APOE-ε4 genes may accelerate the activation of the latent form of HSV-1 which would lead to a faster progression of AD

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Abstract: This study investigates the impact of APOE alleles and latent Herpes Simplex Type 1 virus (HSV-1) activation on Alzheimer's disease (AD) progression using the 5xFAD mouse model. APOE ε4 is recognized as a substantial genetic risk factor for sporadic AD, while HSV-1 has been linked to AD pathogenesis through inflammation and plaque formation. The experimental approach involves the introduction of human neurons carrying latent HSV-1 into 5xFAD mice harboring various APOE alleles (APOE2, APOE3, APOE4), along with stress induction and pharmacological interventions. The study aims to elucidate the combined impact of these variables on AD progression and the formation of Aβ plaques. Our anticipated results suggest that APOE ε4 may accelerate AD development, especially in conjunction with HSV-1 activation, while APOE ε2 may exert a mitigating influence. These findings have the potential to advance our understanding of the intricate mechanisms underpinning AD and provide insights into potential therapeutic approaches. Further exploration of these interactions could offer critical insights into the pursuit of effective AD treatments.

1. Introduction

1.1. Alzheimer's disease

Dementia is a serious and expensive illness that is often caused by Alzheimer's disease (AD), which is becoming increasingly prevalent in our time. AD is a condition that gradually impairs cognitive function and causes memory loss, and emotional disturbances over time. There are two types of AD: early-onset AD (EOAD) and late-onset AD (LOAD). EOAD, characterized by symptom onset before the age of 65, differs significantly from the more common LOAD, and EOAD is associated with genetic mutations in the APP, PSEN1, and PSEN2 genes, while LOAD has no strong genetic links [1]. In addition, a significant proportion of early-onset Alzheimer's disease cases diverge from the usual memory-related symptoms of typical AD, with EOAD patients showing distinctive brain changes and altered neural networks. Hence, even though the management approaches for EOAD and LOAD are similar, it's important to customize treatment strategies for patients with varying types of Alzheimer's disease [2]. There have been recent developments in the diagnosis and research of Alzheimer's disease by Jack and colleagues, who have introduced the ATN framework. The biomarkers are classified into three categories: A for amyloid, T for phosphorylated tau, and N for neurodegeneration measured by total tau. These biomarkers indicate pathological changes that lead to neuronal death, oxidative stress, and neuroinflammation, ultimately resulting in brain atrophy and cognitive impairments [3]. For years, clinical trials have been studying different therapeutic approaches. However, current treatments mainly focus on alleviating symptoms instead of finding a permanent cure. In the case of AD, medication aims to relieve symptoms by rebalancing neurotransmitters. Meanwhile, non-pharmacological and dietary interventions. For example, research is currently being conducted on supplements like omega-3 for their potential to safeguard and defend the brain during the early stages of the illness [4]. Currently, there are several ways to ease the advancement of Alzheimer's disease's pathology, but unfortunately, there is no cure for AD. Nevertheless, it is important that patients are identified as having the disease in the early stage, as this affects the treatment of the disease, and the earlier it is found, the more likely it is to alleviate the progression of the disease and the more time the patient can have to be healthy. In the condition that the treatment is expensive or invasive, like cerebrospinal fluid or positron emission tomography, the blood biomarker amyloid-β (Aβ) can detect prodromal AD, which is a cheaper and non-invasive method [5].

1.2. APOE gene and its alleles

The APOE gene plays a crucial role in various brain functions, specifically in relation to Alzheimer's disease. ApoE affects lipid metabolism, immune response, and more, contributing to amyloid and tau pathology, neurodegeneration, inflammation, and vascular problems. Through extensive research and analysis of the human genome, scientists have discovered The APOE ε4 allele holds the highest significant genetic susceptibility factor for sporadic Alzheimer's disease (SAD). Conversely, the APOE ε2 allele works as a powerful genetic protective

