Alzheimer's Disease Risk Factors: APOE ε4 Allele and Traumatic Brain Injuries

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Article

Keywords:

Posted Date: July 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1883911/v1

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder affecting millions of individuals worldwide. Understanding of the risk factors for Alzheimer's is in its preliminary stages, but researchers have recently made several advancements in searching for treatments for this damaging disorder. This paper aims to garner greater insight into two potential risk factors: APOE ε4 allele and Traumatic Brain injuries (TBIs), specifically their leverage on disease diagnosis through metrics measuring hallmarks of the disease: beta-amyloid plaques and neurofibrillary tangles. Data from the Allen Brain Institute was analyzed through statistical tests that included chi-squared, bar charts, stacked bar charts, two-way ANOVAs, and t-tests to deduce these risk factors' mechanisms in impacting Alzheimer's. From the analyses, it could be understood that the APOE ε4 allele is a significant genetic predisposition for this disease, although a causal relationship could not be determined. On the other hand, TBIs were found to play an additive role in the development of Alzheimer's, which was less significant than the APOE ε4 allele's role since there was a higher correlation between TBIs and non-Alzheimer's dementia, specifically neurofibrillary tangle predominant dementia (NFTPD). Analysis of risk factors provides a means for optimizing therapy by identifying and allocating resources more efficiently to those most vulnerable, as well as improved drug delivery through treatments that target biological pathways which initiate specific biomarkers formation.

Intro

a) The pertinence of Alzheimer's Disease

As the only illness within the top 10 leading causes of death in the United States to not have a means to prevent, cure, or slow progression, Alzheimer's disease (AD) is a significant health complication that cannot be ignored. While other major diseases are all experiencing substantial declines in mortalities, AD experienced a 68% increase from 2000 to 2010, and this upward trend is expected to increase even further in the coming years. Furthermore, AD is placing enormous strain on the economy due to the numerous comorbidities associated with the disease and its prolonged duration. It is projected that AD will cost families up to 1.1 trillion dollars by 2050. As a result of this financial burden, more than 60% of Alzheimer's and dementia caregivers rate their levels of the emotional stress of caregiving as "high" or "very high," with one-third reporting symptoms of depression. The intrusive nature of the disease, coupled with the high levels of stress and finances, make it a pertinent health problem that requires intensive research (Johns, 2013).

b) The fundamentals of Alzheimer's Disease

AD accounts for 60-80% of dementia cases and is associated with memory loss and other areas of cognitive decline to the point where it impedes everyday life. Furthermore, the progressive disease primarily affects adults over the age of 65 and is implicated with neuronal death, resulting in brain regions that appear atrophied ("What is Alzheimer's Disease"). The

destruction of neurons and neuronal connections initially affects brain regions such as the entorhinal cortex and hippocampus, which are associated with memory. This damage later advances to the cerebral cortex, which controls language, reasoning, and social behavior. With this insidious damage, an individual begins to lose their ability to function independently, resulting in death ("What happens to the brain," 2017). There is no determining factor for the disease but rather a myriad of risk factors, which include a family history of dementia, head trauma, specific genotypes, being female, low education level, and many more. In fact, vascular risk factors such as hypertension, diabetes, and hyperhomocysteinemia, have been proved to provide a synergistic or additive effect on AD diagnosis. This is possibly due to a reduced cerebral blood flow (CBF), resulting in hyperperfusion and an earlier onset of AD (Scheffer et al., 2021). Regardless, age is the main factor affecting this disease, as prevalence increases from 3% for ages 65-74 to almost 50% for ages 85 and greater (Castellani et al., 2011).

c) Non-Alzheimer's Disease Dementia

Although AD accounts for many dementia cases, other similar disorders contribute to this statistic. In contrast to specific neurodegenerative diseases, such as AD, dementia is not a disease but a set of symptoms common for these neurogenerative diseases. Dementia encompasses numerous different forms and types, including vascular dementia, dementia with Lewy bodies (DLB), Parkinson's disease dementia, mixed dementia, frontotemporal dementia (FTD), and many more (Martin, 2020). Four features must be scrutinized to distinguish different types of dementia: speed of onset, age of onset, cognitive/neurological profile of the patient, and medical

history that may indicate a non-AD cause. With a rapid and earlier onset of symptoms, the greater the likelihood the disease is not AD (Caselli, 2017).

d) Pathology of Alzheimer's Disease

Two hallmark AD characteristics include the presence of amyloid plaques and neurofibrillary tangles (NFTs). When looking into the formation of amyloid plaques, the amyloid precursor protein (APP) plays a considerable role. APP is a type 1 transmembrane protein that helps neurons undergo growth and repair while also degrading over time. When the protein is spliced by α-secretase and γ-secretase, the segmented peptide becomes soluble and dissolves. However, when β-secretase combines with γ-secretase in slicing the protein, mainly at the N-terminus (contains receptor-binding region: residues 136-150) and C- terminus (lipid-binding region: residues 244-272) of the αβ domain, the leftover peptide becomes insoluble and forms a monomer known as beta-amyloid (αβ) through the amyloidogenic pathway as seen in Figure 1.

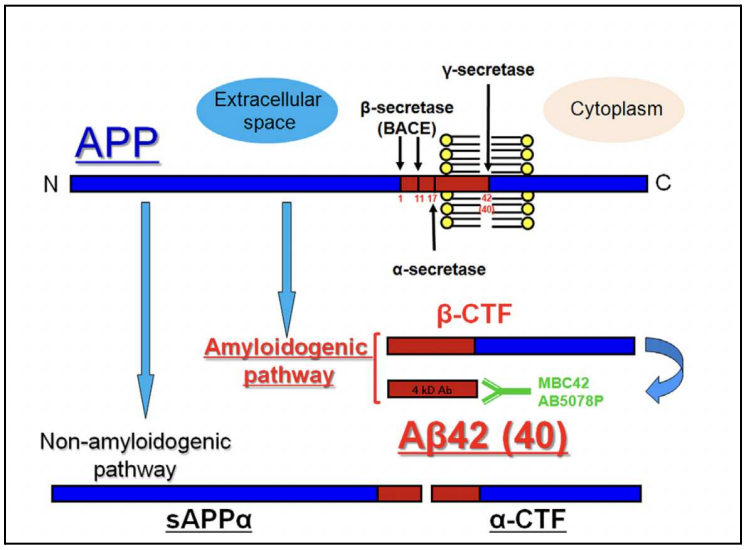

Figure 1. *Secretases splicing APP (Takahashi et al., 2017)*

These monomers are chemically "sticky" and tend to attract one another in the extracellular space, forming insoluble amyloid fibrils of antiparallel-pleated filament sheets 7–9 nm in diameter. These fibrils further assemble into plaques, as seen in Figure 2, which can potentially assemble between neurons to interrupt signaling between the two and thus diminish cognitive function (Takahashi et al., 2017). Some brain regions, such as the hippocampus, are more susceptible to forming plaques. With the hippocampus being localized for the transfer of information from short-term memory (STM) to long-term memory (LTM), its disruption is associated with a symptom of AD (Smith, 2019).

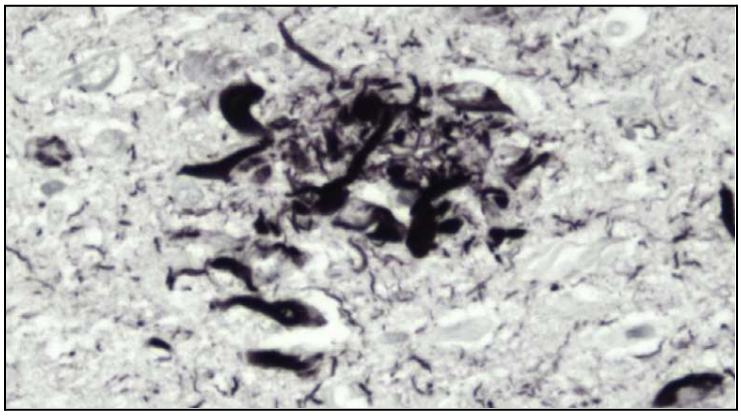

Figure 2. *Neuritic Plaques (Vogels et al., 2019)*

The neuron is held together internally by microtubules, and tau (tubulin-associated unit) protein helps ensure they do not break by providing stability for these microtubules. It is thought that the plaque formation outside of the neuron contributes to pathways where different kinases (ex. ERK2, GSK-3, CDK5, casein kinase, protein kinase A, and MAP/microtubule affinity-regulating kinase 1) are activated and drop off their phosphate group to the tau protein, allowing for hyperphosphorylated tau protein (up to 2-3 time increase in the number of moles per phosphate molecule per mole of protein) (Alonso et al., 2018). This results in a loss of affinity to

microtubules and the self-aggregation of tau proteins into paired helical filaments (PHFs) (loosely intertwined) and straight filaments (SFs) (tightly coiled). These two types of filaments form threads, which eventually get tangled to develop neurofibrillary tangles (NFTs) inside the neurons, as seen in the visuals from Figures 3 and 4. This leads to the neuron's transport system becoming blocked, disrupting the synaptic communication between neurons ("What happens to the brain," 2017).

