APOE4 Interacts With Tau, Amyloid Beta, And Iba1 Pathology In The Middle Temporal Gyrus In Dementia

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 associations between APOE4 and Aβ, tau phosphorylation, and microglial activation in relation to dementia. 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 was linked to greater tau pathology (AT8 immunoreactivity) in individuals with dementia. The APOE4 genotype was associated with differences in microglial activation (Iba1 immunoreactivity) by dementia diagnosis. These findings also suggest that APOE4 may act in concert with neuroinflammation to influence dementia pathology. This study highlights the clinical relevance of the APOE4 genotype in Alzheimer's disease pathology.

Keywords

Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Neuroscience, Dementia, Alzheimer's Disease, and APOE

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 an 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). 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.

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 interaction 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 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 is central in linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor.

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 data set focused on different patients' histological pathology, obtained via immunolabeling and microscopy across cortical layers and protein markers. Along with that, the data set 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.

Dementia Diagnosing

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.).

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.).

Briefly, 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.

Data Retrieval and Organization

The Allen Brain Institute collected and compiled the data for access to the general public. I accessed the data on 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 data sets based on the Donor ID variable. This allowed us to look at neuropathology across clinical status. We generated four groups (APOE4/Dementia, APOE4/No Dementia, No APOE4/Dementia, No APOE4/No Dementia).

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 mean and were generated through Prism.

Results

APOE4 Genotype v. Dementia Diagnosis on AT8 Pathology

A two-way ANOVA was conducted to determine the effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on AT8 pathology (percent area). There was no significant interaction between APOE4 and cognitive status on AT8 expression, $F(1,80)=0.2$, $p = 0.625$. This indicates that the status of APOE4 is independent of the status of dementia in AT8 pathology. The simple effect difference of the interaction was also not statistically significant at 0.3050 (95% Cl of difference: -0.9317 to 1.542). There was a significant main effect of dementia diagnosis independently on 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).

APOE4 Genotype v. Dementia Diagnosis on 6e10 Pathology

The APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) were subjected to a two-way ANOVA on 6e10 pathology (percent area) to determine their effects. There was no significant interaction between APOE4 and cognitive status, $F(1,80)=0.03$, $p = 0.874$. This indicates that the status of APOE4 is independent of the status of dementia in 6e10 pathology. The simple effect difference of the interaction was not statistically significant at 0.1874 (95% Cl of difference: -2.152 to 2.527). There was no significant main effect of dementia diagnosis independently on 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).

APOE4 Genotype v. Dementia Diagnosis on Iba1 Pathology

The effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on Iba1 pathology (percent area) were determined through a two-way ANOVA. There was significant interaction between APOE4 and cognitive status, $F(1,80)=8.5$, $p = 0.004$. This indicates that the status of APOE4 may interact with the status of dementia in Iba1 pathology. The simple effect difference of the interaction was statistically significant at 0.2.918 (95% Cl of difference: 0.9365 to 4.899). There was a significant main effect of dementia diagnosis independently on 6e10 pathology, $F(1,80)=4.5$, $p=0.036$, with dementia diagnosis having greater 6e10 levels (Predicted LS mean = 4.271) than no dementia diagnosis (Predicted LS mean = 3.210).

Post-Hoc Comparisons on AT8 Pathology

Following the two-way ANOVA, a multiple comparisons test was conducted to determine the significant main effects on AT8 pathology from the previous data based on Fisher's LSD post-hoc tests. There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the dementia group with a mean difference of 1.278 (95% Cl of diff: 0.4797 to 2.076, $p = 0.002$). There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the no dementia group with a mean difference of 0.9728 (95% Cl of diff: 0.02813 to 1.917, $p = 0.044$). There was statistically significant interaction between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.264 (95% Cl of diff: 0.2176 to 2.311, $p = 0.019$). There was statistically significant interaction between dementia and no dementia in the no APOE4 allele group with a mean difference of 0.9592 (95% Cl of diff: 0.3005 to 1.618, $p = 0.005$).

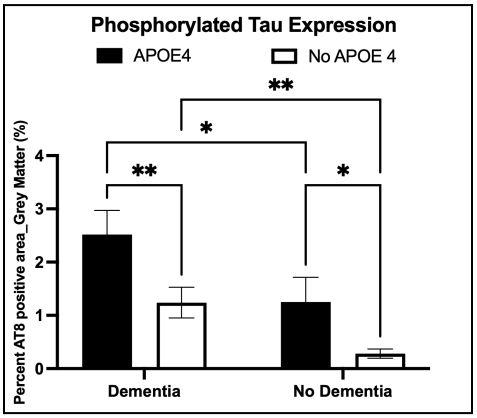

Figure 1: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on AT8 Pathology. Significance was found between APOE4 and no APOE4 Genotype within the dementia group (**p = 0.002). Significance was found between APOE4 and no APOE4 Genotype within the 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).

Post-Hoc Comparisons on 6e10 Pathology

Following the two-way ANOVA, a multiple comparisons test was conducted to determine the significant main effects on 6e10 pathology from the previous data based on Fisher's LSD post-hoc tests. There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers

in the dementia group with a mean difference of 3.118 (95% Cl of diff: 1.608 to 4.628, p < 0.001). There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the no dementia group with a mean difference of 2.931 (95% Cl of diff: 1.143 to 4.718, p = 0.002). There was no statistically significant interaction between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.249 (95% Cl of diff: -0.7313 to 3.229, p = 0.213). There was statistically significant interaction between dementia and no dementia in the no APOE4 allele group with a mean difference of 1.061 (95% Cl of diff: -0.1848 to 2.308, p = 0.094).

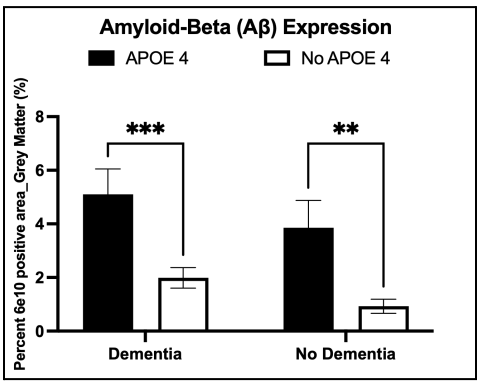

Figure 2: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on 6e10 Pathology. I Significance was found between APOE4 and no APOE4 Genotype within the dementia group (***p < 0.001). Significance was found between APOE4 and no APOE4 Genotype within 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).

Post-Hoc Comparisons on Iba1 Pathology

Following the two-way ANOVA, a multiple comparisons test was conducted to determine the significant main effects on 6e10 pathology from the previous data based on Fisher's LSD post-hoc tests. There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the dementia group, with a mean difference of 1.918 (95% Cl of diff: 0.6392 to 3.197, p < 0.004). There was no statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the no dementia group, with a mean difference of -0.9999 (95% Cl of diff: -2.513 to 0.5136, p = 0.192). There was statistically significant interaction between dementia and no dementia in the APOE4 allele group, with a mean difference of 2.520 (95% Cl of diff: 0.8426 to 4.197, p = 0.004). There was no statistically significant interaction between dementia and no dementia in the no APOE4 allele group with a mean difference of -0.3984 (95% Cl of diff: -1.454 to 0.6571, p = 0.455).

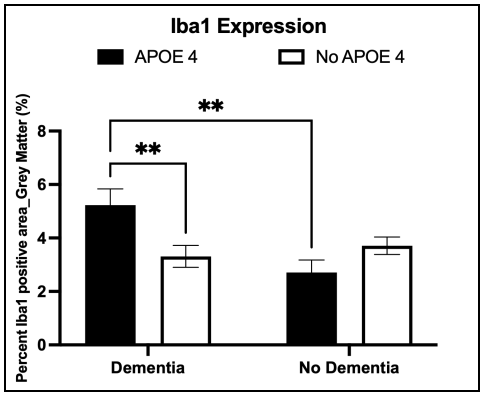

Figure 3: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on Iba1 Pathology. Significance was found between APOE4 and no APOE4 Genotype within the dementia group (**p = 0.004). No significance was found between APOE4 and no APOE4 Genotype within 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).

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 the positive relationship between APOE4 and protein expression of markers associated with dementia in the middle temporal gyrus of the brain.

APOE4 Drives Tau Pathology in 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. Our findings suggested that dementia was prevalent when donors had both the APOE4 genotype and high tau levels. Specifically, donors with the APOE4 allele and a high tau level in the brain had dementia (Figure 1). However, donors with the APOE4 allele and low tau percentage levels did not have dementia. Similarly, donors with the APOE2/3 allele and low tau percentage levels did not have dementia. These results suggest that APOE4 may have an effect with tau expression or at least magnify the effects of tau on dementia. However, one cause for concern from the results demonstrates that donors with the APOE2/3 allele and a low tau percentage did have dementia (Figure 1). This relationship does not align with the hypothesis that APOE4 and tau have an interactive effect on dementia. This could have occurred because factors other than the APOE4 genotype and tau levels resulted in dementia, suggesting that APOE4 and tau levels don't indicate risk to develop dementia. The original data set looks at markers other than AT8, which future research could investigate to determine an explanation.