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factor. Despite this understanding, there are currently no available therapies that target APOE for treating Alzheimer's disease. Various therapeutic methods have shown success in mouse models that exhibit human APOE genes. These include increasing or decreasing the content of APOE levels, enhancing the ability of lipidation, preventing interactions between APOE and A-β peptide, and genetically converting APOE4 to other alleles. However, translating these discoveries into human clinical trials has proven to be a complex endeavor [6]. It is probable that the emergence of this illness is caused by complex interactions between different types of brain cells, and the APOE allele gene is involved in the function of several of these cells. For instance, Neurons with the APOE4 gene have a higher count of synapses and produce a greater amount of Aβ42. Astrocytes with the APOE4 gene variant have difficulty taking up Aβ and accumulating cholesterol, while APOE4 microglia-like cells have altered shapes that affect their ability to eliminate Aβ [7]. In addition, the ApoE Cascade Hypothesis has been proposed by some researchers. They propose that these isoforms trigger a sequence of cellular and systemic events, impacting AD-related conditions. While APOE4 is a major AD risk factor, understanding how cell type, disease status, and isoforms interact remains unclear. Recent findings indicate APOE particle size variations based on isoform and cell type. Further research is needed to explore APOE's structural properties, the protective roles of rare variants, and its peripheral impact [8].

1.3. The 5xFAD mouse model

The 5XFAD mouse model is a frequently used tool to research amyloid deposition in AD. These mice have been genetically altered to possess five familial AD (FAD) mutations, resulting in an increased production of the Aβ42 protein, which is known to have a significant impact on AD pathology. At 1.5 months of age, Aβ42 begins to accumulate within neurons, and by 2 months, amyloid deposits start forming in the brain. At 4 months of age, memory deficits start to appear, making them a valuable model for investigating the development of cognitive impairments associated with AD [9].

1.4. The Herpes simplex type 1 virus (HSV-1 virus)

The HSV-1 virus is a double-stranded DNA virus that mainly infects the epithelial cells found in the nasal and oral mucosa. After infecting these cells, the virus can remain dormant in nerve cells near the face, causing a latent infection. Research indicates that HSV-1 may take effect on the onset of Alzheimer's disease by remaining dormant in the brain and becoming active during specific circumstances, resulting in inflammation. This inflammation could lead to the accumulation of harmful substances such as A-β plaques and tau tangles in the brain, which are associated with AD. Furthermore, ongoing inflammation can damage brain cells and exacerbate memory issues in individuals with Alzheimer's [10].

1.5. The APOE-ε4 and HSV-1

Studies have shown that people who possess the APOE-ε4 allele, a genetic factor linked to Alzheimer's disease, may have an increased risk for the condition and have an increased susceptibility to developing AD when infected with HSV-1. It has been observed that APOE-ε4 can affect the outcome of viral infections and is also a predisposing factor for cold sores. The brain viral load might be impacted by the presence of APOE4 during HSV-1 infection, leading to variations in viral spread and colonization. Furthermore, other genes and proteins associated with AD have been found to interact with the herpes simplex virus genome, indicating a potential synergy between host and pathogen in causing AD-like brain damage. The presence of several genes linked to AD and viral interactions further supports the possibility of a connection between infections and AD-like brain damage [10].

It is not yet clear how APOE4 affects the activation of dormant HSV-1 or if dormant HSV-1 can speed up the progression of AD. This article aims to explore the relationship between HSV-1 and the APOE4 allele in 5xFAD mice. Our main goal is to understand how APOE4 affects the activation of dormant HSV-1 in the 5xFAD mouse model and how these two factors work together to influence Alzheimer's disease.

1.6. Design

As part of our experiment, we will be using 5xFAD mice with different APOE alleles (APOE2, APOE3, APOE4) and introducing human neurons containing latent HSV-1 through xenotransplantation. The mice will be split into experimental and control groups, and we will replicate the experiment to ensure the accuracy of the data. We will provide detailed information about the materials and methods used in the experiment in subsequent sections, and we will follow ethical guidelines throughout the entire experiment.

