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APOE4 Carriers with Dementia Exhibit Increased Tau, Amyloid Beta, and Iba1 Pathology in the Middle Temporal Gyrus

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Abstract

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the accumulation of extracellular amyloid-beta (Aβ) plaques, tau phosphorylation, and neuroinflammation indexed by microglial activation (Iba1 immunoreactivity). The APOE4 allele is also a major risk factor for AD. This study examined how individuals carrying the APOE4 allele with dementia experienced increased Aβ, tau phosphorylation, and microglial activation in the brain. Data was obtained from the ACT Study and the University of Washington ADRC. AT8, 6e10, and Iba1 levels and clinical information were obtained through the Seattle Alzheimer's Disease Data Set. A two-way ANOVA tested main effects and the interaction of the APOE genotype and cognitive status on the percent positive area of phosphorylated tau (AT8 immunoreactivity), Aβ pathology (6e10 immunoreactivity), and microglial activation (Iba1 immunoreactivity). Our analysis revealed that APOE4 carriers with dementia expressed greater levels of tau and neuroinflammation compared to carriers without dementia. We also found that the donors with dementia carrying APOE4 demonstrated greater levels of tau, Aβ, and neuroinflammation compared to dementia donors without APOE4. These findings lead us to hypothesize that APOE4 exacerbates neuropathology to increase the risk of dementia, but more research is needed to confirm this. This study highlights the clinical relevance of the APOE4 genotype in Alzheimer's disease pathology. Furthermore, it builds and expands on the growing literature determining relationships between APOE4 allele and pathology on dementia.

Keywords: APOE4, Alzheimer's disease (AD), dementia, tau pathology, amyloid beta, microglial activation, neuroinflammation

1. Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease that accounts for 60-80% of all dementia cases (Raulin et al., 2022). Dementia is a clinical syndrome defined as a developed loss in several cognitive domains (Arvanitakis et al., 2019). To develop

effective therapeutics, it is essential to understand the disease's biological mechanisms.

A defining hallmark of AD is the accumulation of amyloid-beta (Aβ) plaques in the extracellular space in the brain (Hampel et al., 2021). Aβ peptides form when the amyloid precursor protein (APP), a transmembrane protein localized at the synapses essential for neural function, is cleaved by β-secretase and γ-secretase (Tyan et al., 2012). The resulting protein accumulates to form extracellular neurotoxic amyloid plaques, which inhibit neural function (Iliyasu et al., 2023). These Aβ plaques inhibit synaptic function and initiate neuronal atrophy (Goel et al., 2022).

Prior studies suggest that neuronal dysfunction in AD is also strongly associated with the accumulation of neurofibrillary tangles (NFTs), which form when hyperphosphorylated tau accumulates (Iliyasu et al., 2023). Tau protein stabilizes microtubules to support axonal transport (Gendron & Petrucelli, 2009). When hyperphosphorylated, the tau dissociates from microtubules, leading to defective axonal transport, destabilization, and ultimately neuronal degeneration (Iliyasu et al., 2023; Metaxas & Kempf, 2016). Studies also show Aβ enhances tau pathology specifically in the entorhinal cortex region of the brain (Cicognola et al., 2025). However, while both tau and Aβ are considered essential components in AD pathology, our understanding of their role in dementia is incomplete. New research is needed to facilitate the development of therapeutics that target these pathways.

Genetic risks also promote AD pathology, specifically and most notably through the apolipoprotein E (APOE) gene (Tanzi, 2012). Specifically, these studies have established that those who carry the APOE4 allele have a greater risk of developing dementia as a result of AD (Pirraglia et al., 2023). According to a meta-analysis of articles on APOE4 allele frequency in individuals with AD, 56% of AD-diagnosed patients carried one copy of the gene, and 11% had both copies ("2022 Alzheimer's Disease Facts and Figures," 2022). APOE4 influences Aβ, NFTs, and neuroinflammation to directly affect AD pathology and clinical progression (Parhizkar & Holtzman, 2022). However, the relationship between the gene and these factors on dementia is less understood. It is essential to distinguish between APOE4 carriers and non-APOE4 carriers because those with AD have a higher chance of having the APOE4 variant compared to APOE2 and APOE3 (Liu et al., 2013). Thus, understanding this distinction helps to develop a better understanding of how the gene influences AD.

Neuroinflammation is another hallmark of AD pathology driven by the activation of glial cells known as microglia, along with the expression of inflammatory factors encompassing plaques and degenerated neurons. Previous studies have demonstrated that AD risk genes are related to immune responses and preferentially expressed in the microglia, immune cells in the central nervous system that maintain homeostasis through phagocytic clearance of protein aggregates. As such, these studies found that neuroinflammation may not only be a consequence of AD pathology but also a catalyst (Sobue et al., 2023). Ionized calcium-binding adaptor protein-1 (Iba1) is a structural actin-binding protein expressed in microglia, used to detect inflammation, specifically marking for microglial activation (Hovens et al., 2014). Interestingly, the APOE4 allele has a direct effect on neuroinflammation and microglial activation, linking Iba1 expression with the APOE4 genotype (Major et al., 2024). Due to their potential relationship, this study aims to test and understand the specific associations between APOE4, neuroinflammation, and dementia.

The current study examines the relationships between APOE4 genotype and dementia diagnosis in relation to three indicators of AD: tau, Aβ, and microglial activation. While several studies demonstrate the independent effects of APOE4 and the three indicators on dementia, the interactive effects of the genes and biological mechanisms of AD that go beyond their independent effects are less understood. Thus, this study seeks to determine the relationship between the APOE4 genotype,

dementia, and each of the three indicators, building on previous findings by moving beyond independent effects of genes and protein expression on Alzheimer's Disease and instead elaborating their interaction effects. This current study contributes to expanding the literature evaluating whether APOE4 plays a more active role in linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor that increases the likelihood of developing Alzheimer's disease.