The direct relationship between the APOE4 genotype and tau pathology on 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 regions of the brain entorhinal cortex and the 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). 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, this rationalizes the finding that APOE4 promotes it. APOE4 acts as an indirect catalyst for phosphorylated tau expression, thereby leading to dementia development. On the other hand, APOE2's potential protective effects on tau reduce phosphorylated tau expression, inhibiting the pathway.

Limitations in Assessing APOE4 Interaction with Amyloid-β Pathology in Dementia

Our findings suggest no significant trends between the 6e10 and the APOE4 genotype on dementia, however. APOE4 increases 6e10 levels irrespective of dementia diagnosis. However, there are no differences in 6e10 expression as a result of APOE4 between dementia diagnoses. Also, donors with the APOE4 allele and low levels of Aβ have dementia, contradicting the interaction relationship between these two variables (Figure 2). 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, the donors without dementia, positive for APOE4 and have high levels of Aβ, could, in actuality, have low percent levels of Aβ. This possibility suggests that APOE4 increases Aβ pathology. Since lower percent levels of Aβ with APOE4 alleles lead to no-dementia cases, it can be inferred that high Aβ percent levels drive dementia. Furthermore, this error could also indicate that APOE4 and Aβ don't suggest risk of dementia development. The data set that the current study utilizes looks into several other markers that future studies could investigate to examine the missing explanation.

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, APOE4 allele enhances Aβ expression, leading to AD pathology.

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. However, when comparing the interaction between 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 onset.

Effect of APOE4-Neuroinflammation Interaction on AD Pathology

Neuroinflammation in the brain, an indicator of AD pathology, and the APOE genotype demonstrated a clear relationship on dementia onset. Using the Iba1 protein as a marker for inflammation in the microglial cells, its effect on cognitive status revealed a clear reliance on the APOE4 allele to demonstrate AD pathology, and vice versa. Most donors whose Iba1 percent levels were high and who had the APOE4 allele had dementia (Figure 3). On the other hand, donors who had significantly lower Iba1 levels yet still possessed the APOE4 allele did not have dementia, indicating the reliance of APOE4 on neuroinflammation to lead to dementia (Figure 3). Similarly, donors who had had similar levels of Iba1 with no significant difference while having the APOE2/3 genotype also did not have dementia, suggesting that high Iba1 levels are dependent on APOE4 to indicate AD pathology (Figure 3). The dependence of neuroinflammation levels and the APOE4 allele on each other to result in dementia indicates a potential interactive effect, where one or both variables depend on the other to produce such a result. However, one inconsistency presented in the final analysis was that dementia was still prevalent despite no APOE4 and significantly lower levels of Iba1, countering the original concluding statement of interaction between the two variables (Figure 3). Because the original data set looked into markers other than the ones investigated in the current study, future analysis of the set could determine the unexplained error. Along with this, the result could also suggest that dementia diagnosis is not indicated by APOE4 and neuroinflammation.

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 would have promoted the effects of neuroinflammation. However, while APOE4 enhances neuroinflammation, the results of our study could indicate a more complex relationship. Given the results of the current study, it is 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 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.

Conclusion

Our study highlights that the interactions between the APOE4 allele and phosphorylated tau pathology were associated with the prevalence of AD within the donors in the dataset used. Furthermore, we found that neuroinflammation and the APOE4 allele had interactive effects on dementia, with both dependent on the other to develop AD pathology. However, our study did not observe a clear relationship between Aβ and the APOE4 allele, possibly due to limitations of the marker used in the protocol. These findings suggest there may indeed be interaction between APOE4 and the pathology of neurotoxic proteins which drives AD. Further research could determine whether the region of protein accumulation in the brain has any effect on dementia in relation with 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.

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

Acknowledgements

I would like to thank Dr. Jorge A. Avila for his guidance and mentorship in exploring the SEA-AD dataset and developing this project. I would like to thank him also for his help with statistical analyses, writing, and editing this manuscript.

References:

Arvanitakis, Z., Shah, R. C., & Bennett, D. A. (2019). Diagnosis and Management of Dementia: A Review. JAMA, 322(16), 1589–1599. https://doi.org/10.1001/jama.2019.4782

Cicognola, C., Salvadó, G., Smith, R., Palmqvist, S., Stomrud, E., Betthauser, T. J., Johnson, S. C., Janelidze, S., Mattsson-Carlgren, N., Hansson, O., & Pichet Binette, A. (2025). APOE4 impact on soluble and insoluble tau pathology is mostly influenced by amyloid-β. *Brain*, 148(7), 2373–2383. https://doi.org/10.1093/brain/awaf016

Dias, D., Portugal, C. C., Relvas, J., & Socodato, R. (2025). From Genetics to Neuroinflammation: The Impact of ApoE4 on Microglial Function in Alzheimer's Disease. *Cells*, 14(4), 243. https://doi.org/10.3390/cells14040243

Gabitto, M. I., Travaglini, K. J., Rachleff, V. M., Kaplan, E. S., Long, B., Ariza, J., Ding, Y., Mahoney, J. T., Dee, N., Goldy, J., Melief, E. J., Agrawal, A., Kana, O., Zhen, X., Barlow, S. T., Brouner, K., Campos, J., Campos, J., Carr, A. J., … Lein, E. S. (2024). Integrated multimodal cell atlas of Alzheimer's disease. *Nature Neuroscience*, 27(12), 2366–2383. https://doi.org/10.1038/s41593-024-01774-5

Gendron, T. F., & Petrucelli, L. (2009). The role of tau in neurodegeneration. *Molecular Neurodegeneration*, 4, 13. https://doi.org/10.1186/1750-1326-4-13

Goel, P., Chakrabarti, S., Goel, K., Bhutani, K., Chopra, T., & Bali, S. (2022). Neuronal cell death mechanisms in Alzheimer's disease: An insight. *Frontiers in Molecular Neuroscience*, 15. https://doi.org/10.3389/fnmol.2022.937133

Hampel, H., Hardy, J., Blennow, K., Chen, C., Perry, G., Kim, S. H., Villemagne, V. L., Aisen, P., Vendruscolo, M., Iwatsubo, T., Masters, C. L., Cho, M., Lannfelt, L.,

Cummings, J. L., & Vergallo, A. (2021). The Amyloid-β Pathway in Alzheimer's Disease. *Molecular Psychiatry*, *26*(10), 5481–5503. https://doi.org/10.1038/s41380-021-01249-0

Hovens, I. B., Nyakas, C., & Schoemaker, R. G. (2014). A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: Cell body to cell size ratio. *Neuroimmunology and Neuroinflammation*, *1*(0), 82–88. https://doi.org/10.4103/2347-8659.139719

Iliyasu, M. O., Musa, S. A., Oladele, S. B., & Iliya, A. I. (2023). Amyloid-beta aggregation implicates multiple pathways in Alzheimer's disease: Understanding the mechanisms. *Frontiers in Neuroscience*, *17*. https://doi.org/10.3389/fnins.2023.1081938

Major, C., Rivers, M., Khundmiri, S., & O'neil, J. (2024). IBA1 EXPRESSION IN THE HIPPOCAMPUS OF APOLIPOPROTEIN EPSILON 4 SALT-TREATED MICE. *Innovation in Aging*, *8*(Suppl 1), 690. https://doi.org/10.1093/geroni/igae098.2255

Metaxas, A., & Kempf, S. J. (2016). Neurofibrillary tangles in Alzheimer's disease: Elucidation of the molecular mechanism by immunohistochemistry and tau protein phospho-proteomics. *Neural Regeneration Research*, *11*(10), 1579–1581. https://doi.org/10.4103/1673-5374.193234

Müller, U. C., Deller, T., & Korte, M. (2017). Not just amyloid: Physiological functions of the amyloid precursor protein family. *Nature Reviews Neuroscience*, *18*(5), 281–298. https://doi.org/10.1038/nrn.2017.29

Noguchi, A., Matsumura, S., Dezawa, M., Tada, M., Yanazawa, M., Ito, A., Akioka, M., Kikuchi, S., Sato, M., Ideno, S., Noda, M., Fukunari, A., Muramatsu, S., Itokazu, Y.,

Sato, K., Takahashi, H., Teplow, D. B., Nabeshima, Y., Kakita, A., … Hoshi, M. (2009). Isolation and Characterization of Patient-derived, Toxic, High Mass Amyloid β-Protein (Aβ) Assembly from Alzheimer Disease Brains*. *Journal of Biological Chemistry*, *284*(47), 32895–32905. https://doi.org/10.1074/jbc.M109.000208

Parhizkar, S., & Holtzman, D. M. (2022). APOE mediated neuroinflammation and neurodegeneration in Alzheimer's disease. *Seminars in Immunology*, *59*, 101594. https://doi.org/10.1016/j.smim.2022.101594