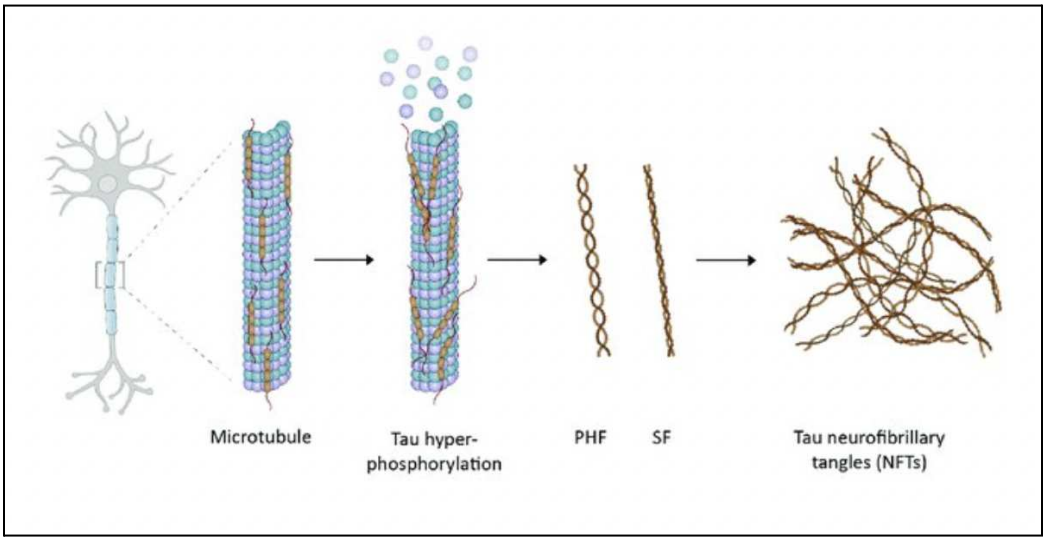

Figure 3. *Process of NFT formation*

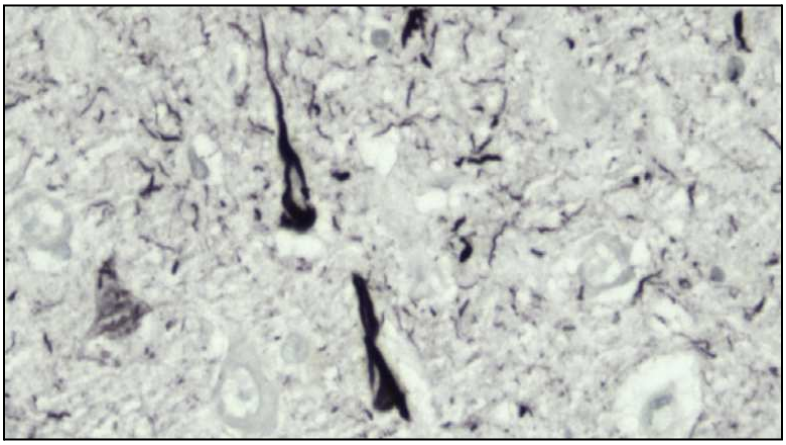

Figure 4. *Neurofibrillary tangles (NFTs) (Vogels et al., 2019)*

These tangles propagate by tau seeds first being directly translocated across the membrane or transported via extracellular vesicles. Then, the neurons take up the tau seeds and damage the endocytic vessels to end up in the cytosolic compartment to seed physiological tau, growing fibrils and starting the aggregation process (Vogels et al., 2019). The process begins at the entorhinal cortex in the medial temporal lobe and slowly progresses to the hippocampal region and then to the neocortex. This resembles the clinical course of AD as it begins with memory deficit and translates into other cognitive deficiencies, pointing toward the idea that tau pathology is intertwined with neurological deficiencies (Takeda, 2019).

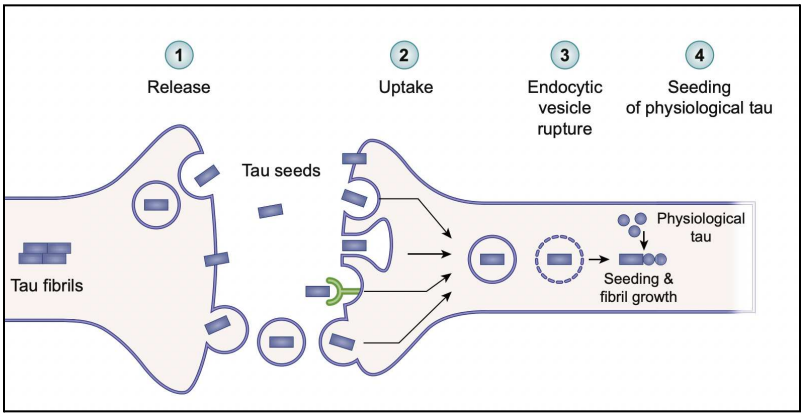

Figure 5. *Propagation of Tau (Vogels et al., 2019)*

e) Pathophysiology of Traumatic Brain Injuries

It was found that older adults with moderate trauma to the head were 2.3 times more likely to develop Alzheimer's disease, and this finding jumped to 4.5 times for those with a history of extensive trauma ("Traumatic Brain Injury (TBI)"). One of the major epigenetic risk factors potentially accelerating the onset of Alzheimer's disease is traumatic brain injury (TBI), which occurs when a sudden external force is exerted on the brain. TBIs can be categorized into

two types of forces: contact and inertial. Contact forces arise directly at the impact site when the brain strikes the skull's inner surface. On the other hand, inertial forces occur without actual head contact (contact force), leading to more diffuse injury, and are divided into two types of acceleration: linear and rotational. Linear acceleration is correlated with peak pressures within a brain. Rotational acceleration is likely to cause shear-induced tissue damage (strains), increasing the likelihood of an unconscious episode. This is possible since brain tissue is malleable as it is composed mainly of water, meaning that a shearing force can easily result in the deformation of the structure (Meaney & Smith, 2011). This leads to unnecessary tension by stretching individual neurons, specifically the axon, resulting in tau protein coming off the cytoskeleton of the neurons. These protein fragments clump up in an individual's brain cells, forming neurofibrillary tangles (NFTs) ("Pathophysiology of Traumatic Brain Injury," 2022). On top of damaging the axons, these external forces produce a sudden depolarization of nerve cells, leading to a flood of neurotransmitters such as glutamate (Kelly, 2001). An increased release of glutamate results in the expenditure of large amounts of energy in the form of ATP to contain this neural activity. However, glucose is not available to this extent, meaning that the brain relies on methods to generate short-term energy, accumulating lactate. This compound can exacerbate neuronal dysfunction and potentially increase the likelihood of damage to neurons should another TBI occur. Furthermore, once glutamate binds to receptors on neurons, it results in an influx of calcium ions in the mitochondria and disrupts the organelle's energy production. This intracellular accumulation of calcium may lead to potentially deleterious effects on the neuron, as it can initiate apoptotic processes due to the lack of ATP production.

Another factor to be considered is the location of the TBI. Due to the localization of function in different brain regions, the region in which a force is exerted on the brain may result in drastically different symptoms and diagnoses, as presented in Figure 6.