2. Materials and Methods

All data were obtained through the Seattle Alzheimer's Disease Brain Cell Atlas Data set (Seattle Alzheimer's Disease Data Set, n.d.). All experimentation and data were conducted by the Kaiser Permanente Washington Health Research Institute ACT Study researchers and the University of Washington ADRC at the UW BioRepository and Integrated Neuropathology (BRaIN) laboratory (Gabitto et al., 2024). The dataset focused on different patients' histological pathology, obtained via immunolabeling and microscopy across cortical layers and protein markers. Along with that, the dataset also specified the patient's clinical characteristics. For this study, we examined previously published data from these studies to examine the specific relationships between cognitive status and APOE genotype on neurodegenerative-causing protein expression, and whether there is an enhanced effect when the two act together or if both only independently affect it.

2.1. Diagnosing Dementia

In the ACT study, dementia was diagnosed in a large cohort based on the CASI scale. Participants determined to have a neuropsychological battery based on this scale were subjected to further evaluation. Diagnosis included an evaluation of clinical data and medical record imaging. The dementia subtype was determined with the diagnostic criteria such as DSM-IV and NINCDS-ADRDA. In the ADRC study, dementia was diagnosed based on medical records, imaging, and genetic testing. The diagnosis process included neurological examination, cognitive testing, and interviews. Both studies determined the diagnosis based on established research criteria at consensus conferences (Seattle Alzheimer's Disease Data Set, n.d.).

2.2. Antigen-dependent Tissue Pathology

A detailed protocol is provided in a previously published paper as well as an online procedural overview (Gabitto et al., 2024; Seattle Alzheimer's Disease Data Set, n.d.).

In brief, the procedures carried out by the Kaiser Permanente Washington Health Research Institute ACT were as follows:

Brain tissues from the middle temporal gyrus were sliced, recorded, blocked, and properly collected for analysis.

The diagnosis consisted of sectioning and staining regions of the brain following the National Institute of Aging–Alzheimer's Association (NIA-AA) criteria to ensure consistent identification of AD pathology. The tissues were embedded in paraffin and sectioned. The sections were processed and stained with duplex immunohistochemical staining (IHC) with antibody markers common for certain neurodegenerative diseases, AT8 (tau marker) and 6e10 (Aβ marker). They used antibodies to detect the markers of interest and secondary polymers conjugated to alkaline phosphatase that were visualized using the intelliPATH Ferangi Blue Chromogen Kit. The final stained slides were processed through a microscope in certain regions where pathology of tau tangles and amyloid plaques typically accumulate to accurately diagnose.

Digital microscopy was used to produce images of slides to store results digitally for future use. The stained slides were

cleaned and scanned at 20x magnification with a Leica Aperio AT2 slide scanner to produce a Whole Slide Image (WSI). The resulting images were imported into a cloud-based image storage system. The images were run through an image analysis algorithm to quantify the pathology of markers, including the percent area.

Quantitative image analysis through the HALO software (v.3.4.2986) was used to analyze whole slide images (WSI) and determine numerical data for statistical analysis. Multiplex HALO modules determined the percent area of markers AT8 (tau marker), Iba1 (microglial activation marker), and 6e10 (Aβ marker). The analysis pipeline to quantify marker expression followed sequential steps, beginning with Color Deconvolution and then Analysis Quantification to determine the percent area of each marker.

2.3. Data Retrieval and Organization

The Allen Brain Institute collected and compiled the data for access to the general public. Data accessed July 5th, 2025, via: https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en.

In an Excel spreadsheet, data from both the Quantitative Neuropathology Summary Data (MTG) and Donor Metadata from the Seattle Alzheimer's Disease Brain Cell Atlas Data set were combined and sorted with Donor IDs.

Data from the Quantitative Neuropathology Summary Data (MTG) and Donor Metadata from the Seattle Alzheimer's Disease Brain Cell Atlas Data set were integrated and aligned into a unified Excel spreadsheet to match both datasets based on the Donor ID variable. This allowed us to look at neuropathology across clinical statuses. We generated four groups: APOE4/Dementia, APOE4/No Dementia, No APOE4/Dementia, and No APOE4/No Dementia.

2.4. Normality and Kruskal-Wallis Test

Each of the four groups was individually tested for normality of their data with a calculator to determine the reliability of two-way ANOVA. Normality of the neuropathology was assessed for each individual group using the Shapiro-Wilk test, but there were only 7 donors within the APOE4 vs. No Dementia group, making normality testing unreliable. Thus, due to the violation of normality, the non-parametric Kruskal-Wallis test was instead conducted separately for the APOE4 allele and Dementia to determine statistical significance between groups. This test determines statistical differences without reliance on normality.

2.5. Statistical Analysis on Graphpad Prism

Data tables from Graphpad Prism (v10; Boston, MA) were organized in a two-way ANOVA setup with multiple comparisons. This ensures analysis of the two independent variables (APOE Genotype and Cognitive Status). We analyzed each protein marker separately, using Fisher's LSD with the independent variables Dementia and APOE and the dependent variable percent positive area. Graphs represent the average and the standard error of the mean, and they were generated through Prism.

3. Results

All results are unadjusted, as they do not account for other variables associated with dementia.

It is necessary to address differences between APOE4 carriers and non-carriers. Carriers are a genetic risk factor of AD, often showing greater levels of neuropathology, including tau, Aβ, and Iba1, and having a greater probability of developing dementia. These differences could demonstrate that APOE4 not only acts as a risk factor that increases the risk of dementia but also influences certain mechanisms and neuropathological pathways.

3.1. Normality Testing

Only the APOE4 vs. Dementia measuring AT8 group and APOE4 vs. Dementia measuring Iba1 group demonstrated normality, with p-values of 0.29873764 and 0.59532366, respectively. All others except the APOE4 vs. No Dementia groups indicated $p<0.05$. This shows that p-values for these groups in the two-way ANOVA could be inaccurate, as they suggested deviations from normality.