Our first purpose is to investigate how different APOE alleles can affect the role of latent HSV-1 in contributing to the progression of AD. In order to accomplish this, we will use 5xFAD mice and 5xFAD mice with different APOE alleles. We will crossbreed 5xFAD mice with mice carrying human APOE [2,3,4] to create a diverse cohort of APOE alleles. Some offspring will receive injections of human neurons containing latent HSV-1 virus in their brains, while others will serve as the control group without HSV-1. After allowing the mice to mature for two months, we will begin the experimental phase. We will use various experimental techniques to detect the formation of Aβ plaques and other markers indicative of AD progression within the mice.

Our second objective is to investigate how different variations of the APOE gene can affect the activation of latent HSV-1. We will use a group of mice for this purpose. Some of these mice will have their latent HSV-1 activated through stress induction, while others will be used as a control group. To confirm the activation of the virus in the mice, we will observe their condition and use herpes

antibody testing to detect specific markers associated with the activated virus. Once the virus activation is confirmed, we will administer a drug treatment to extend the lifespan of the mice, as the activated virus could cause them to die within a few weeks. This extended lifespan will ensure that the mice survive until the experiment is completed. Then, we will use various experimental techniques to identify the formation of Aβ plaques and other markers indicating the progression of AD in the mice's bodies.

2. Material and methods

2.1. Animal

We obtained C57BL/6 APOE2, APOE3, and APOE4 Targeted Replacement mice, as well as C57BL/6 5x FAD mice from Taconic Transgenic Models™ and Jackson Labs, respectively. All mice were between 3-8 weeks of age and were given at least 7 days to adjust to their new environment before any experiments began. Our molecular studies were conducted on mice bred in-house. All the mice were subjected to a 12-hour light/dark cycle.

2.2. Cell culture and cell differentiation

Human Induced Pluripotent Derived Neural Stem Cells (IC-000) from Lifeline cell technology, Herpes Simplex Virus Type 1 (HSV-1) (Strain: MacIntyre) Culture Fluid (1 mL) from ZeptoMetrix. Culturing neural stem cells followed the instructions on the website. (https://www.lifelinecelltech.com/pdf/INC%20iPS-NSC%200119%20v1%20IC-0001.pdf). Inducing the neural stem cell to the neural Progenitor Cell (NPC). These NPCs are cultured in a controlled environment to maintain their viability and growth. Then HSV-1 virus is introduced to the NPC culture. The NPCs are exposed to the virus under specific conditions that favor the establishment of latency rather than viral replication. This step is followed by the previous article [11]. To ensure the establishment of latent infection, monitor the expression of latency-associated transcripts (LATs), which are non-coding RNAs produced during latency. The presence of LATs is an indicator of latent infection and polymerase chain reaction (PCR) to detect the presence of viral DNA in NPCs over time. The persistent presence of viral DNA indicates the successful establishment of latency. After a period of latency, the NPCs are harvested from the culture.

2.3. Xenotransplantation into mice brain

The harvested NPCs with latent HSV-1 are prepared for injection into the mice's brains. Mice are anesthetized to ensure their comfort during the procedure. A stereotactic apparatus is used to precisely target the injection site within the mouse brain. When finished the surgery, the mice are allowed to recover and are carefully monitored for any adverse effects or behavioral changes. Monitor the activation of viruses in mice by using PCR to detect the presence of viral DNA [12]

2.4. Stress induction

The research involved two sets of mice: one was experimental, and the other was a control group. The mice in the experimental group were kept in well-ventilated, loosely fitting 50-ml centrifuge tubes for five cycles. They were restrained for 15 hours starting from 6:00 PM, after the lights were turned off, until 9:00 AM the next day when the lights were turned on again. In contrast, the mice in the control group were not given food or water during the same 15-hour period [13].

2.5. Pharmacological interventions

To administer acyclovir orally, it was dissolved in the drinking water at a concentration of 1.5 mg/ml. Each mouse in the experimental group received approximately 400 mg/kg of the drug per day for seven days, as mice typically drink about 4 to 5 ml of water per day. The control group did not receive acyclovir [14].