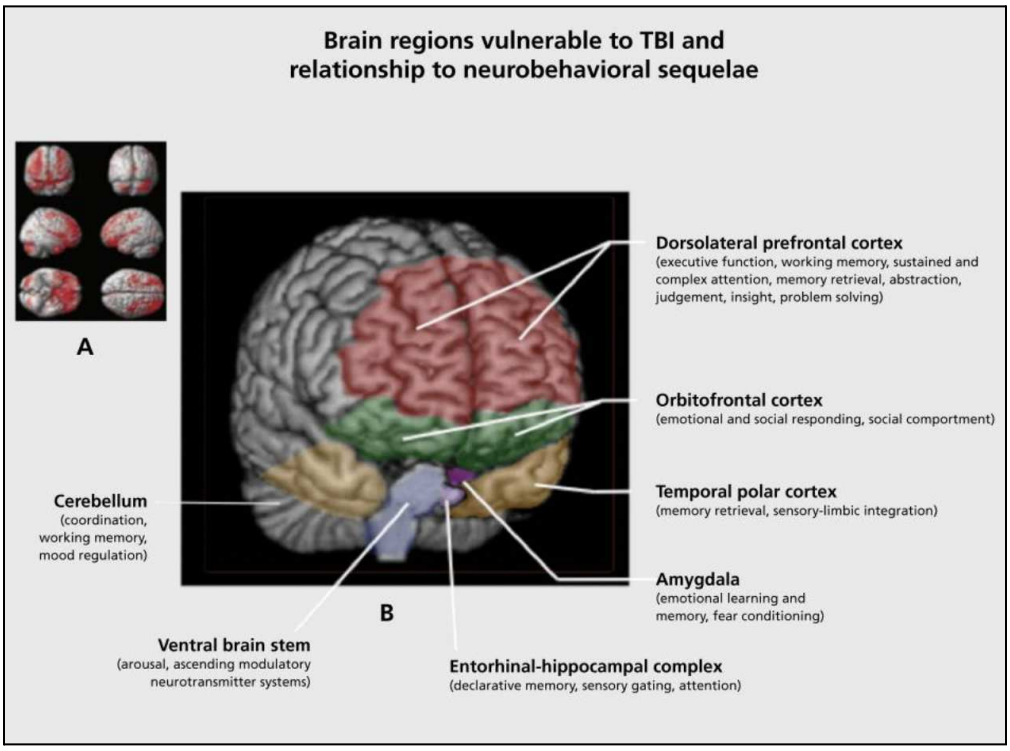

Figure 6. *Susceptible regions of the brain for TBIs (McAllister, 2011)*

f) APOE ε4 Allele

It has been suggested through research that the polymorphism of the apolipoprotein E gene, located on chromosome 19, is the most significant genetic risk factor for AD, influencing its onset. The allele has three versions: APOE ε2, APOE ε3, and APOE ε4. APOE ε4 is associated with reduced cognitive and functional recovery, deposition of β-amyloid, prolonged post-traumatic coma, lower cerebral blood flow, and possibly early signs of symptoms. On the other hand, the ε2 allele is protective, reducing the risk of Alzheimer's disease (Chamelian,

2004). In fact, the relationship has been established with certainty since approximately 40-65% of people diagnosed with AD have the APOE ε4 allele. The risk for developing AD is also heightened if the APOE ε4 allele is inherited from both parents ("Is Alzheimer's Genetic?").

g) Relevance

Although there have been many breakthroughs regarding the known genetic and epigenetic risk factors, many uncertainties have yet to be uncovered. Since the discovery of AD, it has been known that there are plaques and tangles for those with the disease; however, it is still unclear as to what the relationship between these two factors is: if they work together or separately to damage nerve cells, or if they even cause AD or are just a by-product (Smith, 2019). Regardless, discoveries like plaques coming before tangles have been made, but more will be needed to find greater success in uncovering the disease. This is why drugs or medications aimed at reducing a single factor such as tau (for neurofibrillary tangles) may not effectively treat the disease, as there may be a need to reduce other factors like amyloid (for plaques) as AD is multifactorial (Nierenberg, 2016).

h) Aim and Thesis

This paper will observe the neuritic plaques and neurofibrillary tangles development from TBI and APOE ε4 data through Braak and CERAD scores, respectively. Therefore, this paper will examine how these genetic and epigenetic risk factors may influence diagnosis, specifically how they interact with each other. From the background above, TBIs moderately mediate the established genetic risk factor for AD through the accumulation of NFTs.

Method

a) Participants (Age/Sex/Sampling Technique)

Data for this paper was gathered via the Allen Brain Institute "Aging, Dementia, and TBI Study," which utilized a matched-pairs design where a TBI cohort was matched with a control cohort of the same age, sex, year of death, and post-mortem intervals. The following algorithm, seen in Figure 7, was used to produce the most accurate match for each TBI participant to the control:

Algorithm is as follows with comparison to the index TBI case:

- Exact age – yes
  - If exactly 1, then take
  - If more than 1, then closest year of death
    - If exactly 1, then take
    - If more than 1, then closest post-mortem interval
      - If exactly 1, then take
      - If more than 1, then closest date of death
- Exact age – no, then age +/- 1 year and repeat algorithm
- Age +/- 1 year no, then age +/- 2 years and repeat algorithm until a match is identified.

Figure 7. *Selection Algorithm (Allen Brain Atlas)*

Each cohort had 55 participants, with researchers having extensive access to medical testing records and longitudinal neuropsychological tests for dementia as participants were part of a pre-existing study: "Adult Changes in Thought - ACT." Participants were randomly selected from members of Kaiser Permanente Washington, a non-profit healthcare system in the greater Seattle area. The participants' age was recorded linearly until 89 years, which afterward became categorized into 5-year blocks to provide anonymity for the subject: "90-94," "95-99," and "100+".

b) Genotyping & Measurement of Dementia Symptoms

More specifically, each member of the TBI cohort has experienced 1-3 TBIs in their life, having gone through a loss of consciousness from a few seconds to an hour. For greater accuracy, participants were asked, "Have you ever had an injury so severe that you lost consciousness?" If participants responded "yes," they were further questioned on whether it was a brain injury, the age at which it occurred, and the duration. The duration of TBI measured the time an individual was left unconscious, in which the participants were given the following options: "less than 10 seconds," "10 seconds – 1 minute," "1-2 minutes," "3-5 minutes," "6-9 minutes," "10 minutes – 1 hour," and "more than an hour." Data analysis for this paper involved grouping the seven categories mentioned above into the following categories: less than 10 seconds, 10 seconds - 5 minutes, and more than 5 minutes. This ensured equal distribution of participants in each category, maximizing statistical power for data analysis.

Using the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) criteria, data was extracted from the possible outcomes where "no dementia" served as one group while the "dementia group" was individuals who fit under the following diagnoses: "Alzheimer's Disease Type," "Multiple Etiologies," "Vascular," "Other or Unknown Cause," and "Other Medical." While the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria yielded "No Dementia," "Possible Alzheimer's Disease," "Probable Alzheimer's Disease," and "Dementia."

Fixed tissue was stained using immunohistochemistry (IHC) and histochemistry to assess beta-amyloid, tau, and α-synuclein deposition in plaques, tangles, and Lewy bodies, using

formalin-fixed paraffin-embedded (FFPE). Formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissues from each subject were isolated from the temporal neocortex, parietal neocortex, parietal white matter, and the hippocampus to be analyzed for three major data types:

- Quantitative neuropathologic analysis
- Molecular analysis of RNA-sequence
- Analysis of the distribution of different cell types in each tissue sample

In addition, this fresh frozen tissue was used for in situ hybridization (ISH) to gain insight into the cellular makeup of the tissue, to measure the concentrations of various proteins (b-amyloid, tau, and alpha-synuclein, chemokines, cytokines, and interleukins) and to measure oxidative stress in the temporal and parietal regions by observing levels of f2-isoprostanes. For IHC of FFPE tissues, tissue blocks were taken from the parietal cortex, and temporal cortex from the same side of the brain sampled during the rapid autopsy for frozen tissues. FFPE hippocampus for IHC was taken from the opposite side as the entire hippocampus hemisphere was used for frozen tissue processing.