Pirraglia, E., Glodzik, L., & Shao, Y. (2023). Lower mortality risk in APOE4 carriers with normal cognitive ageing. *Scientific Reports*, *13*(1), 15089. https://doi.org/10.1038/s41598-023-41078-5

Raulin, A.-C., Doss, S. V., Trottier, Z. A., Ikezu, T. C., Bu, G., & Liu, C.-C. (2022). ApoE in Alzheimer's disease: Pathophysiology and therapeutic strategies. *Molecular Neurodegeneration*, *17*(1), 72. https://doi.org/10.1186/s13024-022-00574-4

Seattle Alzheimer's Disease Data Set. (n.d.). [Dataset]. https://registry.opendata.aws/allen-sea-ad-atlas. Retrieved July 5, 2025, from https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en

Smith, J. A., Das, A., Ray, S. K., & Banik, N. L. (2012). Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Research Bulletin*, *87*(1), 10–20. https://doi.org/10.1016/j.brainresbull.2011.10.004

Sobue, A., Komine, O., & Yamanaka, K. (2023). Neuroinflammation in Alzheimer's disease: Microglial signature and their relevance to disease. *Inflammation and Regeneration*, *43*(1), 26. https://doi.org/10.1186/s41232-023-00277-3

Tanzi, R. E. (2012). The Genetics of Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, 2(10), a006296. https://doi.org/10.1101/cshperspect.a006296

Therriault, J., Benedet, A. L., Pascoal, T. A., Mathotaarachchi, S., Chamoun, M., Savard, M., Thomas, E., Kang, M. S., Lussier, F., Tissot, C., Parsons, M., Qureshi, M. N. I., Vitali, P., Massarweh, G., Soucy, J.-P., Rej, S., Saha-Chaudhuri, P., Gauthier, S., & Rosa-Neto, P. (2020). Association of Apolipoprotein E ε4 With Medial Temporal Tau Independent of Amyloid-β. *JAMA Neurology*, 77(4), 470–479. https://doi.org/10.1001/jamaneurol.2019.4421

Therriault, J., Benedet, A. L., Pascoal, T. A., Mathotaarachchi, S., Savard, M., Chamoun, M., Thomas, E., Kang, M. S., Lussier, F., Tissot, C., Soucy, J.-P., Massarweh, G., Rej, S., Saha-Chaudhuri, P., Poirier, J., Gauthier, S., & Rosa-Neto, P. (2021). APOEε4 potentiates the relationship between amyloid-β and tau pathologies. *Molecular Psychiatry*, *26*(10), 5977–5988. https://doi.org/10.1038/s41380-020-0688-6

Tyan, S.-H., Shih, A. Y.-J., Walsh, J. J., Murayama, H., Sarsoza, F., Ku, L., Eggert, S., Hof, P. R., Koo, E. H., & Dickstein, D. L. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. *Molecular and Cellular Neurosciences*, *51*(1–2), 43–52. https://doi.org/10.1016/j.mcn.2012.07.009

Young, C. B., Johns, E., Kennedy, G., Belloy, M. E., Insel, P. S., Greicius, M. D., Sperling, R. A., Johnson, K. A., Poston, K. L., Mormino, E. C., for the Alzheimer's Disease Neuroimaging Initiative, & the A4 Study Team. (2023). APOE effects on regional tau in preclinical Alzheimer's disease. *Molecular Neurodegeneration*, *18*(1), 1.

https://doi.org/10.1186/s13024-022-00590-4

https://doi.org/10.1186/s13024-022-00590-4

Title: APOE4 Interacts With Tau, Amyloid Beta, And Iba1 Pathology In The Middle Temporal Gyrus In Dementia

Comments

This paper effectively utilizes a public dataset to explore the associations between APOE4, cognitive status, and pathology related to key molecules. Overall, the manuscript is well written, and the data analysis is appropriately conducted. However, the interpretation of the results is overstated, and some of the conclusions are not directly supported by the data. For example, the current results do not allow for claims about the prevalence of dementia.

While the analysis is technically sound, the presentation is overly focused on statistical details, which makes it difficult to follow the logical flow and fully understand the implications. Moreover, although the background section is adequate, the context is not clearly integrated into the data analysis, which may confuse readers.

Major comments:

1) To support the conclusions made by the authors, additional statistical methods such as logistic regression are highly recommended instead of relying solely on ANOVA. If further analysis is not conducted, I suggest revising the discussion and conclusion sections to better reflect the actual findings.

2) Abstract and Results:

The abstract is very well written and effectively provides the necessary background information. In contrast, the Results section lacks context, background, and rationale. Please consider adding these elements, similar to how they are presented in the abstract.

- Avoid beginning sentences with the name of a statistical test (e.g., "A two-way ANOVA tested the main effects..."). It would be clearer and more informative to first state the purpose and outcome of the analysis, and then briefly mention the statistical method used.For example: *“We analyzed the data to examine the main and interaction effects of XXX (Two-way ANOVA, p = XXX) (Figure XX).”*

3) Introduction

The introduction is well-written. However, this part should clearly explain the difference in APOE4 allele frequency between the general population and individuals with Alzheimer’s disease to better establish its role as a risk factor. Additionally, the manuscript repeatedly compares "APOE4 vs. no APOE4," but for readers unfamiliar with AD research, the rationale

behind this comparison is not clear. Providing more background on why this distinction is important would improve clarity and accessibility.

4) Result section

-The current text focuses almost exclusively on reporting statistical results in a mechanical manner, without providing any context or interpretation. This approach makes it difficult for readers to understand the significance or implications of the findings. Please add more context and explanation for what each factors mean (e.g.) what is the 6e10?)

-Furthermore, there is no explanation of what differentiates the APOE4 carriers from the non-carriers in terms of pathology or dementia risk, nor why these differences matter.

-Each section title (e.g. "Post-Hoc Comparisons on Iba1 Pathology," is too mechanical and overly focused on the statistical method rather than the key findings. In a results section, it's more effective to highlight the main results or conclusions rather than simply stating what type of statistical test was used.

- Have you conducted normality tests to verify if the data are normally distributed within groups. If the data do not meet the normality assumption, then non-parametric alternatives like the Kruskal-Wallis test should be considered instead.

5) Discussion:

Most of the interpretations and conclusions are not supported by the current data and are therefore incorrect.

For example, the comparison in Figure 1 simply shows p-Tau levels across four groups: dementia (APOE4 vs. non-APOE4) and no dementia (APOE4 vs. non-APOE4). This comparison alone does not provide evidence for the prevalence of dementia.

Moreover, the statement "donors with the APOE4 allele and a high tau level in the brain had dementia. However, donors with the APOE4 allele and low tau percentage levels did not have dementia" is not fully supported by the data shown. To make claims about the correlation or prevalence of dementia in relation to APOE4 and tau levels, additional statistical analyses such as logistic regression would be necessary. If further analysis is not feasible, I suggest revising the discussion and conclusion sections to better reflect the actual findings.

6) Conclusion:

The conclusion that the interaction between APOE4 and tau is associated with AD prevalence is not supported by the data. The two-way ANOVA showed no significant interaction between APOE4 and cognitive status. Also, the term "prevalence" is not appropriate without a proper analysis such as logistic regression.

Minor point

Introduction

This current study contributes to expanding the literature evaluating whether APOE4 is central in linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor.-> The distinction between “APOE4 acting as a genetic risk factor” and “APOE4 linking genetic factors to neurodegeneration” is not clearly defined in this sentence.

Result section

Please indicate in parentheses which content corresponds to each figure.

Final Recommendation

- Accept with major revisions (acceptance conditional on satisfactory major revisions)

EDITOR COMMENTS AND RECOMMENDATION:

The reviewers commend the student for a well-written manuscript focused on the relationship between APOE4, Amyloid Beta, phosphorylated Tau, and microglial activation in the pathology of dementia. The reviewers do, however, raise important concerns which should be remediated prior to manuscript acceptance. First, Reviewer 1 notes over-interpretation of the results, as well as over-stated conclusions not fully supported by the presented data, and asks that the student edit these aspects of the manuscript in concordance with the data analyzed. Additionally, Reviewer 2 raises concerns about the novelty of the manuscript in the current body of literature, and suggests evaluating interaction effects between these different variables, as well as editing the manuscript to make the description of the statistical analyses more precise and accurate. Given these feedback, the editor recommends a major revision prior to acceptance.

REVIEWER 1:

This paper effectively utilizes a public dataset to explore the associations between APOE4, cognitive status, and pathology related to key molecules. Overall, the manuscript is well written, and the data analysis is appropriately conducted. However, the interpretation of the results is overstated, and some of the conclusions are not directly supported by the data. For example, the current results do not allow for claims about the prevalence of dementia.

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Minor points:

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Result section

Please indicate in parentheses which content corresponds to each figure.