2.6. Herpes antibody testing

Serum collection, Preparation of serum panels, HA antigen preparation, HA assay, Serum inactivation, and HI assay. The article contains information about the process, but it's important to note that certain conditions may differ in the actual experiment [15].

2.7. Histology

Sample Collection and Euthanasia, Perfusion and Brain Removal, Fixation, Sucrose Gradient and Embedding, Sectioning, and Storage. The article contains all the necessary details, and certain conditions may vary based on the results of our actual experiment [16].

2.8. Western blot

Sample Collection, Sample Homogenization, Protein Concentration Determination, SDS-PAGE Gel Electrophoresis, Transfer to Nitrocellulose Membranes, Membrane Preparation, Primary and Secondary Antibody Incubation, Detection and Imaging, Quantification, Molecular Mass, Determination. The process outlined in the article will serve as a foundation, but certain conditions may be subject to modification based on the actual experiment [16].

2.9. Quantification of Aβ load

Sample Preparation, Immunohistochemical Staining, Microscopy and Imaging, Image Analysis, Quantitative Measurements, Interpreting Results. The article outlines the specific reagents and process details, but it's important to note that real-world experiments might involve some modifications [17].

2.10. Statistical analysis

The statistical analysis was conducted using GraphPad Prism version 9.5.0 for Mac. The data are presented as mean ± SEM, and the significance levels were indicated as follows: ***(P<0.001), **(P<0.01), *(P<0.05). The analysis involved t-test column analyses followed by t-tests.

Experiment 1

a. Get the target mice.

Crossbreeding the mice as Table 1 shows.

Table 1. Grouping of experimental mice and target mice produced after the experiment

Parental female mice n=20	Parental male mice n=20	Target offspring (mice) n=80
5xFAD mice	APOE2 mice	5xFAD /APOE2 mice
5xFAD mice	APOE3 mice	5xFAD /APOE3 mice
5xFAD mice	APOE4 mice	5xFAD /APOE4 mice

To identify the offspring that meet the desired criteria, PCR will be used. After identifying the target mice, they will be fostered for a period of 2 months in preparation for the next step.

b. Inject the latent HSV-1 in the target mice's brain.

To begin, the neural stem cell is cultured and transformed into neural progenitor cells (NPC). Next, the NPCs are exposed to HSV-1 to establish a state of latency. The NPCs with latent HSV-1 are then harvested and injected into the brains of mice using Xenotransplantation. The mice are then divided into different groups as outlined in Table 2. N=20

Table 2. Experimental and Control Group Compositions

Experimental group n=20	Control group n=20
5xFAD /APOE2 mice with latent HSV-1	5xFAD /APOE2 mice
5xFAD /APOE3 mice with latent HSV-1	5xFAD /APOE3 mice
5xFAD /APOE4 mice with latent HSV-1	5xFAD /APOE4 mice
5xFAD mice with latent HSV-1	5xFAD mice

c. Monitor the reactivation of HSV-1 in mice and detect the formation of A-β plaque in the brain.

Periodically record observations of Aβ42 oligomer accumulation via fMRI while feeding the mice 3 times a month. When the mice reach the age of 4-5 months, assess their behavior using the Y maze. Then, dissect the mice to obtain brain tissue and use immunofluorescence to observe the Aβ plaque.

Experiment 2

a. Activation of HSV-1

Mice are divided into experimental and control groups, as shown in Table 3.