The transcriptome was analyzed by RNA sequencing in tissue isolated from four brain regions: the neocortex from the posterior superior temporal gyrus and the inferior parietal lobule, white matter underlying the parietal neocortex, and the hippocampus. APOE ε4 allele was identified in which those with at least one allele of the APOE gene were categorized into the “yes” condition, whereas those without any allele for this gene were categorized into the “no” condition.

Furthermore, tissue from the regions mentioned above was collected by manual macrodissection. Using an annotated Nissl-stained section as a guide, samples were excised, and RNA was isolated and processed for sequencing, producing a minimum of 30 million 50bp paired-end clusters per sample.

This data set consists of one data set from fresh frozen tissue and one data set from FFPE tissue.

- **Fresh frozen tissue:** Histological staining consisted of Nissl staining (for anatomical reference) and Thioflavin-S (for visualization of plaques and tangles). Immunohistochemical staining was used to visualize amyloid, tau, and alpha-synuclein pathology within the fresh frozen tissue.
- **FFPE tissue:** Tissue was processed for standard neuropathology and stained by IHC and histochemistry to assess αβ, tau, and α-synuclein deposition in plaques, tangles, and Lewy bodies.

Pathology in both fixed and fresh frozen tissue was measured and quantified with IHC using automated image analysis to generate proxy values for the severity of pathology. To generate quantitative image metrics for the IHC on fresh frozen tissue, the macrodissection sites (for the samples for RNA-Seq) as delineated on the Nissl images were used to guide the identification and annotation of equitable regions of interest (ROIs) on each of the near-adjacent IHC images. For quantitative image metrics for the IHC on fixed tissue, ROIs for image analysis were selected on images of IBA1 stained tissue, using the same criteria used for determining macrodissection sites on fresh frozen tissue. The expression density, defined as the percentage of the area within the ROI occupied by the IHC reaction product, was then assessed algorithmically.

c) Data Analysis Metrics

i. Braak Scores

Discovered by Heiko Braak, Braak staging depicts neurofibrillary tangle (NFT) pathology, specifically its distribution and severity, and is divided into six stages. Silver stain is used to visualize the NFTs in the frontal, temporal, parietal, entorhinal cortex, and hippocampus. The variation in topological distribution patterns of neurofibrillary lesions allows for the classification of a case to be between 1 and 6, where the greater the number, the greater the scale Alzheimer's disease has spread and severity, as seen through NFTs (Braak, 2006).

Braak stages I and II are used when neurofibrillary tangle involvement is confined mainly to the transentorhinal region of the brain, stages III and IV when there is also the involvement of limbic regions such as the hippocampus, and V and VI when there is severe and extensive cortical involvement, as seen in Figures 8 and 9 (“Braak Stage”).

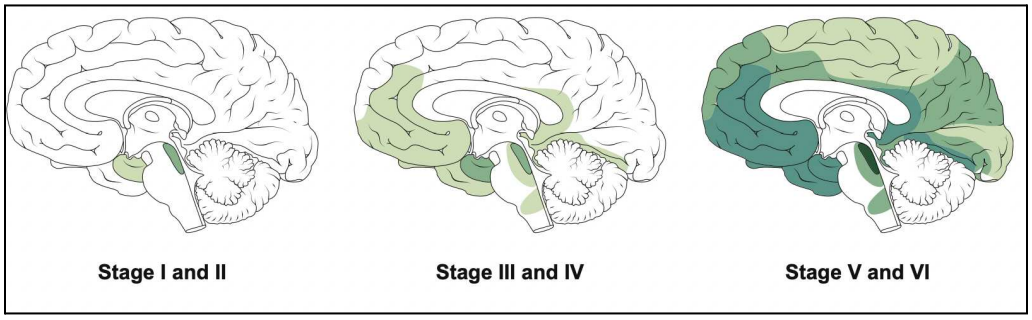

Figure 8. *Visual progression of tau pathology in AD is described using the Braak staging method (1-6) (Vogels et al., 2019)*

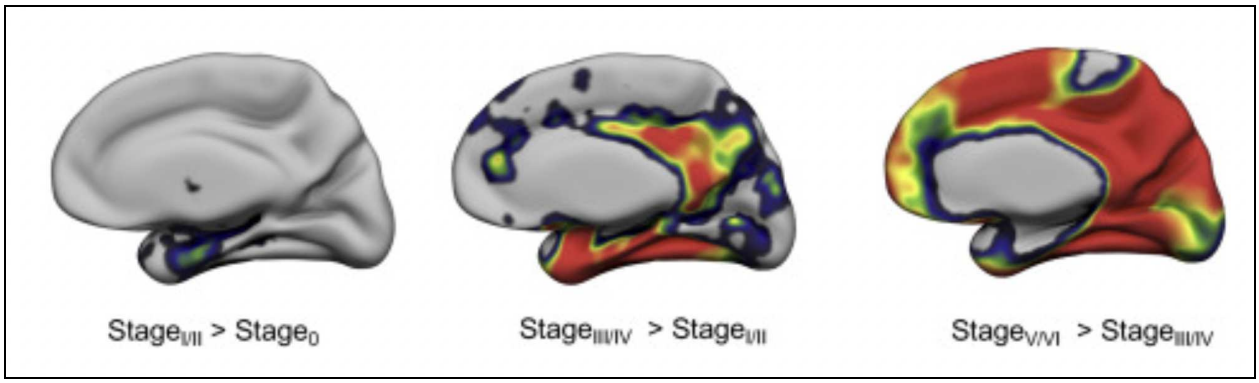

Figure 9. *PET (Positron Emission Tomography) regarding the progression of tau pathology in AD (Vogels et al., 2019)*

ii. CERAD Scores

CERAD scores measure the staging of severity of amyloid deposition on a scale of 0 (none) to 3 (severe) ("CERAD score"). Plaques vary in size but often measure 50-100 μm and are spherical. They are identified through the microscopic examination of silver-stained slides (Naha, 2021).

iii. NIA Reagan Scores

Another metric used was the NIA Reagan score, which measured the likelihood of developing AD. The criteria were calculated by taking into account Braak scores (metric for NFTs) and CERAD scores (metric for neuritic plaques) and are measured on a scale of 0 to 3, where 0 = no AD, 1 = low, 2 = intermediate, and 3 = high (Allen Brain Atlas).

d) Other Relevant Variables

i) # TBIs: the number of self-reported TBIs (ranging from 0-3)

ii) Education Years: the number of years of self-reported education (ranging from 9-21)

iii) Sex: male or female

iv) TBI?: whether an individual self-reported a TBI (yes or no)

v) Age at 1st TBI: the age at which first TBI with loss of consciousness was reported

