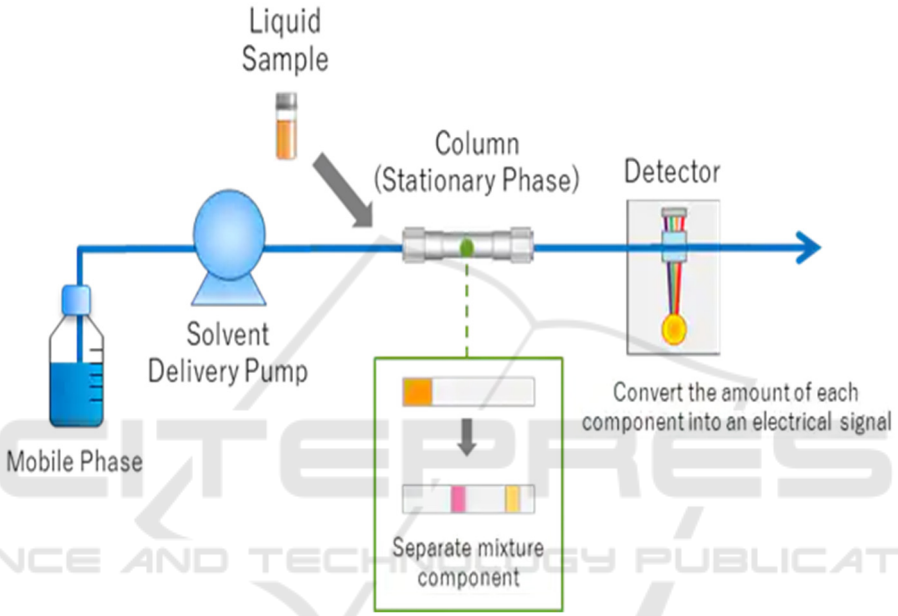

Figure 10:Process of HPLC (Shimadzu.com. Retrieved 21 September 2021).

3.2.8 Procedure

Experimental group

Transgenic mice with wild-type FAD expression in the brain

Transgenic mice with E693Q mutation FAD expression only in the hippocampus

Firstly, gene strand is imported using virus-mediated gene delivery into all 60 mice's brains at the embryo stage. After one day, the wild-type FAD gene and terminator are removed in the hippocampus of 30 mice (15 female and 15 male) using the Cre-loxP system.

By 2, 6, 9, 12 months of age, six strong, healthy mice (three males and three females) were taken from each group. The brain of each mouse is taken and cutting slices of each mouse's hippocampus and cortex are made. Brown-dye antibody is added to the cutting slices and observes under the microscope. The amyloid beta plaques in the cortex region of mice with E693Q mutated FAD expression only in the hippocampus are extracted using IP and elution. The presence of the E693Q mutated FAD gene is tested using HPLC.

4 RESULTS

4.1 Amyloid Beta Progression Pathway

There are three possible results for the first experiment. Firstly, neither the hippocampus nor cortex has plaques present. Secondly, plaques are not present in the hippocampus but present in the cortex. Lastly, both hippocampus and cortex have plaques present.

4.2 Mechanism of Amyloid Beta Progression

There are two possible results for the first experiment. Firstly, E693Q mutated FAD gene is present in the amyloid beta from the cortex. Secondly, E693Q mutated FAD gene is not present in the amyloid beta from the cortex

Discussion

For the first experiment, it is designed to determine whether amyloid beta originates from the hippocampus, and there is one result that corresponds to the hypothesis. If the result shows that there are no amyloid beta plaques present in both hippocampus and cortex after FAD gene knockout in the hippocampus, this indicates amyloid beta plaques are originally produced in the hippocampus.

The other two results are not consistent with the hypothesis, and both indicate that amyloid beta originates from the cortex. One of the results is that amyloid beta is present in the cortex but cannot be seen in the hippocampus. The other result is that amyloid beta is present in both areas, which shows that amyloid beta is initiated from other parts of the brain and spread to the hippocampus region.

For the second experiment, it is designed to testify whether amyloid beta diffuses from the hippocampus to the cortices. This corresponds to the result that the same Dutch mutated gene in the amyloid beta from the cortex is found as that in the hippocampus, which is consistent with the gene imported into the hippocampus. This implies that amyloid beta is produced in the hippocampus and diffuses out to the cortex from the hippocampus.

The second result is that the wild-type amyloid beta is present in the cortex, which differs from the mutated amyloid beta in the hippocampus. This indicates that signals were sent to the cortex to activate the β and γ secretase and thus the production of amyloid beta. Hence, this does not match what have speculated.

5 EVALUATION

This work tried to design an experiment of RNA sequencing previously to testify the second possible result of the second experiment, which is assumed to be signaling from the hippocampus. But it was weeded out because no effective and pragmatic way was found to do it. It is hard to determine the signal in one simple experiment because the possible signal can vary from Herpes Virus to small proteins. Therefore, it has been ruled out as details were considered to practice it.

The Cre-loxP system used in both experiments allows us to knock out specific genes between two loxP sites. It is very useful and reliable to cut the specific site wanted and precede as is expect. However, only genes in the hippocampus region are designed to be knocked out in both experiments. This work has been checked whether there is a specific promoter that only activates the Cre line in the hippocampus region and it turns out there are only promoters that work in subunits in the hippocampus. To perform the experiments, a specific promoter is assumed that activates the Cre line in the whole hippocampus region, which may not exist.

In the second experiment, Dutch mutation is used for us to track and distinguish the origin of amyloid beta proteins. This mutation changes the 693rd amino acid on APP from glutamic acid to glutamine. Dutch mutation are specifically chosen because it does not affect the function of APP, and the mutation site is on the amyloid beta section. Therefore, different amyloid beta can be produced, which indicates no inconsistency with our experimental design.

Our hypothesis will determine the direction and mechanism of amyloid beta spreading, which can provide clues for limiting the area amyloid beta spread, and possibly control dementia. If the first half of the hypothesis is consolidated, the next step will be to control the amount of amyloid beta plaques and clear them in the hippocampus. If amyloid beta diffuses to the cortex, restricting amyloid beta diffusion to control dementia would be important.

6 CONCLUSIONS

This paper provides two designed experiments on transgenic C57BL/6J wild type mice to investigate the pathway and mechanism of amyloid beta progression of Alzheimer's Disease. Cre-LoxP system were used to introduce or remove gene segment of Human FAD gene and APP E693Q into the mice's brains and specifically the hippocampus region. The research significance lies on the pathology and possible treatment of Alzheimer's Disease. If the amyloid beta progression can be controlled or eliminated, we are one step closer to the cure of Alzheimer's Disease.

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