There was a significant difference in AT8 across APOE4 vs. No APOE4 according to the Kruskal-Wallis test ($H(1)=12.508$, $p=0.00041$). There was a significant difference in AT8 across Dementia vs. No Dementia according to the Kruskal-Wallis test ($H(1)=17.0824$, $p=0.00004$). This indicates that the APOE4 allele and dementia diagnosis are individually associated with differences in AT8 pathology. There was a significant difference in 6e10 across APOE4 vs. No APOE4 according to the Kruskal-Wallis test ($H(1)=27.9616$, $p<0.00001$). There was a significant difference in 6e10 across Dementia vs. No Dementia according to the Kruskal-Wallis test ($H(1)=11.6786$, $p=0.00063$). This indicates that the APOE4 allele and dementia diagnosis are individually associated with differences in 6e10 pathology. There was no significant difference in Iba1 across APOE4 vs. No APOE4 according to the Kruskal-Wallis test ($H(1)=2.4656$, $p=0.11636$). There was no significant difference in Iba1 across Dementia vs. No Dementia according to the Kruskal-Wallis test ($H(1)=0.6339$, $p=.42592$). This indicates that neither APOE4 carriers nor dementia diagnosis is individually associated with differences in Iba1 pathology.

Since the Kruskal-Wallis test does not account for interaction between APOE4 and dementia, interpretations should thus be analyzed with caution. Interpretations of two-way ANOVA should be investigated with caution due to violations of normality. As a result, future research should consider larger samples and non-parametric factorial ANOVA methods.

3.2. APOE4 Genotype and Dementia Diagnosis Differentially Impact Tau Pathology

We determined the effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on AT8 expression (percent area). AT8 is a monoclonal antibody marker that detects phosphorylated tau. Thus, we wanted to investigate whether APOE4 or dementia status is associated with AT8 expression. There was no significant interaction between APOE4 and cognitive status on AT8 expression (two-way ANOVA, $F(1,80)=0.2$, $p=0.625$). This indicates that the status of APOE4 is independent of the status of dementia on AT8 pathology. In other words, carrying the APOE4 allele does not appear to modify the relationship between dementia and tau accumulation in this sample. The simple effect difference of the interaction was also not statistically significant at 0.3050 (95% CI of difference: -0.9317 to 1.542), further emphasizing that finding. However, there was a significant main effect of dementia diagnosis independently of AT8 pathology ($F(1,80)=12$, $p<0.001$), with dementia diagnosis having greater AT8 levels (Predicted LS mean = 1.879) than no dementia diagnosis (Predicted LS mean = 0.7668). This finding demonstrates that dementia diagnosis is associated with increased tau pathology.

Amyloid-β Pathology is Elevated in APOE4 Carriers Regardless of Dementia

The APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) effect on 6e10 expression (percent area) was examined. 6e10 is a monoclonal antibody marker that detects Aβ. Thus, we wanted to investigate whether APOE4 or dementia status is associated with 6e10 expression. There was no significant interaction between APOE4 and cognitive status (two-way ANOVA, $F(1,80)=0.03$, $p=0.874$). This indicates that the status of APOE4 is independent of the status of dementia in 6e10 pathology. In other words, carrying the APOE4 allele does not modify the relationship between dementia and Aβ accumulation in this sample. The simple effect difference of the interaction was not statistically significant at 0.1874 (95% CI of difference: -2.152 to 2.527), further emphasizing this finding. There was also no significant main effect of dementia diagnosis independently of 6e10 pathology ($F(1,80)=3.8$, $p=0.053$), with dementia diagnosis having greater 6e10 levels (Predicted LS mean = 3.547) than no dementia diagnosis (Predicted LS mean = 2.392). This reveals that dementia was not significantly associated with pathology in this sample.

3.3. APOE4 Genotype and Dementia Diagnosis Influences Iba1 Pathology

The effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on Iba1 expression (percent area) were determined. Iba1 is a protein marker for the microglia whose expression increases when there is neuroinflammation in the brain. Thus, we wanted to investigate whether APOE4 or dementia status is associated with Iba1 expression. There was significant interaction between APOE4 and cognitive status (two-way ANOVA, $F(1,80)=8.5$, $p=0.004$). This indicates that the status of APOE4 may interact with the status of dementia in Iba1 pathology. In other words, carrying the APOE4 may modify the relationship between dementia and Iba1 accumulation, such that there is enhanced expression. The simple effect difference of the interaction demonstrates this point further, being statistically significant at 0.2.918 (95% CI of difference: 0.9365 to 4.899). There was a significant main effect of dementia diagnosis independently of Iba1 pathology, $F(1,80)=4.5$, $p=0.036$, with dementia diagnosis having greater Iba1 levels (Predicted LS mean = 4.271) than no dementia diagnosis (Predicted LS mean = 3.210). This conveys that dementia diagnosis is associated with increased Iba1 in this sample.

3.4. APOE4 Increases Tau Pathology in Individuals With and Without Dementia

A multiple comparisons test was conducted to determine the significant main effects on AT8 pathology from the previous data, as shown in Figure 1 (Two-way ANOVA, Fisher's LSD post hoc tests). There was a statistically significant difference between APOE4 carriers and non-APOE4 carriers in the dementia group, with a mean difference of 1.278 (95% CI of diff: 0.4797 to 2.076, $p=0.002$). This indicates that for individuals in the sample who have dementia, carrying APOE4 is associated with greater tau levels than not carrying it. There was a statistically significant difference between APOE4 carriers and non-APOE4 carriers in the no dementia group, with a mean difference of 0.9728 (95% CI of diff: 0.02813 to 1.917, $p=0.044$). This conveys that for individuals in the sample who don't have dementia, carrying APOE4 is linked to greater tau levels than not carrying it. There was a statistically significant difference between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.264 (95% CI of diff: 0.2176 to 2.311, $p=0.019$). This reveals that for individuals in the sample who carry the APOE4 allele, having dementia is linked to greater tau levels than not having dementia. There was a statistically significant difference between dementia and no dementia in the no APOE4 allele group, with a mean difference of 0.9592 (95% CI of diff: 0.3005 to 1.618, $p=0.005$). This reveals that for individuals in the sample who don't carry the APOE4 allele, having dementia is linked to greater tau levels than not having dementia.