REVIEWER 2

The authors have constructed a well-written article evaluating the relationship between APOE4 gene expression and Dementia, while characterizing the levels of Tau, Amyloid Beta, and Iba1 as possible interaction effects. Alzheimer’s disease is an ever relevant and important topic, and the rationale for the study is warranted. I think the introduction is very well written and easy to follow, and at the appropriate level for an advanced highschool student/first year college student. Summarization of the methods is good (e.g. how cases of dementia were defined, and

how the tissue was harvested). The discussion does a good job broadening the topic while addressing limitations of the study.

I think a major limitation is that the current investigation is not necessarily novel (a number of studies have evaluated the allele frequencies of APOE4 in the context of Alzheimer's). Furthermore, while the paper does perform statistical analyses, the analyses have already been done by the group who organized the dataset. I think the novelty could lie primarily in the evaluation for interaction effects, but please see my comments below regarding effect modification vs. interaction. Also, cognitive status should be treated as a dependent variable, whereas APOE genotype is an independent variable. It doesn't make as much sense to conduct the analyses using cognitive status as an independent variable when in reality it is being driven by changes in APOE and various inflammatory pathways such as those related to AT8 expression and 6e10. I think the paper would benefit from restructuring the analyses where cognitive status is the outcome, and evaluating how various combinations of APOE expression and AT8, 6e10, and Iba1 affect dementia. Authors should also note that these are not adjusted for various demographic and biological variables known to be associated with dementia, so these results should be presented as unadjusted. I think overall the student does well to dive into statistical analyses and specifically start to learn about post hoc comparisons.

Note: The authors confuse the concepts of effect modification and interaction. I would argue that measuring levels of Dementia based on allele expression is an example of effect modification. Furthermore, if the expression of APOE4 drives increases in AT8 which in turn promotes Dementia, then this is more so a causal pathway analysis as opposed to interaction.

APOE4 Carriers With Dementia Exhibit Increased ~~Interacts With~~ Tau, Amyloid Beta, And Iba1 Pathology In The Middle Temporal Gyrus ~~In Dementia~~

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. ~~associations between APOE4 and Aβ, tau phosphorylation, and microglial activation in relation to dementia~~. 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. ~~Our analysis revealed that APOE4 was linked to greater tau pathology (AT8 immunoreactivity) in individuals with dementia. The APOE4 genotype was associated with differences in microglial activation (Iba1 immunoreactivity) by dementia diagnosis. These findings also suggest that APOE4 may act in concert with neuroinflammation to influence dementia pathology.~~ 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

Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Neuroscience, Dementia, Alzheimer's Disease, and APOE

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 an 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 on 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~~interaction~~ 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~~APOE4 is plays~~ a more active role in~~role in~~central in~~ in~~ linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor that increases the likelihood of developing Alzheimer's disease.

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 data set focused on different patients' histological pathology, obtained via immunolabeling and microscopy across cortical layers and protein markers. Along with that, the data set 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.

Dementia Diagnosing

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.).

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.).

Briefly, 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.

Data Retrieval and Organization

The Allen Brain Institute collected and compiled the data for access to the general public. I accessed the data on 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 data sets based on the Donor ID variable. This allowed us to look at neuropathology across clinical status. We generated four groups (APOE4/Dementia, APOE4/No Dementia, No APOE4/Dementia, No APOE4/No Dementia).

Normality and Kruskal-Wallis Test

Each of the four groups were individually tested for normality of their data with a calculator to determine reliability of two-way ANOVA. Data was run through the Kruskal-Wallis test separately for APOE4 and dementia to determine statistical significance between groups.

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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 mean and were generated through Prism.

Results

All results are unadjusted since 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 does not only act as a risk factor that increases risk of dementia but also influences certain mechanisms and neuropathological pathways.

Normality Testing

Normality of the neuropathology was assessed for each individual group using the Shapiro-Wilk test. 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, since they were suggesting deviations from normality. Along with that, there are 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 APOE4 allele and Dementia. This test determines statistical differences without reliance on 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 ($H(1)=27.9616$, $p< .00001$). 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 ($H(1)=2.4656$, $p=.11636$). 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 and dementia diagnosis are 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 violation of normality. As a result, future research should consider larger samples and non-parametric factorial ANOVA methods.

~~*APOE4 Genotype v. Dementia Diagnosis on AT8 Pathology*~~ *APOE4 Genotype and Dementia Diagnosis Differentially Impact Tau Pathology*

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We ~~A two-way ANOVA was conducted to~~ determined the effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on AT8 expression~~pathology~~ (percent area). AT8 is a monoclonal antibody marker which 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~~in~~ AT8 pathology. In other words, carrying the APOE4 allele does not appear to modify dementia and tau accumulation's relationship in this sample. The simple effect difference of the interaction was also not statistically significant at 0.3050 (95% CI~~Cl~~ of difference: -0.9317 to 1.542), further emphasizing that finding. ~~,~~ ~~However,~~ t~~T~~here was a significant main effect of dementia diagnosis independently on 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 Onset~~APOE4 Genotype v. Dementia Diagnosis on 6e10 Pathology~~

The APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) effect on 6e10 expression (percent area) was examined. ~~were subjected to a two-way ANOVA on 6e10 pathology (percent area) to determine their effects~~. 6e10 is a monoclonal antibody marker which 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~~Cl~~ of difference: -2.152 to 2.527), further emphasizing this finding. There was also no significant main effect of dementia diagnosis independently on 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.

APOE4 Genotype and Dementia Diagnosis Influences Iba1 Pathology ~~APOE4 Genotype v. Dementia Diagnosis on Iba1 Pathology~~

The effects of APOE4 allele status (APOE4 vs No APOE4) and cognitive status (Dementia vs. No Dementia) on Iba1 expression~~pathology~~ (percent area) were determined ~~(Two-way ANOVA,through a two-way ANOVA~~. 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~~was~~ statistically significant at 0.2.918 (95% CI~~Cl~~ of difference: 0.9365 to 4.899). There was a

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significant main effect of dementia diagnosis independently on Iba1~~6e10~~ pathology, F(1,80)=4.5, p=0.036, with dementia diagnosis having greater Iba1~~6e10~~ 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.

APOE4 Increases Tau Pathology in Individuals With and Without Dementia ~~Post-Hoc Comparisons on AT8 Pathology~~

~~Following the two-way ANOVA, a~~ 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). ~~based on Fisher's LSD post-hoc tests~~. There was a statistically~~was statistically~~ significant difference~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the dementia group with a mean difference of 1.278 (95% CI~~Cl~~ 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 ~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the no dementia group with a mean difference of 0.9728 (95% CI~~Cl~~ 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 ~~interaction~~ between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.264 (95% CI~~Cl~~ 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 ~~interaction~~ between dementia and no dementia in the no APOE4 allele group with a mean difference of 0.9592 (95% CI~~Cl~~ 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.

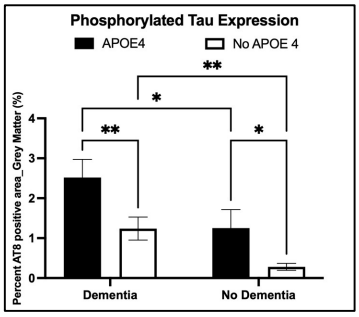

Figure 1: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on AT8 Pathology. Significance was found between APOE4 and no APOE4 Genotype within the dementia group (**p = 0.002). Significance was found between APOE4 and no APOE4 Genotype within the 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).

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APOE4 Influences Amyloid-β Pathology Independently of Dementia Diagnosis~~Post-Hoc Comparisons on 6e10 Pathology~~

~~A~~Following the two-way ANOVA, a multiple comparisons test was conducted to determine the significant main effects on 6e10 pathology from the previous data, as shown in Figure 2 ~~based on Fisher's LSD post-hoc tests~~ (Two-way ANOVA, Fisher's LSD post hoc tests). There was a statistically significant difference~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the dementia group with a mean difference of 3.118 (95% CI~~Cl~~ 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~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the no dementia group with a mean difference of 2.931 (95% CI~~Cl~~ 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~~interaction~~ between dementia and no dementia in the APOE4 allele group, with a mean difference of 1.249 (95% CI~~Cl~~ 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~~statistically~~ significant difference~~interaction~~ between dementia and no dementia in the no APOE4 allele group with a mean difference of 1.061 (95% CI~~Cl~~ 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.

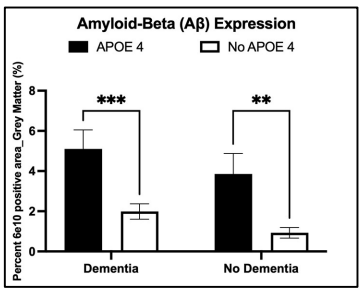

Figure 2: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on 6e10 Pathology. I Significance was found between APOE4 and no APOE4 Genotype within the dementia group (***$p < 0.001$). Significance was found between APOE4 and no APOE4 Genotype within 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 ($p = 0.094$).