Table 3. Experimental and Control Group Compositions

Experimental group n=20	Control group n=20
5xFAD /APOE2 mice with latent HSV-1	5xFAD /APOE2 mice with latent HSV-1
5xFAD /APOE3 mice with latent HSV-1	5xFAD /APOE3 mice with latent HSV-1
5xFAD /APOE4mice with latent HSV-1	5xFAD /APOE4mice with latent HSV-1
5xFAD mice with latent HSV-1	5xFAD mice with latent HSV-1

The study will have an experimental group that will be exposed to stress induction, while the control group will not be exposed to any stress. The experimental group will undergo stress induction for a duration of 3 weeks. During this period, the time it takes for the virus to be excited in each mouse after stress induction will be recorded. Additionally, the physiological condition and behavior of the mice will be monitored, including any loss of appetite, skin diseases, and behavioral disorders. The activation of the virus in the mice will be tracked by using PCR to detect the presence of viral DNA. After the stress induction cycle is complete, WB will be used to identify specific proteins of HSV-1 activation to confirm virus activation.

b. Pharmacological Interventions

Mice are divided into experimental and control groups, as shown in Table 4.

Table 4. Experimental and Control Group Compositions

Experimental group n=20	Control group n=20
5xFAD /APOE2 mice with activated HSV-1	5xFAD /APOE2 mice with latent HSV-1
5xFAD /APOE3 mice with activated HSV-1	5xFAD /APOE3 mice with latent HSV-1
5xFAD /APOE4mice with activated HSV-1	5xFAD /APOE4mice with latent HSV-1
5xFAD mice with activated HSV-1	5xFAD mice with latent HSV-1

As part of the experiment, Acyclovir will be added to the drinking water of some mice. Meanwhile, the control group will receive drug-free water. The purpose of this is to increase the chances of the mice surviving for the duration of the experiment. We will keep track of their behavior, weight changes, and skin conditions. Additionally, we will monitor mortality rates and watch out for the possibility of activated mice reverting to a latent state. This could provide valuable information on how gene activation affects the virus.

c. Observe the formation of Aβ in the brain and detect the special markers of Aβ plaque.

Record the Observation of Aβ42 oligomers accumulation via fMRI periodically (3 times a month). When the mice get to the age of 4-5 months, using Y maze to assess mice behavior. Then, dissect the mice to get the brain tissue and then use immunofluorescence to observe the Aβ plaque.

3. Excepted results

3.1. The accelerated development of Alzheimer's disease is influenced by both APOE alleles and latent HSV-1 activation, acting synergistically.

This study investigated how APOE alleles and latent HSV-1 activation affect the progression of Alzheimer's disease (AD). The onset of AD was found to be faster in APOE4-carrying mice, while APOE2 mice showed delayed

behavioral effects. (See Figure 1) Interestingly, APOE4 mice with latent HSV-1 infection developed AD more quickly than non-infected mice. The use of fMRI showed increased Aβ plaque aggregation in APOE4 mice with HSV-1 (although the data was not shown). Additionally, APOE4 mice had faster plaque formation compared to wild-type mice. These findings suggest that APOE4 and HSV-1 have a synergistic effect on promoting Aβ plaque formation and accelerating AD progression, although the exact mechanism is still unknown.

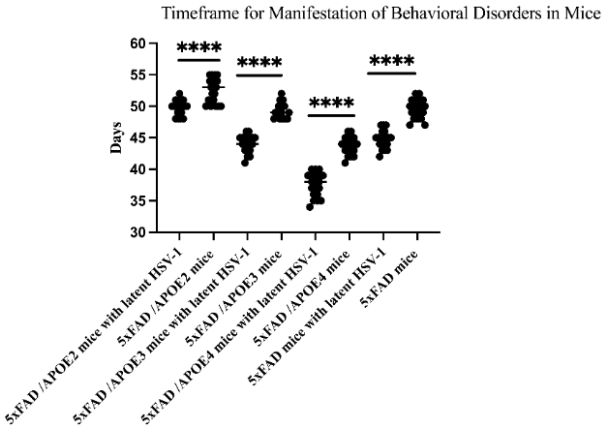

Figure 1. The onset of AD Behavioral Symptoms in Mice

This picture illustrates the time it took for mice with different APOE alleles to manifest symptoms of AD behavioral disorders, categorized by the presence or absence of latent HSV-1 virus. APOE2 mice showed the longest delay in symptom onset, while APOE4 mice were the quickest to exhibit symptoms, and APOE3 mice had a similar onset time to 5xFAD mice. Additionally, mice with latent HSV-1 virus demonstrated an earlier initiation of behavioral disorders when compared to those without the virus. The data is expressed in days." ****(P<0.0001), ***(P<0.001), **(P<0.01), *(P<0.05).