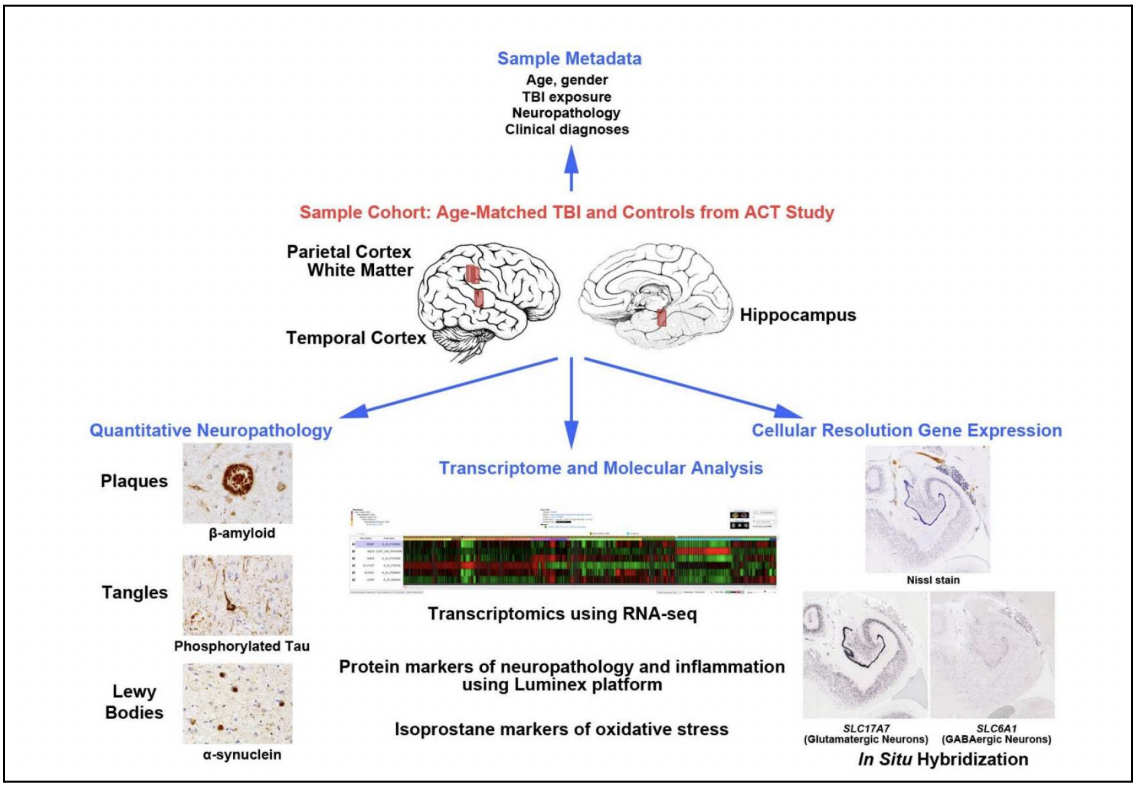

Figure 10. *Allen Brain Institute Aging, Dementia, and TBI Study data collection process (Allen Brain Atlas)*

e) Data Analysis Procedure

Data from this study was analyzed through a vast assortment of tests. First, a chi-squared test was used to determine if there was a statistical relationship between the two binary, categorical data: APOE ε4 allele and dementia. The dementia diagnosis metric used for this experiment will be DSM-IV. Alzheimer's diagnosis could not be done through this statistical procedure due to the multifactorial outcomes within the data set (ex. "vascular," "multiple etiologies," etc.). A null hypothesis (H_0) - assuming no association - and an alternate hypothesis (H_1) - assuming an association - were devised for this statistical test. If the chi-squared value is greater than the chi-square critical value for $p = 0.05$ and a degree of freedom (df) $= 1$, then the null hypothesis is rejected, meaning there is significant evidence supporting that APOE ε4 is associated with the development of dementia. Whereas, if the chi-squared value is smaller than the chi-square critical value for $p = 0.05$ and a degree of freedom (df) $= 1$, then the null hypothesis is accepted, meaning there is no evidence supporting the relationship mentioned above.

Second, a series of bar graphs were designed by grouping the duration of TBI into three categories (less than 10 seconds, 10 seconds - 5 minutes, and more than 5 minutes) for an equal representation of participants in each category. This duration was plotted against average Braak, CERAD, and NIA Reagan scores, representing the likelihood of developing Alzheimer's disease or its effects on the brain. Additionally, a similar series of bar graphs were produced where the combination of environmental (TBI presence/absence) and genetic (APOE ε4 presence/absence) factors and their effect on Braak, CERAD, and NIA Reagan scores were seen. The independent variable for this graph had four possible outcomes (TBI + APOE ε4, TBI + No APOE ε4, NO

TBI + APOE ε4, NO TBI + NO APOE ε4), which allowed for four possible bar graphs. The average scores for the three tests for AD within these four groups determined the height of these bars (y-axis). These bar graphs were analyzed through the error bars from the standard error of the mean, which can be calculated on excel and google spreadsheets using the function "=STDEV(sampling range)/SQRT(COUNT(sampling range))" to quantify the preciseness of the mean, taking into account the standard deviation and sample size. However, statistical significance cannot be deduced through this method. If the error bars overlap, it indicates that the p-value is much greater than 0.05; however, it is unclear if the error bars do not overlap as the p-value can be either greater or less than 0.05. For this reason, a t-test was conducted to recognize any statistically significant difference between any of the groups, which will be compiled later in the discussion section for a more holistic analysis of the risk factors' effect on AD diagnosis, specifically using the standard error of the mean for each group as well as t-tests to affirm the results. The procedure, as mentioned earlier, was done on TBI duration graphs as well.

In addition, a 100% stacked bar chart was used to aid in the visualization of the four possible IV outcomes concerning the diagnostic results: "no dementia," "possible Alzheimer's, "probable Alzheimer's," and "dementia." Through this, inferences can be made as to which risk factor correlates to a specific diagnosis, which will be elaborated on in the discussion.

Moreover, 2 x 2 factorial design graphs investigated the two variables of interest (TBI presence/absence APOE ε4 presence/absence) against Braak, CERAD, and NIA Reagan to

visualize any interactive effects. The main effect of either of the two individual variables was determined using a two-way ANOVA through p-values and t-tests.

Lastly, a series of two continuous variables (ex. Education years & NIA Reagan Scores) were plotted on a scatterplot, in which a line of best fit and Pearson's correlation coefficient was determined to understand the relationship's strength and direction better.

Results

a) Chi-squared test: APOE ε4 vs. Dementia

H_1 / Alternative hypothesis: The APOE ε4 allele affects Dementia

H_0 / Null hypothesis: The APOE ε4 allele does not affect Dementia

Table of observed frequencies

	APOE E4 (Yes)	APOE E4 (No)	Row Totals
Dementia (Yes)	13	32	45
Dementia (No)	7	48	55
Column Totals	20	80	100

Table of expected frequencies

	APOE E4 (Yes)	APOE E4 (No)
Dementia	9	36
No Dementia	11	44

$X^2 = 4.\overline{04} > 3.841$

Table 1. *Chi-squared: testing the presence of an association between APOE ε4 and Dementia*

b) Chi-squared test: TBI vs. Dementia

Table of observed frequencies

	TBI (Yes)	TBI (No)	Row Totals
Dementia (Yes)	25	21	46
Dementia (No)	25	29	54
Column Totals	50	50	100

Table of expected frequencies

	APOE E4 (Yes)	APOE E4 (No)
Dementia	23	23
No Dementia	27	27

$X^2 = 0.6441 < 3.841$

Table 2. *Chi-squared: testing the presence of an association between TBI and Dementia*

The critical chi-square value comes from accounting for two values: degrees of freedom and p-value. The degree of freedom for the chi-squared test was calculated using the equation: $df = (rows - 1)\, x\, (columns - 1)$. For this case, there are two rows and two columns, meaning that the degrees of freedom for this particular case will be 1. The p-value (the probability that a phenomenon will occur by chance) was 0.05 since it serves to differentiate

statistical significance (any deviation with a chance probability greater than 5% is not statistically significant while it being lower than 5% is statistically significant). The conclusion could be drawn that there is an association between the APOE ε4 allele and dementia as the null hypothesis (H_0) is rejected since the calculated chi-square value ($4.\overline{04}$) is greater than the chi-square critical value (3.841). On the other hand, there is no association between TBIs and dementia as the null hypothesis (H_0) is rejected since the calculated chi-square value (0.6441) is greater than the chi-square critical value (3.841).