Microglial Activation Increases in APOE4 Carriers with Dementia~~Post-Hoc Comparisons on Iba1 Pathology~~

~~A~~Following the two-way ANOVA, 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)~~based on Fisher's LSD post-hoc tests~~. There was a statistically significant difference~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the dementia

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group, with a mean difference of 1.918 (95% CI~~CI~~ of diff: 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~~interaction~~ between APOE4 carriers and non-APOE4 carriers in the no dementia group, with a mean difference of -0.9999 (95% CI~~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~~interaction~~ between dementia and no dementia in the APOE4 allele group, with a mean difference of 2.520 (95% CI~~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~~interaction~~ between dementia and no dementia in the no APOE4 allele group with a mean difference of -0.3984 (95% CI~~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.

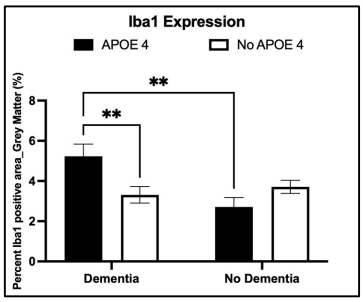

Figure 3: Multiple Comparisons Between APOE4 Genotype and Dementia Diagnosis on the Significance of Main Effects on Iba1 Pathology. Significance was found between APOE4 and no APOE4 Genotype within the dementia group (**p = 0.004). No significance was found between APOE4 and no APOE4 Genotype within 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).

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 than carriers without dementia. Furthermore, our study found that donors with dementia similarly showed increased neuropathology when carrying the APOE4 allele than not carrying it.

~~the positive relationship between APOE4 and dementia~~protein expression of markers ~~on protein expression of markers associated with dementia in the middle temporal gyrus of the brain~~.

Tau Pathology in Participants with APOE4 and Dementia ~~APOE4 Drives Tau Pathology in 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 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 relationship. Along with that, while post-hoc comparisons suggested that APOE4 carriers with dementia indicated greater neuropathological levels than carriers without dementia, interaction between dementia and APOE4 were not statistically significant. Thus, causal pathways and effect modification is speculative. Future research should examine correlation and logistic regressions to test the strength of this relationship between dementia, APOE4, and these protein markers.

~~Our findings suggested that dementia was prevalent when donors had both the APOE4 genotype and high tau levels. Specifically, donors with the APOE4 allele and a high tau level in the brain had dementia (Figure 1). However, donors with the APOE4 allele and low tau percentage levels did not have dementia. Similarly, donors with the APOE2/3 allele and low tau percentage levels did not have dementia. These results suggest that APOE4 may have an effect with tau expression or at least magnify the effects of tau on dementia. However, one cause for concern from the results demonstrates that donors with the APOE2/3 allele and a low tau percentage did have dementia (Figure 1). This relationship does not align with the hypothesis that APOE4 and tau have an interactive effect on dementia. This could have occurred because factors other than the APOE4 genotype and tau levels resulted in dementia, suggesting that APOE4 and tau levels don't indicate risk to develop dementia. The original data set looks at markers other than AT8, which future research could investigate to determine an explanation.~~

The direct relationship between the APOE4 genotype and tau pathology on 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 regions of the brain entorhinal cortex and the hippocampus without an interaction with Aβ (Therriault et al., 2020). These effects were significantly greater compared to those with APOE2, which

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demonstrated reduced and protective effects of regional tau (Young et al., 2023). This demonstrates a causal pathway, where APOE4 allele potentiate 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. ~~greater rationalizes the finding that APOE4 promotes it. According to this theoretical reasoning, APOE4 would act~~acts as an indirect catalyst for phosphorylated tau ~~expression, thereby leading to greater frequency of~~ dementia development. ~~On the other hand, APOE2's potential protective effects on tau reduce phosphorylated tau expression, inhibiting the pathway.~~

Limitations in Assessing Amyloid-β Pathology in APOE4 Carriers with Dementia ~~*APOE4 Interaction with Amyloid-β Pathology in 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 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 finding. 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.

~~Our findings suggest no significant trends between the 6e10 and the APOE4 genotype on dementia, however. APOE4 increases 6e10 levels irrespective of dementia diagnosis. However, there are no differences in 6e10 expression as a result of APOE4 between dementia diagnoses. Also, donors with the APOE4 allele and low levels of Aβ have dementia, contradicting the interaction relationship between these two variables (Figure 2).~~ 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 ~~the donors without dementia~~ could have actually had significantly lower levels of Aβ compared to APOE4 carriers with dementia.

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If this possibility were true and there was indeed 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, 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 which 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 were not statistically significant, indicating that these associations are speculative.~~, positive for APOE4 and have high levels of Aβ, could, in actuality, have low percent levels of Aβ. This possibility suggests that APOE4 increases Aβ pathology. Since lower percent levels of Aβ with APOE4 alleles lead to no-dementia cases, it can be inferred that high Aβ percent levels drive dementia. Furthermore, this error could also indicate that APOE4 and Aβ don't suggest risk of dementia development. The data set that the current study utilizes looks into several other markers that future studies could investigate to examine the missing explanation.~~

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, 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. W~~However, w~~hen 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 ~~onset~~. 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 demonstrates their effect modification to enhance dementia frequency.

~~*Effect of APOE4-Neuroinflammation Interaction on AD Pathology*~~ *Neuroinflammation in APOE4 Carriers with Dementia*

~~Neuroinflammation in the brain, an indicator of AD pathology, and the APOE genotype demonstrated a clear relationship on dementia onset. Using the Iba1 protein as a marker for inflammation in the microglial cells, its effect on cognitive status revealed a clear reliance on the APOE4 allele to demonstrate AD pathology, and vice versa. Most donors whose Iba1 percent levels were high and who had the APOE4 allele had dementia (Figure 3). On the other hand, donors who had significantly lower Iba1 levels yet still possessed the APOE4 allele did not have dementia, indicating the reliance of APOE4 on neuroinflammation to lead to dementia (Figure 3). Similarly,~~

~~donors who had had similar levels of Iba1 with no significant difference while having the APOE2/3 genotype also did not have dementia, suggesting that high Iba1 levels are dependent on APOE4 to indicate AD pathology (Figure 3). The dependence of neuroinflammation levels and the APOE4 allele on each other to result in dementia indicates a potential interactive effect, where one or both variables depend on the other to produce such a result. However, one inconsistency presented in the final analysis was that dementia was still prevalent despite no APOE4 and significantly lower levels of Iba1, countering the original concluding statement of interaction between the two variables (Figure 3). Because the original data set looked into markers other than the ones investigated in the current study, future analysis of the set could determine the unexplained error. Along with this, the result could also suggest that dementia diagnosis is not indicated by APOE4 and neuroinflammation.~~

In determining neuroinflammation, protein marker Iba1 was used to detect it. 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 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 was significantly greater Iba1 levels in carriers with dementia than 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.

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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~~would have promoted~~ 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 ~~However, while APOE4 enhances neuroinflammation, the results of our study could indicate a more complex relationship. Given the results of the current study, it is~~ 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.

Conclusion

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 the two increase parallel to 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 with 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.

~~Our study highlights that the interactions between the APOE4 allele and phosphorylated tau pathology were associated with the prevalence of AD within the donors in the dataset used. Furthermore, we found that neuroinflammation and the APOE4 allele had interactive effects on dementia, with both dependent on the other to develop AD pathology. However, our study did not observe a clear relationship between Aβ and the APOE4 allele, possibly due to limitations of the marker used in the protocol. These findings suggest there may indeed be interaction between APOE4 and the pathology of neurotoxic proteins which drives AD. Further research could determine whether the region of protein accumulation in the brain has any effect on dementia in relation with 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

Acknowledgements

I would like to thank [mentor name redacted by Managing Editor] for his guidance and mentorship in exploring the SEA-AD dataset and developing this project. I would like to thank him also for his help with statistical analyses, writing, and editing this manuscript.