3.2. HSV-1 Activation in APOE4 Mice Accelerates Alzheimer's Progression and Aβ formation.

The activation of HSV-1 is observed earlier in APOE4 mice compared to other mouse types. APOE4 mice with the activated HSV-1 virus show a faster progression towards AD than those with the latent virus form (Figure 2), exhibiting early symptoms like skin disease, anorexia, and behavioral disorders. In contrast, APOE2 mice might have a moderating effect on delaying AD progression. Using fMRI, Aβ plaque aggregation is more pronounced in mice with activated HSV-1 than in those with the latent form. These findings suggest that the activated virus form accelerates Aβ plaque formation, thereby promoting the advancement of AD.

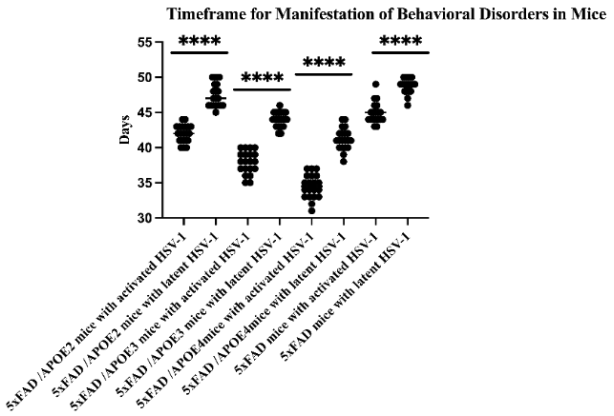

Figure 2. The onset of AD Behavioral Symptoms in Mice

This picture illustrates the time it took for mice with different APOE alleles to manifest symptoms of AD behavioral disorders, categorized by the presence of latent HSV-1 virus or activated HSV-1 virus. APOE2 mice showed the longest delay in symptom onset, while APOE4 mice were the quickest to exhibit symptoms, and APOE3 mice had a similar onset time to 5xFAD mice. Additionally, mice with activated HSV-1 virus demonstrated an earlier initiation of behavioral disorders when compared to the latent HSV-1 virus. The data is expressed in days." ****(P<0.0001), ***(P<0.001), **(P<0.01), *(P<0.05).

4. Discussion

This study suggests that APOE allele and HSV-1 activation could play a role in speeding up the progression of AD. Further investigation is needed to determine the precise molecular mechanisms behind this interaction, which could lead to the discovery of new therapeutic targets. Using the 5xFAD mouse model allowed researchers to study A-β plaque formation, a key characteristic of AD. The study successfully explored how the interaction between APOE alleles and viral activation affects AD progression by introducing human neurons carrying potential HSV-1 into mouse brains. The ability to manipulate variables such as APOE alleles and HSV-1 activation is a significant advantage when studying animal models. By utilizing this approach, researchers can delve into the causality and underlying mechanisms of observed effects. The study design also allows for controlled interventions and exploration of complex interactions that may not be possible in human studies. To increase the relevance of research results, combining animal studies with human clinical data and in vitro models provides a more comprehensive understanding of complex interactions in Alzheimer's disease pathology. Exploring the intricacies of Alzheimer's disease (AD) requires a deep dive into the cellular and molecular changes that occur during its progression. Advanced technologies such as single-cell RNA sequencing and proteomics can provide us with detailed insights into these changes. However, it's important to view these findings within the context of the limitations that exist in animal models. Only then can we truly understand the complexities of AD and develop effective treatments. It is important to note that while the

5xFAD mouse model is used to study Alzheimer's disease (AD), it does not fully capture the complexity of the human disease. AD is a multifactorial disease that involves several genetic, environmental, and epigenetic factors that may not be entirely reproducible in animal models. Furthermore, transferring the results of mouse research to humans requires caution due to species variations. There are genetic, physiological, and behavioral differences between mice and humans that could impact the outcomes and restrict the direct applicability of the results to human AD patients. Additionally, the ethical considerations surrounding the use of animals in research must be acknowledged. While animal models provide valuable insights, the ethical issues necessitate a thoughtful approach to experimental design and execution. It is essential to keep in mind that the use of animals in research raises some ethical issues, and we must be kind to animals while learning from them.