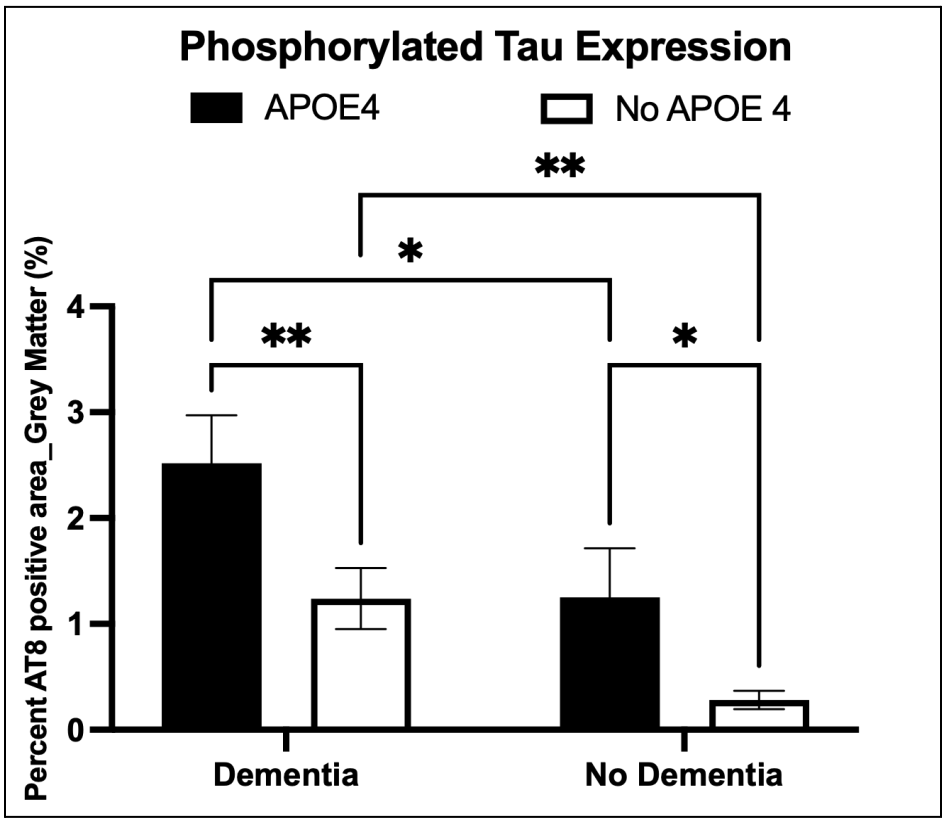

Figure 1: Multiple comparisons between APOE4 genotype and dementia diagnosis on the significance of main effects on AT8 pathology

Note: Significance was found between APOE4 and no APOE4 genotype within the dementia group (**p=0.002). Significance was found between APOE4 and the no APOE4 genotype within the no dementia group (*p=0.044). Significance was found between dementia and no dementia within the APOE4 group (*p=0.019). Significance was found between dementia and no dementia within the no APOE4 group (**p=0.005).

3.5. APOE4 Influences Amyloid-β Pathology Independently of Dementia Diagnosis

A multiple comparisons test was conducted to determine the significant main effects on 6e10 pathology from the previous data, as shown in Figure 2 (Two-way ANOVA, Fisher's LSD post hoc tests). There was a statistically significant difference between APOE4 carriers and non-APOE4 carriers in the dementia group, with a mean difference of 3.118 (95% CI of diff: 1.608 to 4.628, p<0.001). This shows that for individuals in the sample who have dementia, carrying APOE4 is linked to greater Aβ levels than not carrying it. There was a statistically significant difference between APOE4 carriers and non-APOE4 carriers in the no dementia group, with a mean difference of 2.931 (95% CI of diff: 1.143 to 4.718, p=0.002). This indicates that for individuals in the sample who don't have dementia, carrying APOE4 is linked to greater Aβ levels than not carrying it. There

was no statistically significant difference between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.249 (95% CI of diff: -0.7313 to 3.229, p=0.213). This reveals that for individuals in the sample who carry the APOE4 allele, there is no difference in Aβ levels between donors with and without dementia. There was no statistically significant difference between dementia and no dementia in the no APOE4 allele group, with a mean difference of 1.061 (95% CI of diff: -0.1848 to 2.308, p=0.094). This demonstrates that for individuals in the sample who do not carry the APOE4 allele, having dementia is not linked to greater Aβ accumulation compared to not having dementia.