References:

2022 Alzheimer's disease facts and figures. (2022). *Alzheimer's & Dementia*, *18*(4), 700–789. https://doi.org/10.1002/alz.12638

ApoE-calypse tau: ApoE–tau synergy in Alzheimer's disease | Journal of Experimental Medicine | Rockefeller University Press. (n.d.). Retrieved October 4, 2025, from https://rupress.org/jem/article/222/10/e20250965/278284/ApoE-calypse-tau-ApoE-tau-synergy-in-Alzheimer-s

Arvanitakis, Z., Shah, R. C., & Bennett, D. A. (2019). Diagnosis and Management of Dementia: A Review. *JAMA*, *322*(16), 1589–1599. https://doi.org/10.1001/jama.2019.4782

Cicognola, C., Salvadó, G., Smith, R., Palmqvist, S., Stomrud, E., Betthauser, T. J., Johnson, S. C., Janelidze, S., Mattsson-Carlgren, N., Hansson, O., & Pichet Binette, A. (2025). APOE4 impact on soluble and insoluble tau pathology is mostly influenced by amyloid-β. *Brain*, *148*(7), 2373–2383. https://doi.org/10.1093/brain/awaf016

Dias, D., Portugal, C. C., Relvas, J., & Socodato, R. (2025). From Genetics to Neuroinflammation: The Impact of ApoE4 on Microglial Function in Alzheimer's Disease. *Cells*, *14*(4), 243. https://doi.org/10.3390/cells14040243

Gabitto, M. I., Travaglini, K. J., Rachleff, V. M., Kaplan, E. S., Long, B., Ariza, J., Ding, Y., Mahoney, J. T., Dee, N., Goldy, J., Melief, E. J., Agrawal, A., Kana, O., Zhen, X., Barlow, S. T., Brouner, K., Campos, J., Campos, J., Carr, A. J., … Lein, E. S. (2024). Integrated multimodal cell atlas of Alzheimer's disease. *Nature Neuroscience*, *27*(12), 2366–2383. https://doi.org/10.1038/s41593-024-01774-5

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Gendron, T. F., & Petrucelli, L. (2009). The role of tau in neurodegeneration. *Molecular Neurodegeneration*, *4*, 13. https://doi.org/10.1186/1750-1326-4-13

Goel, P., Chakrabarti, S., Goel, K., Bhutani, K., Chopra, T., & Bali, S. (2022). Neuronal cell death mechanisms in Alzheimer's disease: An insight. *Frontiers in Molecular Neuroscience*, *15*. https://doi.org/10.3389/fnmol.2022.937133

Hampel, H., Hardy, J., Blennow, K., Chen, C., Perry, G., Kim, S. H., Villemagne, V. L., Aisen, P., Vendruscolo, M., Iwatsubo, T., Masters, C. L., Cho, M., Lannfelt, L., Cummings, J. L., & Vergallo, A. (2021). The Amyloid-β Pathway in Alzheimer's Disease. *Molecular Psychiatry*, *26*(10), 5481–5503. https://doi.org/10.1038/s41380-021-01249-0

Hovens, I. B., Nyakas, C., & Schoemaker, R. G. (2014). A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: Cell body to cell size ratio. *Neuroimmunology and Neuroinflammation*, *1*(0), 82–88. https://doi.org/10.4103/2347-8659.139719

Iliyasu, M. O., Musa, S. A., Oladele, S. B., & Iliya, A. I. (2023). Amyloid-beta aggregation implicates multiple pathways in Alzheimer's disease: Understanding the mechanisms. *Frontiers in Neuroscience*, *17*. https://doi.org/10.3389/fnins.2023.1081938

Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: Risk, mechanisms, and therapy. *Nature Reviews. Neurology*, *9*(2), 106–118. https://doi.org/10.1038/nrneurol.2012.263

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Major, C., Rivers, M., Khundmiri, S., & O'neil, J. (2024). IBA1 EXPRESSION IN THE HIPPOCAMPUS OF APOLIPOPROTEIN EPSILON 4 SALT-TREATED MICE. *Innovation in Aging*, *8*(Suppl 1), 690. https://doi.org/10.1093/geroni/igae098.2255

Metaxas, A., & Kempf, S. J. (2016). Neurofibrillary tangles in Alzheimer's disease: Elucidation of the molecular mechanism by immunohistochemistry and tau protein phospho-proteomics. *Neural Regeneration Research*, *11*(10), 1579–1581. https://doi.org/10.4103/1673-5374.193234

Müller, U. C., Deller, T., & Korte, M. (2017). Not just amyloid: Physiological functions of the amyloid precursor protein family. *Nature Reviews Neuroscience*, *18*(5), 281–298. https://doi.org/10.1038/nrn.2017.29

Noguchi, A., Matsumura, S., Dezawa, M., Tada, M., Yanazawa, M., Ito, A., Akioka, M., Kikuchi, S., Sato, M., Ideno, S., Noda, M., Fukunari, A., Muramatsu, S., Itokazu, Y., Sato, K., Takahashi, H., Teplow, D. B., Nabeshima, Y., Kakita, A., … Hoshi, M. (2009). Isolation and Characterization of Patient-derived, Toxic, High Mass Amyloid β-Protein (Aβ) Assembly from Alzheimer Disease Brains*. *Journal of Biological Chemistry*, *284*(47), 32895–32905. https://doi.org/10.1074/jbc.M109.000208

Parhizkar, S., & Holtzman, D. M. (2022). APOE mediated neuroinflammation and neurodegeneration in Alzheimer's disease. *Seminars in Immunology*, *59*, 101594. https://doi.org/10.1016/j.smim.2022.101594

Pirraglia, E., Glodzik, L., & Shao, Y. (2023). Lower mortality risk in APOE4 carriers with normal cognitive ageing. *Scientific Reports*, *13*(1), 15089. https://doi.org/10.1038/s41598-023-41078-5

Formatted: Font: (Default) Lora

Raulin, A.-C., Doss, S. V., Trottier, Z. A., Ikezu, T. C., Bu, G., & Liu, C.-C. (2022). ApoE in Alzheimer's disease: Pathophysiology and therapeutic strategies. *Molecular Neurodegeneration*, *17*(1), 72. https://doi.org/10.1186/s13024-022-00574-4

Seattle Alzheimer's Disease Data Set. (n.d.). [Dataset]. https://registry.opendata.aws/allen-sea-ad-atlas. Retrieved July 5, 2025, from https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en

Smith, J. A., Das, A., Ray, S. K., & Banik, N. L. (2012). Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Research Bulletin*, *87*(1), 10–20. https://doi.org/10.1016/j.brainresbull.2011.10.004

Sobue, A., Komine, O., & Yamanaka, K. (2023). Neuroinflammation in Alzheimer's disease: Microglial signature and their relevance to disease. *Inflammation and Regeneration*, *43*(1), 26. https://doi.org/10.1186/s41232-023-00277-3

Tanzi, R. E. (2012). The Genetics of Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(10), a006296. https://doi.org/10.1101/cshperspect.a006296

Therriault, J., Benedet, A. L., Pascoal, T. A., Mathotaarachchi, S., Chamoun, M., Savard, M., Thomas, E., Kang, M. S., Lussier, F., Tissot, C., Parsons, M., Qureshi, M. N. I., Vitali, P., Massarweh, G., Soucy, J.-P., Rej, S., Saha-Chaudhuri, P., Gauthier, S., & Rosa-Neto, P. (2020). Association of Apolipoprotein E ε4 With Medial Temporal Tau Independent of Amyloid-β. *JAMA Neurology*, *77*(4), 470–479. https://doi.org/10.1001/jamaneurol.2019.4421

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Therriault, J., Benedet, A. L., Pascoal, T. A., Mathotaarachchi, S., Savard, M., Chamoun, M., Thomas, E., Kang, M. S., Lussier, F., Tissot, C., Soucy, J.-P., Massarweh, G., Rej, S., Saha-Chaudhuri, P., Poirier, J., Gauthier, S., & Rosa-Neto, P. (2021). APOEε4 potentiates the relationship between amyloid-β and tau pathologies. *Molecular Psychiatry*, *26*(10), 5977–5988. https://doi.org/10.1038/s41380-020-0688-6

Tyan, S.-H., Shih, A. Y.-J., Walsh, J. J., Murayama, H., Sarsoza, F., Ku, L., Eggert, S., Hof, P. R., Koo, E. H., & Dickstein, D. L. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. *Molecular and Cellular Neurosciences*, *51*(1–2), 43–52. https://doi.org/10.1016/j.mcn.2012.07.009

Young, C. B., Johns, E., Kennedy, G., Belloy, M. E., Insel, P. S., Greicius, M. D., Sperling, R. A., Johnson, K. A., Poston, K. L., Mormino, E. C., for the Alzheimer's Disease Neuroimaging Initiative, & the A4 Study Team. (2023). APOE effects on regional tau in preclinical Alzheimer's disease. *Molecular Neurodegeneration*, *18*(1), 1. https://doi.org/10.1186/s13024-022-00590-4 https://doi.org/10.1186/s13024-022-00590-4

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Dear Reviewers and Convergence Editors,

Thank you very much for taking the time and effort to read through my manuscript and provide pivotal feedback. I have thus thoroughly reviewed your comments and outlined my responses to those suggestions. The reviewers had provided me with major revisions, among which included a lack of connection between the results and discussion along with other suggestions such as terminology and phrasing. The reviewers also emphasized a question on the novelty of the manuscript. I have responded to these comments thoroughly and have revised the manuscript as such. I have provided a one-by-one response to each of the comments and suggestions which the reviewer has provided, with page numbers given as a reference to the types of edits I have made in the manuscript. Furthermore, all reviewer's comments are bolded and my responses to those comments are underlined. As there are 2 reviewers, I have highlighted which reviewer's comments are which to make more clear.