c) TBI Duration

i) TBI Duration vs. Braak

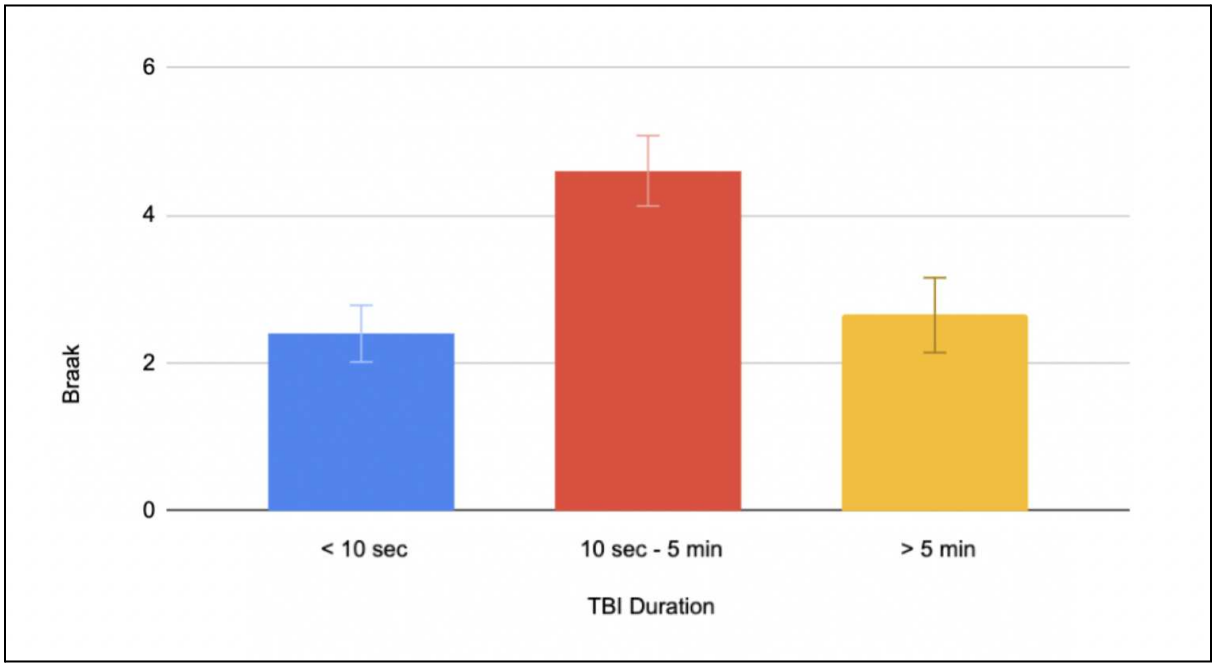

Figure 11. *Bar Graph (TBI Duration vs Braak). Height of the bar graph indicates mean Braak scores and the error bars represents the standard error of mean for each TBI duration.*

From Figure 11, a TBI duration of 10 seconds to 5 minutes has the highest average Braak score of 4.60. For durations less than 10 seconds, the average Braak score was 2.51, while for durations greater than 5 minutes, the average Braak score was 2.65.

There is a statistical significance for the conditions:

1. “10 sec - 5 min” and “> 5 min” (p-value ≈ 0.0162, t-value ≈ 2.57)

There is no statistical significance for the condition:

1. “< 10 sec” and “> 5 min’ (p-value ≈ 0.126, t-test ≈ 1.57)
2. “< 10 sec” and “10 sec - 5 min” (p-value ≈ 0.138, t-value ≈ -1.53)

ii) TBI Duration vs. CERAD

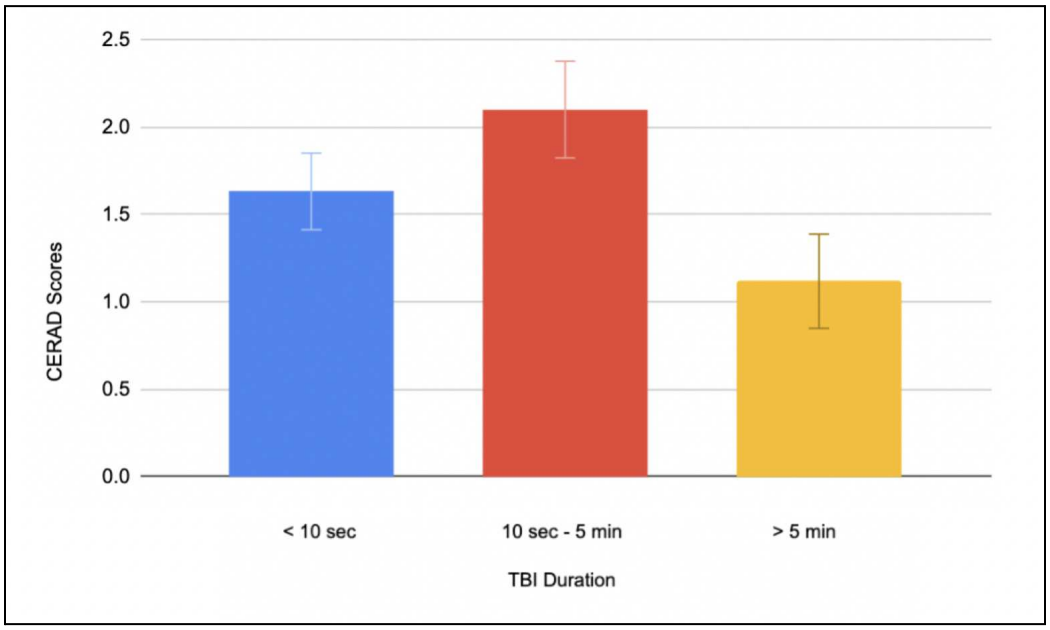

Figure 12. *Bar Graph (TBI Duration vs. CERAD). The height of the bar graph indicates mean CERAD scores, and the error bars represent the standard error of the mean for each TBI duration.*

From Figure 12, CERAD scores resembled similarities to Braak scores, with the 10-second to 5-minute duration having the highest average score of 2.10. For durations less than 10 seconds, the average CERAD score was 1.63, while for durations greater than 5 minutes, the average CERAD score was 1.12.

There is a statistically significant difference for the following conditions:

1. “10 sec - 5 min” and “> 5 min” (p-value ≈ 0.025, t-value ≈ 2.39)

There is no statistically significant difference for the following condition:

1. “< 10 sec” and “10 sec - 5 min” (p-value ≈ 0.208, t-value ≈ -1.29)
2. “<10 sec” and “> 5 min” (p-value ≈ 0.145, t-value ≈ 1.49)

iii) TBI Duration vs. NIA Reagan

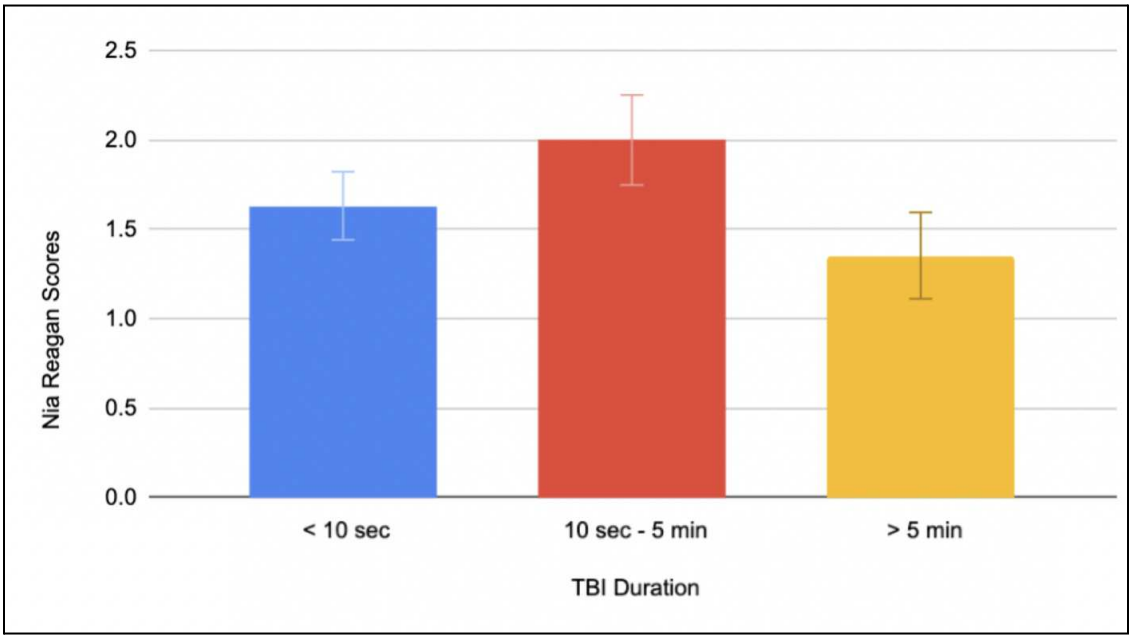

Figure 13. *Bar Graph (TBI Duration vs. NIA Reagan). The height of the bar graph indicates mean NIA Reagan scores, and the error bars represent the standard error of the mean for each TBI duration.*

From Figure 13, NIA Reagan scores resembled CERAD and Braak graphs, which is compatible with literature as this metric measures AD likelihood by considering CERAD and Braak scores. The highest average NIA Reagan score was between 10 seconds and 5 minutes, with a score of 2.00. For durations less than 10 seconds, the average NIA Reagan score was 1.63, while for durations greater than 5 minutes, the average score was 1.35.