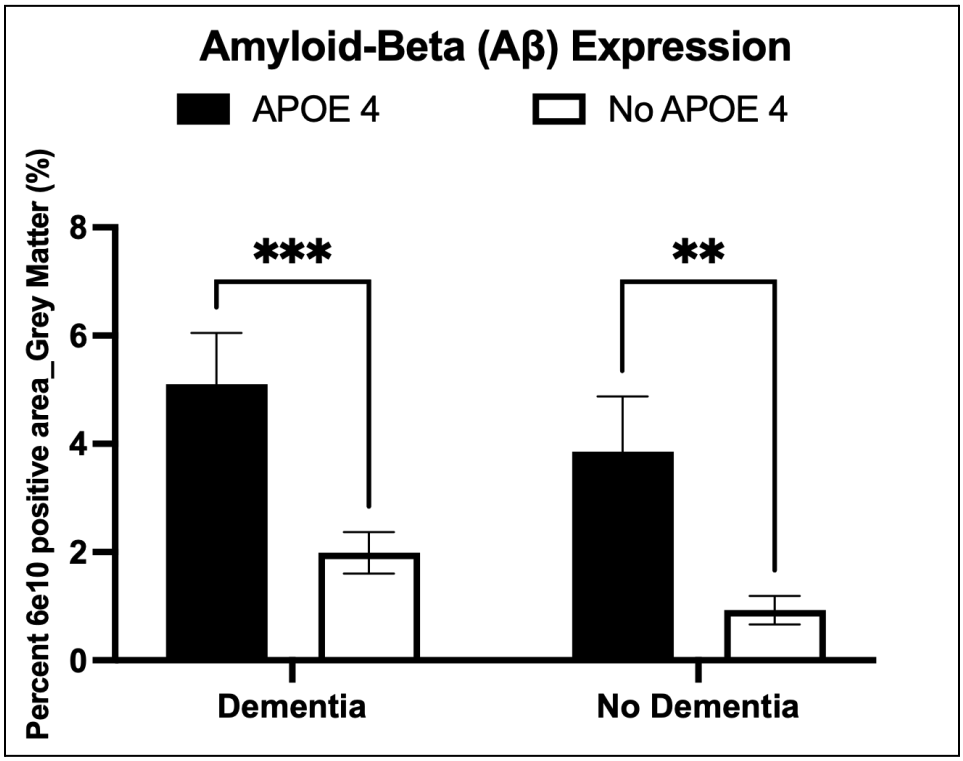

Figure 2: Multiple comparisons between APOE4 genotype and dementia diagnosis on the significance of main effects on 6e10 pathology

Note: Significance was found between APOE4 and no APOE4 genotype within the dementia group (***p<0.001). Significance was found between APOE4 and no APOE4 genotype within the no dementia group (**p=0.002). No significance was found between dementia and no dementia within the APOE4 group (p=0.213). No significance was found between dementia and no dementia within the no APOE4 group (p=0.094).

3.6. Microglial Activation Increases in APOE4 Carriers with Dementia

A multiple comparisons test was conducted to determine the significant main effects on 6e10 pathology from the previous data, as shown in Figure 3 (Two-way ANOVA, Fisher's LSD post hoc tests). There was a statistically significant difference between APOE4 carriers and non-APOE4 carriers in the dementia group, with a mean difference of 1.918 (95% CI of diff:

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0.6392 to 3.197, $p<0.004$). This indicates that for individuals in the sample who have dementia, carrying APOE4 is linked to greater Iba1 levels than not carrying it. There was no statistically significant difference between APOE4 carriers and non-APOE4 carriers in the no dementia group, with a mean difference of -0.9999 (95% CI of diff: -2.513 to 0.5136, $p=0.192$). This indicates that for individuals in the sample who don't have dementia, there is no difference in Iba1 levels between APOE4 carriers and non-APOE4 carriers. There was a statistically significant difference between dementia and no dementia in the APOE4 allele group, with a mean difference of 2.520 (95% CI of diff: 0.8426 to 4.197, $p=0.004$). This shows that for individuals in the sample who carry the APOE4 allele, having dementia is linked to greater Iba1 levels than not having dementia. There was no statistically significant difference between dementia and no dementia in the no APOE4 allele group, with a mean difference of -0.3984 (95% CI of diff: -1.454 to 0.6571, $p=0.455$). This shows that for individuals in the sample who do not carry the APOE4 allele, there is no difference in Iba1 levels between donors with and without dementia.

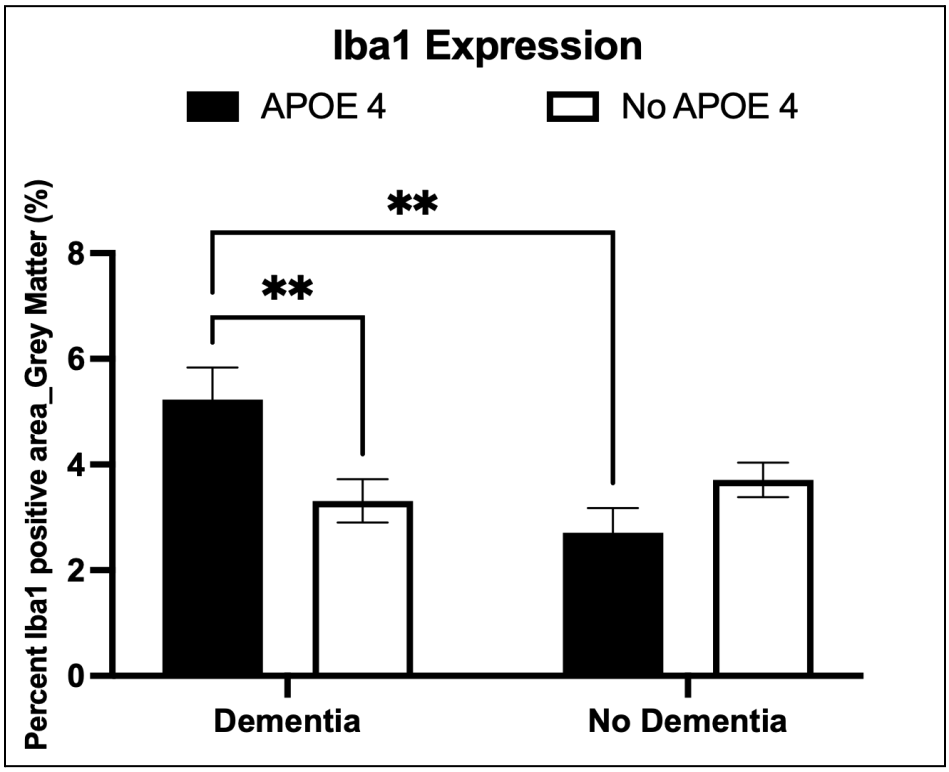

Figure 3: Multiple comparisons between APOE4 genotype and dementia diagnosis on the significance of main effects on Iba1 pathology

Note: Significance was found between APOE4 and no APOE4 genotype within the dementia group (**$p=0.004$). No significance was found between APOE4 and no APOE4 genotype within the no dementia group ($p=0.192$). Significance was found between dementia and no dementia within the APOE4 group (**$p=0.004$). No significance was found between dementia and no dementia within the no APOE4 group ($p=0.455$).