5. Conclusion

In conclusion, this research explores the interaction between APOE alleles and the activation of latent Herpes Simplex Type 1 virus (HSV-1) in the context of Alzheimer's disease (AD) progression, utilizing the 5xFAD mouse model. Our findings indicate that APOE ε4 may indeed accelerate the development of AD, especially when combined with HSV-1 activation, while APOE ε2 may exert a mitigating effect. These results suggest a synergistic relationship between APOE ε4 and HSV-1 activation in promoting the formation of Aβ plaques and hastening AD progression. While these findings offer crucial insights into the complex mechanisms underlying AD, it is important to acknowledge the limitations of using animal models and the need for further investigation into the molecular mechanisms at play. This research advances our understanding of the intricate factors contributing to AD and opens avenues for potential therapeutic interventions. The implications of this study may pave the way for novel treatment strategies that target the interaction between APOE alleles and viral activation, ultimately improving the lives of individuals affected by AD. As we move forward, it is imperative to explore these interactions further, bridging the gap between animal models and human clinical data to gain a comprehensive understanding of AD pathogenesis. Additionally, the ethical considerations surrounding animal research should guide our approach as we continue to unravel the complexities of Alzheimer's disease.

Reference

1. Christensen, D. Z., Kraus, S. L., Flohr, A., Cotel, M.-C., Wirths, O., & Bayer, T. A. (2008). Transient intraneuronal Aβ rather than extracellular plaque pathology correlates with neuron loss in the frontal cortex of APP/PS1KI mice. Acta Neuropathologica, 116(6), 647–655. https://doi.org/10.1007/s00401-008-0451-6
2. Eimer, W. A., & Vassar, R. (2013). Neuron loss in the 5XFAD mouse model of Alzheimer's disease correlates with intraneuronal Aβ42 accumulation and Caspase-3 activation. Molecular Neurodegeneration, 8(1), 2. https://doi.org/10.1186/1750-1326-8-2
3. Fan, R., DeFilippis, K., & Van Nostrand, W. E. (2007). Induction of complement proteins in a mouse model for cerebral microvascular Aβ deposition. Journal of Neuroinflammation, 4(1), 22. https://doi.org/10.1186/1742-2094-4-22
4. Kern, E. R., Richards, J. T., Glasgow, L. A., Overall, J. C., & Miranda, D. (n.d.). Optimal Treatment of Herpes Simplex Virus Encephalitis in Mice with Oral Acyclovir.
5. Knopman, D. S., Haeberlein, S. B., Carrillo, M. C., Hendrix, J. A., Kerchner, G., Margolin, R., Maruff, P., Miller, D. S., Tong, G., Tome, M. B., Murray, M. E., Nelson, P. T., Sano, M., Mattsson, N., Sultzer, D. L., Montine, T. J., Jack, C. R., Kolb, H., Petersen, R. C., … Siemers, E. (2018). The National Institute on Aging and the Alzheimer's Association Research Framework for Alzheimer's disease: Perspectives from the Research Roundtable. Alzheimer's & Dementia, 14(4), 563–575. https://doi.org/10.1016/j.jalz.2018.03.002
6. Lin, Y.-T., Seo, J., Gao, F., Feldman, H. M., Wen, H.-L., Penney, J., Cam, H. P., Gjoneska, E., Raja, W. K., Cheng, J., Rueda, R., Kritskiy, O., Abdurrob, F., Peng, Z., Milo, B., Yu, C. J., Elmsaouri, S., Dey, D., Ko, T., … Tsai, L.-H. (2018). APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. Neuron, 98(6), 1141-1154.e7. https://doi.org/10.1016/j.neuron.2018.05.008
7. Mancuso, R., Van Den Daele, J., Fattorelli, N., Wolfs, L., Balusu, S., Burton, O., Liston, A., Sierksma, A., Fourne, Y., Poovathingal, S., Arranz-Mendiguren, A., Sala Frigerio, C., Claes, C., Serneels, L., Theys, T., Perry, V. H., Verfaillie, C., Fiers, M., & De Strooper, B. (2019). Stem-cell-derived human microglia transplanted in mouse brain to study human disease. Nature Neuroscience, 22(12), 2111–2116. https://doi.org/10.1038/s41593-019-0525-x
8. Martens, Y. A., Zhao, N., Liu, C.-C., Kanekiyo, T., Yang, A. J., Goate, A. M., Holtzman, D. M., & Bu, G. (2022). ApoE Cascade Hypothesis in the pathogenesis of Alzheimer's disease and related dementias. Neuron, 110(8), 1304–1317. https://doi.org/10.1016/j.neuron.2022.03.004
9. Mendez, M. F. (2017). Early-Onset Alzheimer Disease. Neurologic Clinics, 35(2), 263–281. https://doi.org/10.1016/j.ncl.2017.01.005
10. Nabers, A., Perna, L., Lange, J., Mons, U., Schartner, J., Güldenhaupt, J., Saum, K., Janelidze, S., Holleczek, B., Rujescu, D., Hansson, O., Gerwert, K., & Brenner, H. (2018). Amyloid blood biomarker detects Alzheimer's disease. EMBO Molecular Medicine, 10(5), e8763. https://doi.org/10.15252/emmm.201708763