1. **Reviewer 1: This current study contributes to expanding the literature evaluating whether APOE4 is central in linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor.-> The distinction between "APOE4 acting as a genetic risk factor" and "APOE4 linking genetic factors to neurodegeneration" is not clearly defined in this sentence. (Introduction)**

To begin with, reviewer one mentions that the sentence "This current study contributes to expanding the literature evaluating whether APOE4 is central in linking genetic factors to neurodegeneration rather than acting just as a genetic risk factor" does not clearly define "APOE4 acting as a genetic risk factor" and "APOE4 linking genetic factors to neurodegeneration." I completely agree with this statement and appreciate the suggestion, as I can see that I have not clearly defined those two phrases. As a result, I altered the sentence to instead state "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" (Page 3). This clearly defines the role that APOE4 plays and how I want to present it.

2. **Reviewer 1: Please indicate in parentheses which content corresponds to each figure. (Results)**

Reviewer one also states that I should "indicate in parentheses which content corresponds to each figure." I agree with this completely, and have done so accordingly in all my post-hoc analysis sections, which refer to the images.

3. **Reviewer 1: However, the interpretation of the results is overstated, and some of the conclusions are not directly supported by the data. For example, the current results do not allow for claims about the prevalence of dementia.**

   To support the conclusions made by the authors, additional statistical methods such as logistic regression are highly recommended instead of relying solely on ANOVA. If further analysis is not conducted, I suggest revising the discussion and conclusion sections to better reflect the actual findings.

   Most of the interpretations and conclusions are not supported by the current data and are therefore incorrect.

   For example, the comparison in Figure 1 simply shows p-Tau levels across four groups: dementia (APOE4 vs. non-APOE4) and no dementia (APOE4 vs. non-APOE4). This comparison alone does not provide evidence for the prevalence of dementia.

   Moreover, the statement "donors with the APOE4 allele and a high tau level in the brain had dementia. However, donors with the APOE4 allele and low tau percentage levels did not have dementia" is not fully supported by the data shown. To make claims about the correlation or prevalence of dementia in relation to APOE4 and tau levels, additional statistical analyses such as logistic regression would be necessary. If further analysis is not feasible, I suggest revising the discussion and conclusion sections to better reflect the actual findings.

   The conclusion that the interaction between APOE4 and tau is associated with AD prevalence is not supported by the data. The two-way ANOVA showed no significant interaction between APOE4 and cognitive status. Also, the term "prevalence" is not appropriate without a proper analysis such as logistic regression.

   Reviewer 2: Also, cognitive status should be treated as a dependent variable, whereas APOE genotype is an independent variable. It doesn't make as much sense to conduct the analyses using cognitive status as an independent variable when in reality it is being driven by changes in APOE and various inflammatory pathways such as those related to AT8 expression and 6e10. I think the paper would benefit from restructuring the analyses where cognitive status is the outcome, and evaluating how various combinations of APOE expression and AT8, 6e10, and Iba1 affect dementia.

   (Results and Discussion)

Both reviewer one and reviewer two commented on the lack of correlation between the conclusion I made in my discussion and the results and analysis. Reviewer one stated that "the interpretation of the results is overstated, and some of the conclusions are not directly supported by the data." Reviewer one goes further into saying that many of the claims regarding correlation

or prevalence of dementia are not backed by the results, and that, therefore, it would be better to run additional analyses or adjust the discussion and conclusion to match the actual results. Reviewer two stated that the cognitive status should be treated as the dependent variable since the discussion emphasizes how dementia is the outcome and measurement. Both reviewers emphasize the incorrect interpretations in the results and discussion. I fully agree with this assertion, since my analysis does not have the capacity to demonstrate a direct correlation or interaction between marker level and APOE4 alleles on dementia. Also, I agree that, with my primary outcome being whether this interaction causes or does not cause dementia, it would be better to have dementia as a dependent variable rather than an independent variable. Thus, I have attempted to resolve this issue by changing the discussion so that my findings align with the two-way ANOVA. I deleted previous paragraphs heavily interpreting interaction and replaced them with the finding that APOE4 allele carriers with dementia demonstrate either a higher or lower marker expression than carriers without dementia. Along with that, I added the finding that dementia donors carrying the APOE4 allele expressed either a higher or lower marker expression compared to dementia donors without the allele. Specifically, I state that, "This study identifies how donors carrying the APOE4 allele with dementia have increased neuropathology than carriers without dementia. Furthermore, our study found that donors with dementia similarly showed increased neuropathology when carrying the APOE4 allele than not carrying it" (Page 9). This conclusion aligns more with my findings with the two-way ANOVA. I also rationalized that APOE4 carriers with dementia had greater neuropathology compared to carriers without dementia, possibly because of an association between the APOE4 allele and pathology that increases dementia risk. For example, I state that "there may be both a causal pathway and effect modification" which links the APOE4 allele with neuropathology (Page 12). I also back each of these justifications for the final conclusion with prior research that indicates this relationship. However, I heavily emphasize that this is hypothetical since "analysis with a two-way ANOVA is insufficient in determining the strength and direction of this association relationship" (Page 10) and that "while post-hoc comparisons suggested that APOE4 carriers with dementia indicated greater neuropathological levels than carriers without dementia, interaction between dementia and APOE4 were not statistically significant" (Page 10). I thus state that future research should "examine correlation and logistic regressions to test the strength of this relationship between dementia, APOE4, and these protein markers" (Page 10). I also adjusted my conclusion section by deleting the previously overinterpreted conclusion and replacing it with the adjusted findings.

4. **Reviewer 1: The introduction is well-written. However, this part should clearly explain the difference in APOE4 allele frequency between the general population and individuals with Alzheimer's disease to better establish its role as a risk factor. (Introduction)**

Reviewer one emphasized that while "the introduction well-written," it would benefit from clearly explaining the role that APOE4 plays as a risk factor for Alzheimer's. Specifically, they

state that I should explain “the difference in APOE4 allele frequency between the general population and individuals with Alzheimer’s disease to better establish its role as a risk factor.” I thank the editor and reviewer for this comment. I agree that the original introduction does not do as well to clearly establish APOE4 as a risk factor. As such, I have adjusted this original manuscript by incorporating a statistic comparing the APOE4 allele frequency in patients with AD in the introduction. This statistic emphasized how individuals with AD were more likely to have at least one APOE4 allele, stating that “56% of AD-diagnosed patients carried one copy of the gene and 11% had both copies” (Page 2). This statement elaborates more on what role APOE4 plays by demonstrating the risk percentages.

5. **Reviewer 1: Additionally, the manuscript repeatedly compares "APOE4 vs. no APOE4," but for readers unfamiliar with AD research, the rationale behind this comparison is not clear. Providing more background on why this distinction is important would improve clarity and accessibility. (Introduction)**

Furthermore, reviewer one mentions that the relevance of the distinction between APOE4 carriers and non-APOE4 carriers is not clear and well established, stating that “for readers unfamiliar with AD research, the rationale behind this comparison is not clear” and that “providing more background on why this distinction is important would improve clarity and accessibility.” I thank the reviewer for the suggestion and completely agree with this assertion, since the paragraph explaining the APOE4 risks doesn’t emphasize this distinction and instead only briefly touches upon it. I only state that “studies have established that those who carry the APOE4 allele have a greater risk of developing dementia as a result of AD” (Page 2). As such, I have addressed the comment by adding information that demonstrates that most AD cases carry the APOE4 variant rather than the APOE2 and APOE3 to emphasize the difference. In that introduction paragraph, I write that “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 on how the gene influences AD” (Page 2). This elaborates on the distinction and its importance.

6. **Reviewer 1: The abstract is very well written and effectively provides the necessary background information. In contrast, the Results section lacks context, background, and rationale. Please consider adding these elements, similar to how they are presented in the abstract.**

   Moreover, although the background section is adequate, the context is not clearly integrated into the data analysis, which may confuse readers.

While the analysis is technically sound, the presentation is overly focused on statistical details, which makes it difficult to follow the logical flow and fully understand the implications.

The current text focuses almost exclusively on reporting statistical results in a mechanical manner, without providing any context or interpretation. This approach makes it difficult for readers to understand the significance or implications of the findings. Please add more context and explanation for what each factors mean (e.g.) what is the 6e10?)

(Results)

Reviewer one also stated that the text within the results was reported in a "mechanical manner, without providing any context or interpretation." I thank the reviewer for bringing this to light, and agree that it can be confusing to some readers without writing an interpretation of the specific data and graphs. As a result, for each section of the results, I have added a sentence that reminds the readers of what each marker represents to establish context. For example, I write in the results analysis on the two-way ANOVA on AT8 that "AT8 is a monoclonal antibody marker which detects phosphorylated tau. Thus, we wanted to investigate whether APOE4 or dementia status is associated with AT8 expression" (Page 5). This sentence establishes context on what AT8 is and provides a reason why we wanted to investigate it. There is a similar context for the other two sections on 6e10 and Iba1. Furthermore, as a way to address the lack of interpretation in the results, I incorporated a short analysis after the statistical references. For example, originally, there was a sentence in the post-hoc comparisons on AT8 pathology section which stated that "There was statistically significant interaction between APOE4 carriers and non-APOE4 carriers in the no dementia group with a mean difference of 0.9728 (95% Cl of diff: 0.02813 to 1.917, p = 0.044)" (Page 7). This sentence can be confusing for some readers, who can have difficulty following the "logical flow and fully understand the implications," as stated by reviewer one. As a result, I added additional analysis to demonstrate the meaning of this finding, saying that "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" (Page 7). This format is found throughout the results section, where results findings are extended with additional interpretation that supports and addresses the overall thesis.