4. Discussion

Carriers of the APOE4 allele are at high risk of developing Alzheimer's dementia (Pirraglia et al., 2023). Researchers have hypothesized that AD and the resulting dementia are linked to neuroinflammation along with the accumulation of phosphorylated tau and amyloid-beta oligomers. This study identifies how donors carrying the APOE4 allele with dementia have increased neuropathology compared to carriers without dementia. Furthermore, our study found that donors with dementia similarly showed increased neuropathology when carrying the APOE4 allele than when not carrying it.

4.1. Tau Pathology in Participants with APOE4 and Dementia

Based on the previously established relationship between the APOE genotype and the hyperphosphorylated tau's independent effects on dementia, we developed a two-way ANOVA to investigate the genotype's effects on AT8 pathology. We found that APOE4 carriers with dementia had higher levels of phospho-tau compared to carriers without dementia (Figure 1). Along with that, APOE4 carriers with dementia exhibited greater levels of tau compared to non-carriers with dementia (Figure 1). This indicates that APOE4 is a pivotal factor in the tau-neuropathology observed in dementia.

Based on the finding that APOE4 carriers with dementia had a greater tau pathology compared to carriers without dementia, there may be a causal pathway and effect modification linking the APOE4 allele and tau expression on dementia. Since APOE4 carriers with dementia had higher tau levels than APOE4 carriers without dementia donors, this could mean that the APOE4 allele influenced greater dementia risk through its effects on tau pathology, rationalizing a causal pathway. This relationship could also be justified as a result of an effect modification between tau pathology and the APOE4 allele, which leads to dementia risk. However, analysis with a two-way ANOVA is insufficient in determining the strength and direction of this association. Along with that, while post-hoc comparisons suggested that APOE4 carriers with dementia indicated greater neuropathological levels than carriers without dementia, the interaction between dementia and APOE4 was not statistically significant. Thus, causal pathways and effect modification are speculative. Future research should examine correlation and logistic regressions to test the strength of this relationship between dementia, APOE4, and these protein markers.

The direct relationship between the APOE4 genotype and tau pathology in dementia is not concrete, with ongoing debate on whether the allele exerts influence only through interaction with Aβ. Some previous studies have shown that the presence of the APOE4 allele alone causes an increase in tau pathology independent of Aβ and other covariates. These studies show that APOE4 carriers saw greater tau in the entorhinal cortex and hippocampus without an interaction with Aβ (Therriault et al., 2020). These effects were significantly greater compared to those with APOE2, which demonstrated reduced and protective effects of regional tau (Young et al., 2023). This demonstrates a causal pathway, where the APOE4 allele potentiates the expression of tau, thus increasing dementia risk. Studies have also highlighted a potential interaction between tau and the APOE4 allele, where they may have a complex relationship that contributes to AD. Specifically, these studies have hypothesized that the APOE4 may direct the spread of tau or directly interact with tau by colocalizing in NFTs in the form of APOE4 fragments (ApoE-Calypse Tau: ApoE–Tau Synergy in Alzheimer's Disease | Journal of Experimental Medicine | Rockefeller University Press, n.d.). This indicates the possible effect modification between the APOE4 allele and tau pathology in addition to their causal pathway. Some studies have also shown that the APOE4 allele has minimal independent effects on tau pathology, having a more indirect effect (Cicognola et al., 2025). However, the APOE4 genotype nonetheless demonstrates a positive relationship with tau pathology. Since APOE4 enhances the expression of phosphorylated tau and may also synergize with it to increase dementia risk, this supports both the possible causal pathway-based rationale and effect

modification rationale behind the finding that APOE4 carriers with dementia have greater tau levels than APOE4 carriers without dementia.

4.2. Limitations in Assessing Amyloid-β Pathology in APOE4 Carriers with Dementia

Our findings demonstrate that Aβ levels were higher in carriers of the APOE4 allele with dementia. Carriers of the APOE4 allele with dementia demonstrated greater levels of tau compared to non-APOE4 carriers with dementia (Figure 2). This result agrees with the deduction that neuropathology will be greater in dementia donors who carry the APOE4 allele. However, the results do raise certain contrasts against parts of the findings. Specifically, carriers of APOE4 with dementia had no significant difference in Aβ levels compared to carriers without dementia. This diverges from the idea that the neuropathology is greater in APOE4 carriers with dementia compared to carriers without dementia. However, possible errors could have conflicted with the actual results. The marker used to detect Aβ, 6e10, also acts as a marker for APP (Noguchi et al., 2009). APP, unlike Aβ, is an essential component in regular neural physiology, contributing to nervous system development and synaptic functioning (Müller et al., 2017). Thus, it is possible that 6e10 marked APP, which does not have neurotoxic effects, along with Aβ, which does have neurotoxic effects. As a result, carriers of the APOE4 allele without dementia could have actually had significantly lower levels of Aβ compared to APOE4 carriers with dementia.

If this possibility were true and there was indeed an error in methodology, there may be both a causal pathway and effect modification between the APOE4 allele and greater Aβ levels, leading to greater dementia risk. With APOE4 carriers with dementia having greater Aβ levels than carriers without dementia, greater Aβ levels could interact with the APOE4 allele to lead to dementia risk. Along with that, this finding could be justified because APOE4 enhances Aβ pathology, increasing dementia risk and revealing a causal pathway. However, due to this speculation being based on an error and on a two-way ANOVA, future research should focus on logistic regression and the use of another protein marker that focuses solely on Aβ to determine more precisely the relationship between the APOE4 allele, dementia, and Aβ. Furthermore, interaction between dementia and APOE4 on Aβ was not statistically significant, indicating that these associations are speculative.