There is no statistically significant difference for the following conditions:

1. “<10 sec” and “>5 min” (p-value ≈ 0.367, t-value ≈ 0.915)
2. “<10 sec” and “10 sec - 5 min” (p-value ≈ 0.264, t-value ≈ -1.41)

3. “10 sec - 5 min” and “> 5 min” (p-value ≈ 0.0950, t-value ≈ 1.74)

d) Gene & Environment Combination

i) Braak Score vs Genotype & Environment Combination

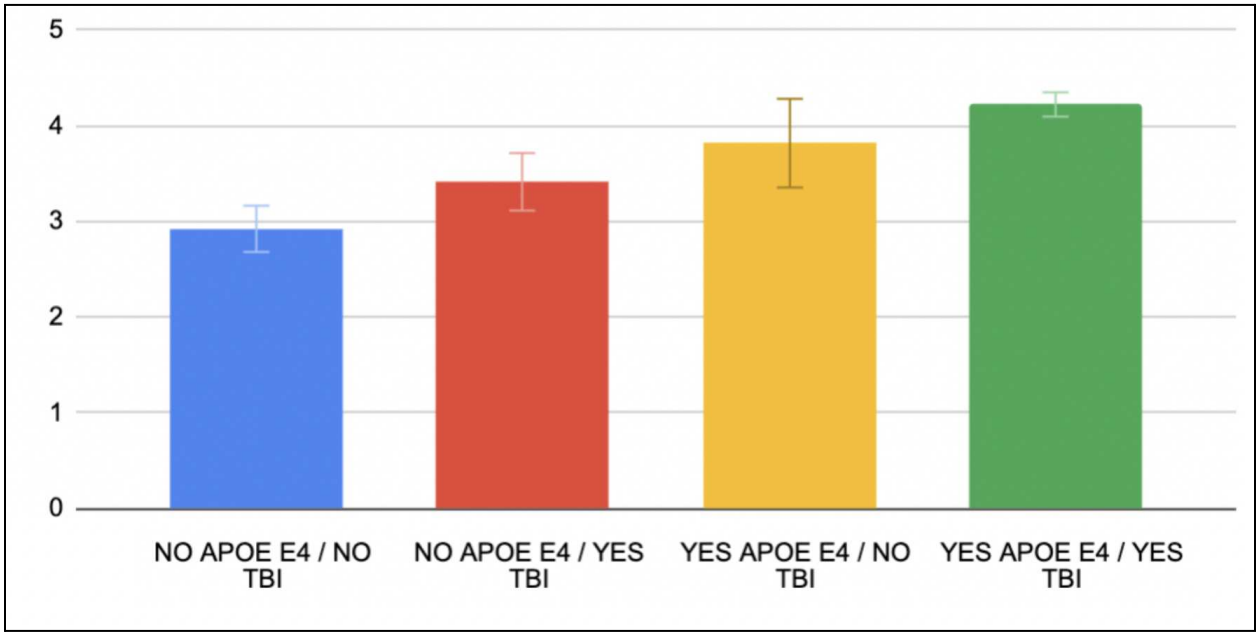

Figure 14. *Bar Graph (Braak Score vs. Genotype & Environment Combination). The height of the bar graph indicates mean Braak scores, and the error bars represent the standard error of the mean for each environment/genotype combination.*

When viewing the combinatory effects of genetics and epigenetics on Braak scores, Figure 14 demonstrates the four different combinations. Individuals without the APOE ε4 allele and those that have not experienced a TBI in their lifetime have an average Braak score of 2.92. Individuals without the APOE ε4 allele but who have experienced a TBI in their lifetime have an average Braak score of 3.41. Individuals with the APOE ε4 allele but who have not experienced

a TBI in their lifetime have an average Braak score of 3.82. Lastly, individuals with the APOE ε4 allele who have experienced a TBI in their lifetime have an average Braak score of 4.22.

There is a statistically significant difference for the following conditions:

1. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.0216, t-test ≈ -2.38)

There is no statistically significant difference for the following conditions:

1. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.0903, t-test ≈ -1.73)
2. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.238, t-test ≈ -1.20)
3. “NO APOE ε4 / NO TBI” and “NO APOE ε4 / YES TBI” (p-value ≈ 0.209, t-test ≈ -1.27)
4. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.524, t-test ≈ -0.642)
5. “YES APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.540, t-test ≈ -0.625)

ii) CERAD Score vs. Genotype & Environment Combination

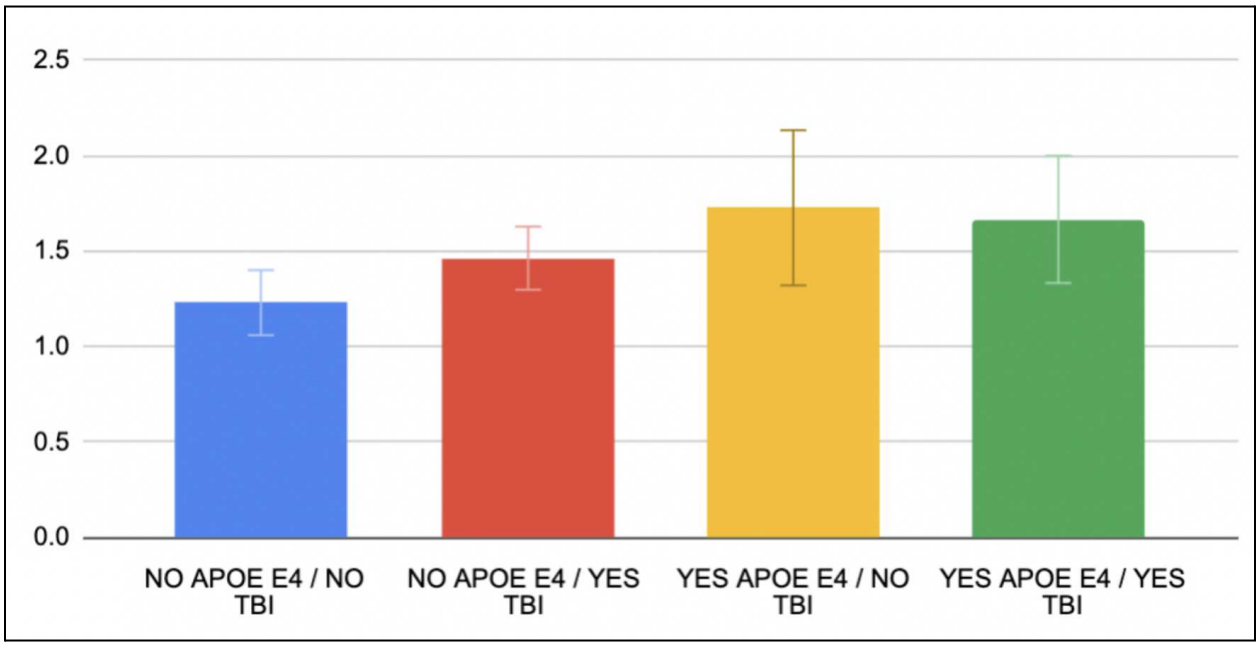

Figure 15. *Bar Graph (CERAD Score vs. Genotype/Environment Combination). The height of the bar graph indicates mean CERAD scores, and the error bars represent the standard error of the mean for each environment/genotype combination.*

Similar to the Braak score bar graphs, Figure 15 shows four combinations that can be created for epigenetic and genetic factors with respect to determining CERAD scores. Individuals without the APOE ε4 allele and those that have not experienced a TBI in their lifetime have an average CERAD score of 1.23. Individuals without the APOE ε4 allele but who have experienced a TBI in their lifetime have an average CERAD score of 1.46. Individuals with the ε4 allele who have not experienced a TBI in their lifetime have an average CERAD score of ε4 allele and have experienced a TBI in their lifetime have an average CERAD score of 1.67.