11. Ortiz, G. C., Sheridan, J. F., & Marucha, P. T. (2003). Stress-induced changes in pathophysiology and interferon gene expression during primary HSV-1 infection. Brain, Behavior, and Immunity, 17(5), 329–338. https://doi.org/10.1016/S0889-1591(03)00027-8
12. Passeri, E., Elkhoury, K., Morsink, M., Broersen, K., Linder, M., Tamayol, A., Malaplate, C., Yen, F. T., & Arab-Tehrany, E. (2022). Alzheimer's Disease: Treatment Strategies and Their Limitations. International Journal of Molecular Sciences, 23(22), 13954. https://doi.org/10.3390/ijms232213954
13. Piacentini, R., De Chiara, G., Li Puma, D. D., Ripoli, C., Marcocci, M. E., Garaci, E., Palamara, A. T., & Grassi, C. (2014). HSV-1 and Alzheimerâ€TMs disease: More than a hypothesis. Frontiers in Pharmacology, 5. https://doi.org/10.3389/fphar.2014.00097
14. Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & Van Der Flier, W. M. (2021). Alzheimer's disease. The Lancet, 397(10284), 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4
15. Serrano-Pozo, A., Das, S., & Hyman, B. T. (2021). APOE and Alzheimer's disease: Advances in genetics, pathophysiology, and therapeutic approaches. The Lancet Neurology, 20(1), 68–80. https://doi.org/10.1016/S1474-4422(20)30412-9
16. Trybala, E., Bergström, T., Olofsson, S., Svennerholm, B., & Jeansson, S. (1995). An evaluation of a hemagglutination-inhibition test for the detection of antibodies to herpes simplex virus type 1. Clinical and Diagnostic Virology, 3(2), 191–201. https://doi.org/10.1016/0928-0197(94)00030-X
17. Zheng, W., Benner, E. M., Bloom, D. C., Muralidaran, V., Caldwell, J. K., Prabhudesai, A., Piazza, P. A., Wood, J., Kinchington, P. R., Nimgaonkar, V. L., & D'Aiuto, L. (2022). Variations in Aspects of Neural Precursor Cell Neurogenesis in a Human Model of HSV-1 Infection. Organogenesis, 18(1), 2055354. https://doi.org/10.1080/15476278.2022.2055354