Previous research has demonstrated that the APOE4 allele drives Aβ deposition with greater and earlier onset compared to isoforms E2 and E3. Specifically, these studies have shown that an APOE4 allele increases toxicity, neuronal death, and synaptic damage through greater accumulation of these proteins by demonstrating that suppressing the alleles in vivo reduces these effects (Raulin et al., 2022). Thus, the APOE4 allele enhances Aβ expression, leading to AD pathology. This affirms the possibility of a causal pathway linking APOE4 and Aβ expression.

The interaction effect between APOE4 and Aβ also increases tau concentration. When comparing the interaction between one allele and Aβ, it resulted in greater tau concentration locally. When comparing the interaction between two APOE alleles and Aβ, there was a more widespread increase in tau concentration for APOE4-positive individuals. Thus, APOE4 is thought to drive tau pathology through interactive effects with Aβ (Therriault et al., 2021). As a hallmark of dementia, a greater concentration of phosphorylated tau leads to AD. This also illustrates the rationale that there is effect modification between the allele and the protein. Specifically, since phosphorylated tau has been shown to increase the risk and frequency of dementia as levels increase, increased tau levels as a result of associations between Aβ levels and an APOE4 allele demonstrate their effect modification to enhance dementia frequency.

4.3. Neuroinflammation in APOE4 Carriers with Dementia

Protein marker Iba1 was used to detect and determine neuroinflammation. Our findings showed that APOE4 carriers with dementia expressed significantly higher levels of Iba1 compared to APOE4 carriers without dementia (Figure 3). Similarly, carriers with dementia also expressed significantly higher levels of Iba1 compared to non-carriers with dementia (Figure 3).

It is possible that the finding that APOE4 carriers with dementia had a greater Iba1 pathology compared to carriers without dementia occurred due to a causal pathway linking the APOE4 allele and neuroinflammation. According to the two-way ANOVA, APOE4 and dementia demonstrated statistically significant interaction on Iba1, suggesting that APOE4 could possibly enhance neuroinflammation in dementia. Since there were significantly greater Iba1 levels in carriers with dementia than in carriers without dementia, there could be a pathway where the APOE4 allele influences greater neuroinflammation levels, enhancing the risk of dementia. However, analysis of this relationship with a two-way analysis is inappropriate because it does not determine the strength and direction of the relationship. Thus, other analysis tools such as logistic regression and correlation should be utilized in future research to determine a more concrete conclusion regarding the actual relationship between APOE4, neuroinflammation, and dementia.

Microglia in the brain maintain homeostasis by engaging in phagocytosis and regulating neuronal conditions under normal conditions. However, studies have demonstrated that with the appearance of APOE4, the microglia become activated. In this activated state, these glial cells produce pro-inflammatory cytokine factors, inducing neuroinflammation (Dias et al., 2025; Smith et al., 2012). Thus, the presence of the APOE4 allele in donors promotes the effects of neuroinflammation. This affirms the possibility of a causal pathway between APOE4 and neuroinflammation. Specifically, the APOE4 allele potentiates greater neuroinflammation, thus increasing dementia risk. If the causal pathway between the allele and dementia marker is true, it could also be plausible that APOE4 and neuroinflammation are involved in a positive feedback loop. Specifically, as APOE4 increases neuroinflammation, this greater neuroinflammation triggers further APOE4 activity. This may lead to a cascade of further inflammation and APOE4 expression, leading to dementia. However, further research would also need to be conducted regarding neuroinflammation's effect on APOE4 activation. Specifically, in vivo experimentation could manipulate microglial activation in APOE4 carrier mice to determine a relationship.

5. Discussion

Our study highlights that APOE4 carriers with dementia showed greater neuropathology of tau and neuroinflammation compared to carriers without dementia. However, this effect was not demonstrated in levels of Aβ, possibly due to limitations of the marker used in the protocol. Furthermore, we found that carriers with dementia showed greater neuropathology than non-carriers with dementia. These findings suggest that there may be a causal pathway or effect modification between APOE4, marker levels, and AD, where dementia risk increases when in the presence of APOE4 and higher levels of a marker. However, the results do not suggest whether increased neuropathology causes increased dementia risk or if the two increase in parallel with one another. Future research should consider the use of logistic regression or correlation analysis to determine the strength and direction of this relationship. If this rationale is confirmed, further research could also determine whether the region of protein accumulation in the brain has any effect on dementia in relation to the APOE4 genotype and independently. This will narrow the scope of AD pathology and provide insight that will help in the pharmaceutical efforts to develop therapeutics against dementia.

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Data Availability

All datasets analyzed and methodology are available through the Allen Brain Map in the Seattle Alzheimer's Disease Brain Cell Atlas:
https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en

Two-way ANOVA and post-hoc tests were performed using Graphpad Prism (v10; Boston, MA). Shapiro-Wilk and Kruskal-Wallis tests were calculated manually by calculator. All raw data used for analyses are also provided in the Appendix.

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Author Biography

William Wang is a young researcher from the United States of America. His specialty spans neuroscience and infectious diseases. At his secondary school, his passion for medicine and science has led him to guide his team in competitions such as the HOSA and Science Olympiad conferences. Beyond his neuroscience research, Wang has conducted work in infectious disease, authoring a pharmacological review on the efficacy of Artemisia compounds against multidrug-resistant pulmonary tuberculosis under the mentorship of Dr. Pascal Gisenya.

Mentor Contribution Statement

Dr. Jorge A. Avila provided key mentorship and support in developing and completing this project.