7. **Reviewer 1: Avoid beginning sentences with the name of a statistical test (e.g., "A two-way ANOVA tested the main effects..."). It would be clearer and more informative to first state the purpose and outcome of the analysis, and then briefly mention the statistical method used.For example: "We analyzed the data to examine the main and interaction effects of XXX (Two-way ANOVA, p = XXX) (Figure XX)."**

Reviewer one also mentions that it would be "clearer and more informative to first state the purpose and outcome of the analysis, and then briefly mention the statistical method used." I thank the reviewer for this suggestion and completely agree with the assertion, as doing so can contribute to better flow in the paper. As a result, I have altered sentences that state the exact statistical method utilized and instead just focus on the finding. For example, I removed "two-way ANOVA" in the beginning sentences and incorporated it after stating the statistical finding. Specifically, I state in the first section in the results that "There was no significant interaction between APOE4 and cognitive status on AT8 expression, (two-way ANOVA, $F(1,80)=0.2$, $p = 0.625$)" (Page 6). I write similarly for the other two markers as well.

8. **Reviewer 1: Furthermore, there is no explanation of what differentiates the APOE4 carriers from the non-carriers in terms of pathology or dementia risk, nor why these differences matter. (Results Section)**

Reviewer one also stated that "there is no explanation of what differentiates the APOE4 carriers from the non-carriers in terms of pathology or dementia risk, nor why these differences matter." I fully agree with this statement, as doing so can allow readers to understand the context of the results before interpreting them. As such, I added at the beginning of the results section that "It is necessary to address differences between APOE4 carriers and non-carriers. Carriers often show greater levels of neuropathology (tau, Aβ, Iba1) along with greater probability of developing dementia. These differences could demonstrate that APOE4 does not only act as a risk factor that increases risk of dementia but also influences certain mechanisms and neuropathological pathways" (Page 4). This sentence brings readers context before diving into the results, which allows them to understand why the APOE4 factor is important.

9. **Reviewer 1: Each section title (e.g. "Post-Hoc Comparisons on Iba1 Pathology," is too mechanical and overly focused on the statistical method rather than the key findings. In a results section, it's more effective to highlight the main results or conclusions rather than simply stating what type of statistical test was used. (Results)**

Reviewer one also stated that in the results section, the titles of each section "is too mechanical and overly focused on the statistical method rather than the key findings." They stated that it would be better if it focused more on the main conclusion. I thank the reviewer and fully agree with this assertion that the titles are too mechanical. As a result, for each of the titles in the results section, I removed mechanical aspects such as "APOE4 Genotype v. Dementia Diagnosis on Iba1 Pathology" and replaced them with one that encapsulates the main finding, such as "APOE4 Genotype and Dementia Diagnosis Influences Iba1 Pathology" (Page 6).

10. **Reviewer 1: Have you conducted normality tests to verify if the data are normally distributed within groups. If the data do not meet the normality assumption, then non-parametric alternatives like the Kruskal-Wallis test should be considered instead. (Results)**

Reviewer one also wanted "normality tests to verify if the data are normally distributed within groups" and that if the data violated normality, "then non-parametric alternatives like the Kruskal-Wallis test should be considered instead." I fully agree with this assertion and thank the reviewer for the suggestion. I ran normality tests on each of the four groups with the Shapiro-Wilk test. I wrote that "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" (Page 5). As a result, I conducted the Kruskal-Wallis test separately for dementia and APOE4. When writing about the normality and Kruskal-Wallis test, I also incorporated elaborations and interpretations of the results to strengthen the reader's comprehension and understanding of what each result meant as a way to address reviewer one's previous comments to write interpretations along with each statistic. I also incorporated my procedure for the tests in the methods section.

11. **Reviewer 2: I think a major limitation is that the current investigation is not necessarily novel (a number of studies have evaluated the allele frequencies of APOE4 in the context of Alzheimer's). Furthermore, while the paper does perform statistical analyses, the analyses have already been done by the group who organized the dataset. I think the novelty could lie primarily in the evaluation for interaction effects, but please see my comments below regarding effect modification vs. interaction.**

Reviewer two also showed concerns regarding the novelty of the findings and the paper, stating that "a number of studies have evaluated the allele frequencies of APOE4 in the context of Alzheimer's", and that the original research group had already done the same analysis in their paper. We acknowledge that this is true and that several studies, such as the original research group, have touched upon APOE4 allele frequencies. However, our study does differ from these studies and provides novelty in its own unique way. Our study extends further than the original study focused on and looks into the data in more depth. Also, unlike the original research group, we heavily focused on separating and making groups out of select variables. Our paper is focused on finding a potential association between the APOE4 allele, protein neuropathology (tau, Aβ, Iba1), and dementia. (1) The original research paper looked at all participants as almost one. (2) Our study is still relevant because it extends and provides new insight, whereas other studies approach analyses through a multivariate but weakly hypothesis-driven approach. (3) Our study has a clear hypothesis, which is that APOE4 may drive neuropathology, which may increase risk for dementia. Our data investigates whether there are any observed neuropathological changes within the APOE4 and dementia groups, which is important because, through understanding how much of a role these groups play in pathology, we can better

understand the link and relationship between the allele, pathology, and dementia. Furthermore, several recent studies have already begun to investigate this link, such as the August 2025 paper by the Rockefeller University Press titled *ApoE-calypse tau: ApoE–tau synergy in Alzheimer's disease.* This paper investigates the potential interaction relationship and synergistic effects between the allele and tau expression and how it affects AD. The study also heavily emphasizes that "targeting the APOE4-tau axis may offer promising therapeutic strategies to address the molecular mechanisms driving AD and tauopathies." This demonstrates how the concept is still novel and still has the potential to make an impact. Since the paper was published recently, it further illustrates how this concept is still worthy of further investigation and that others agree with our conclusion.

12. **Reviewer 2: Authors should also note that these are not adjusted for various demographic and biological variables known to be associated with dementia, so these results should be presented as unadjusted.**

Reviewer two stated, "Authors should also note that these are not adjusted for various demographic and biological variables known to be associated with dementia, so these results should be presented as unadjusted." I completely agree with this statement and thank the reviewer for bringing this up, as this can establish a better context for readers on the results. I have thus added a sentence at the beginning of my results section to emphasize this point, stating "All results are unadjusted since they do not account for variables associated with dementia" (Page 5).

13. **Reviewer 2: The authors confuse the concepts of effect modification and interaction. I would argue that measuring levels of Dementia based on allele expression is an example of effect modification. Furthermore, if the expression of APOE4 drives increases in AT8 which in turn promotes Dementia, then this is more so a causal pathway analysis as opposed to interaction.**

Reviewer two also mentions that in the original discussion, there is a misuse of the word interaction, and that some are actually examples of "effect modification" and "causal pathway." I thank the reviewer for catching this error and bringing this to light. I agree that in many parts of my original conclusion, I overemphasize the term "interaction" when, in fact, many of the explanations reflect a causal pathway or effect modification. Thus, I adjusted these rationales to emphasize a causal pathway or effect modification when appropriate. For example, in my conclusion section, I state that "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 increased neuropathology. However, the results do not suggest whether neuropathology causes dementia or dementia increases parallel to neuropathology" (Page 13). This adjusts the previous error and replaces it with correct terminology. I also state throughout the discussion that this rationale is purely speculative while

also backing the rationale with previous studies. Overall, my changes in terminology highlight and align with the conceptual distinction which the reviewer suggests.

I sincerely thank the reviewers and editors for the constructive feedback they have provided me. Overall, I have utilized these comments to the fullest extent, adjusting issues between my main discussion and results, and addressing other issues such as novelty and terminology. I believe that these revisions, such as improved contextualization, interpretation, and conclusions, have improved the manuscript as a whole. I hope that the changes I have made to the manuscript do their best to address the concerns from the reviewers and enhance its contribution to the field.

Thank you for your time, feedback, and thoughtful consideration.

Sincerely,

[Author name redacted by Managing Editor]

Referee 1:

Overall, the manuscript has improved significantly and the author has addressed the reviewers' comments appropriately.

The responses to minor comments are well-handled, and the manuscript appears suitable for acceptance with minor changes.

One minor comment:

In the Results section, there is a "Normality Testing" subsection. I recommend moving the detailed description of this part to the Method section, and if they want to mention results, please merge it into the existing Results subsection for better flow.

Referee 2:

I find the paper suitable for publication. Extensive work was done by the student to address the major issues appropriately.