Dr. Avila provided initial access to the datasets, procedures, and instructions on software protocols to conduct the secondary data analyses. Through his guidance, the author was able to organize, analyze, visualize, and interpret the data used in this study.

Second, Dr. Avila provided critical feedback on the analyses, interpretation, and reporting in the author's manuscript. Through his guidance, the manuscript improved tremendously and helped develop the author's scientific voice.

Finally, Dr. Avila provided critical guidance in revisions, further improving this manuscript before publication. His mentorship is greatly appreciated, and this manuscript would not have been possible without his guidance.

Appendix

Table 1: Donor AT8 Percent positive area_Grey Matter Raw

APOE4 with Dementia	APOE4 without Dementia	No APOE4 with Dementia	No APOE4 without Dementia
0.011590787	1.419477191	0.076823969	0.024581404
1.888201808	0.043412911	0.033161347	0.009936062
0.9100716	2.837355384	0.127550188	0.113147487
0.903202772	0.072875885	1.978833129	0.214959723
2.011315673	0.677142237	1.683492812	0.022147665
2.588241585	0.0080518	0.093844431	0.015822854
2.837021516	2.992553952	1.605939852	0.045866304
0.650423677	3.173003567	1.200098662	0.011863017
2.497302798	0.055242071	3.455456081	0.028657524
3.857216337		0.960635601	0.454189024
6.924529518		0.05606376	0.017713481
1.395571711		0.680889755	0.053114626
1.011173608		1.666672955	0.118152115
4.062182723		0.365801476	0.048465869
4.22259745		5.382839956	0.235346855
4.509271665		0.400661396	0.278156957
		2.887391692	0.592027496

		0.081182247	0.101750643
		0.474951992	1.91768683
		0.007338071	0.013502549
		3.296115657	0.274923388
		0.019594538	0.007572086
		4.201246422	0.023134551
		0.028482753	0.266903579
		0.929310786	0.711325686
		0.536235038	0.228219981
			0.090378507
			0.041366857

Table 2: Donor 6e10 Percent positive area_Grey Matter Raw

APOE4 with Dementia	APOE4 without Dementia	No APOE4 with Dementia	No APOE4 without Dementia
0.09800745	2.520692565	1.869931936	0.233687244
9.060768751	2.116395548	0.003928574	0.000806054
1.678467763	2.776826055	1.299187906	0.168045591
6.44630744	6.975808464	2.357342442	6.546090886
3.000509274	3.242628319	0.377332871	0.000532484
3.362636896	1.130799083	1.198885779	0.000191268
3.514674635	10.75596125	3.162984639	2.889335118
3.582815402	3.044747366	6.495166629	0.026073154
3.707978047	2.153060603	1.536472839	0.000203861
2.60783311		0.34648185	0.119973881
3.261146895		1.505708265	0.001294962
15.29570291		1.66063891	0.12824423
6.760132171		2.107489042	0.000764286

10.3937098		0.7896283	2.249172799
4.201598271		1.472427711	1.383656429
4.727165529		2.50434235	1.877288571
		5.553367866	3.225659769
		0.001530732	0.622260668
		0.577381515	2.207219434
		0.630034814	0.001108222
		8.195152303	0.570678226
		1.290035963	0.000168494
		1.291883589	0.001272704
		1.577091086	0.242039104
		1.307675521	3.217809436
		2.584244759	0.001626802
			0.003521124
			0.002016322
			0.08114139
			1.736206567
			3.048089522
			0
			0.001590433

Table 3: Donor Iba1 Percent positive area_Grey Matter Raw

APOE4 with Dementia	APOE4 without Dementia	No APOE4 with Dementia	No APOE4 without Dementia
3.464914626	1.423919869	1.378781196	2.076656194
8.936774274	3.86842721	3.213022413	2.475174768
5.348567263	1.179175425	5.072148371	3.799753301

7.445298151	4.362645253	5.778139261	6.884660471
4.495065804	1.167843406	6.49415367	7.091355926
4.998530112	1.370065824	1.953493903	1.222678946
9.513231434	3.937066762	2.48339243	5.816597407
3.110123941	3.13064423	0.889626131	2.524858341
5.7200832	3.951066127	1.195535503	2.706449592
1.10601287		0.696723748	3.444246513
6.44983744		3.705694053	6.048013586
8.657872802		1.830671577	4.510429237
3.567180647		1.591359154	2.567704324
2.645538686		2.740128909	4.123424188
4.17471966		4.49607682	1.00351526
4.040954188		3.319630794	3.528520219
		3.63883636	1.140085506
		2.286564722	2.851270194
		1.239221399	2.299509752
		3.842750143	2.995392424
		10.14681443	6.097387985
		3.86278641	4.23694088
		3.724230816	1.372365783
		2.552773483	3.975261562
		5.702373748	3.780249904
		2.26740914	3.724480169
			4.896030473
			8.930063233
			2.403797472
			2.47034273

			5.947291923
			2.938929588
			2.546147942

Table 4: Normality Shapiro-Wilk p-Value Test Statistics

Marker	APOE4 vs Dementia	APOE4 vs No Dementia	No APOE4 vs Dementia	No APOE4 vs No Dementia
AT8	0.29873764	–	0.00025023	0.00000002
6e10	0.01855652	–	0.00005096	0.00000034
Iba1	0.59532366	–	0.00948454	0.03820368

Table 5: Kruskal-Wallis Test Statistics

Marker	Comparison	H Statistic	N	p-Value	Significance
AT8	Dementia vs No	17.0824	84	0.00004	Yes
AT8	APOE4 vs No	12.508	84	0.00041	Yes
6e10	Dementia vs No	11.6786	84	0.00063	Yes
6e10	APOE4 vs No	27.9616	84	<0.00001	Yes
Iba1	Dementia vs No	0.6339	84	0.42592	No
Iba1	APOE4 vs No	2.4656	84	0.11636	No