There is no statistically significant difference for the following conditions:

1. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.204, t-test ≈ -1.29)

2. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.268, t-test ≈ -1.12)

3. “NO APOE ε4 / NO TBI” and “NO APOE ε4 / YES TBI” (p-value ≈ 0.328, t-test ≈ -0.984)

4. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.490, t-test ≈ -0.696)

5. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.599, t-test ≈ -0.530)

6. “YES APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.912, t-test ≈ 0.112)

iii) NIA Reagan Score vs. Genotype & Environment Combination

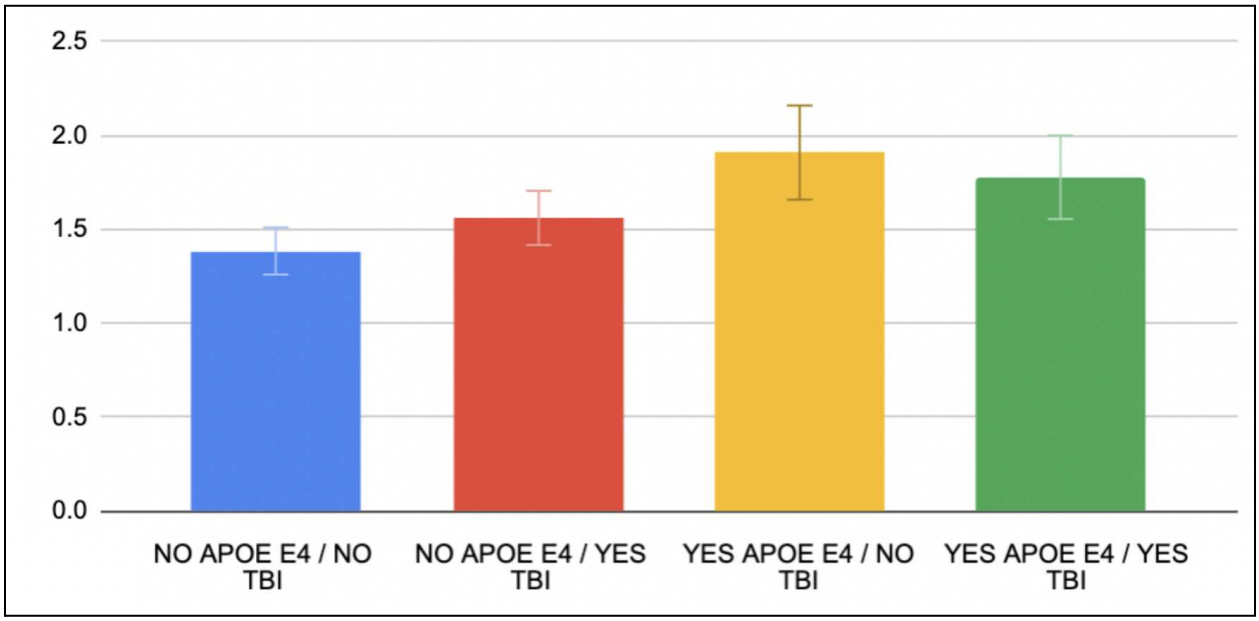

Figure 16. *Bar Graph (NIA Reagan Score vs. Genotype/Environment Combination). The height of the bar graph indicates mean NIA Reagan scores, and the error bars represent the standard error of the mean for each environment/genotype combination.*

Again, for Figure 16, four combinations can be created for epigenetic and genetic factors with respect to determining NIA Reagan scores. Individuals without the APOE ε4 allele and those that have not experienced a TBI in their lifetime have an average NIA Reagan score of

1.38. Individuals without the APOE ε4 allele but who have experienced a TBI in their lifetime have an average NIA Reagan score of 1.56. Individuals with the APOE ε4 allele who have not experienced a TBI in their lifetime have an average NIA Reagan score of 1.91. Lastly, individuals with the APOE ε4 allele and who have experienced a TBI in their lifetime have an average NIA Reagan score of 1.77.

There is no statistically significant difference for the following conditions:

1. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.0584, t-test ≈ -1.94)
2. “NO APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.170, t-test ≈ -1.39)
3. “NO APOE ε4 / NO TBI” and “NO APOE ε4 / YES TBI” (p-value ≈ 0.361, t-test ≈ -0.920)
4. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / NO TBI” (p-value ≈ 0.263, t-test ≈ -1.13)
5. “NO APOE ε4 / YES TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.509, t-test ≈ -0.665)
6. “YES APOE ε4 / NO TBI” and “YES APOE ε4 / YES TBI” (p-value ≈ 0.706, t-test ≈ 0.383)

e) Alzheimer's Disease Diagnosis vs. Genotype & Environment Combination

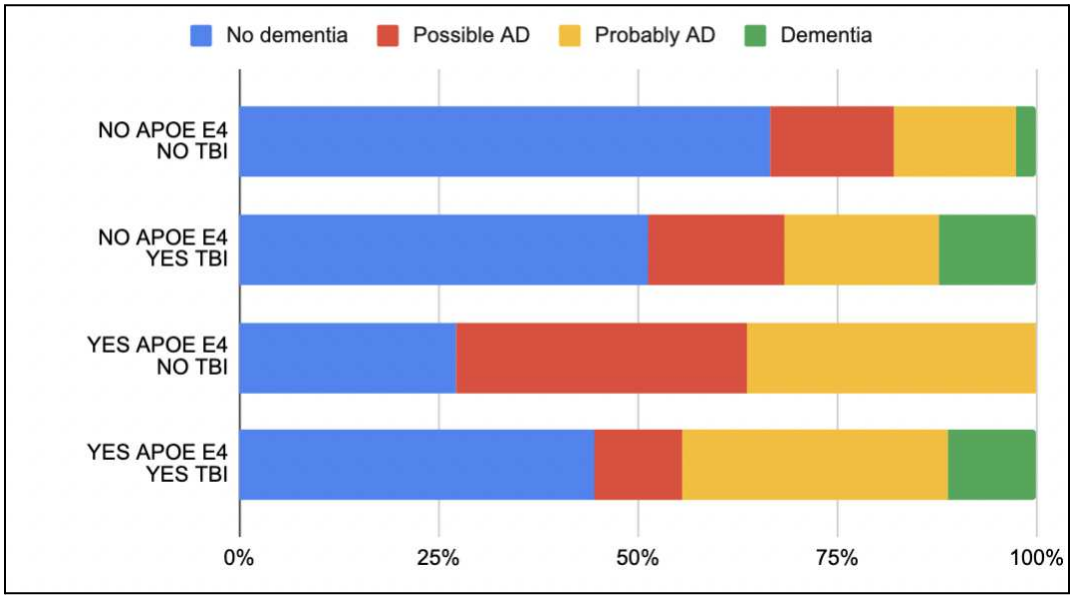

Figure 17. *100% stacked bar chart (Alzheimer's disease diagnosis vs. Genotype & Environment Combination). The color-coded region represents the percent (%) of individuals falling under the highlighted category.*

In Figure 17, the four combinations of genotype and epigenetics were compared based on an individual's NINCDS-ARDA Alzheimer's disease diagnosis. For the "NO APOE / NO TBI" group, the diagnosis was: 66.6% no dementia, 15.4% possible AD, 15.4% probable AD, 2.6% dementia. For the "NO APOE ε4 / YES TBI" group, the diagnosis was: 51.2% no dementia, 17.1% possible AD, 19.5% probable AD, 12.2% dementia. For the "YES APOE ε4 / NO TBI," the diagnosis was: 27.2% no dementia, 36.4% possible AD, and 36.4% probable AD. Lastly, for the "YES APOE ε4 / YES TBI", the diagnosis was: 44.4% no dementia, 11.1% possible AD, 33.3% probable AD, and 11.1% dementia.

f) 2 x 2 factorial design graphs

i) APOE ε4 presence/absence & TBI occurrence on Braak scores

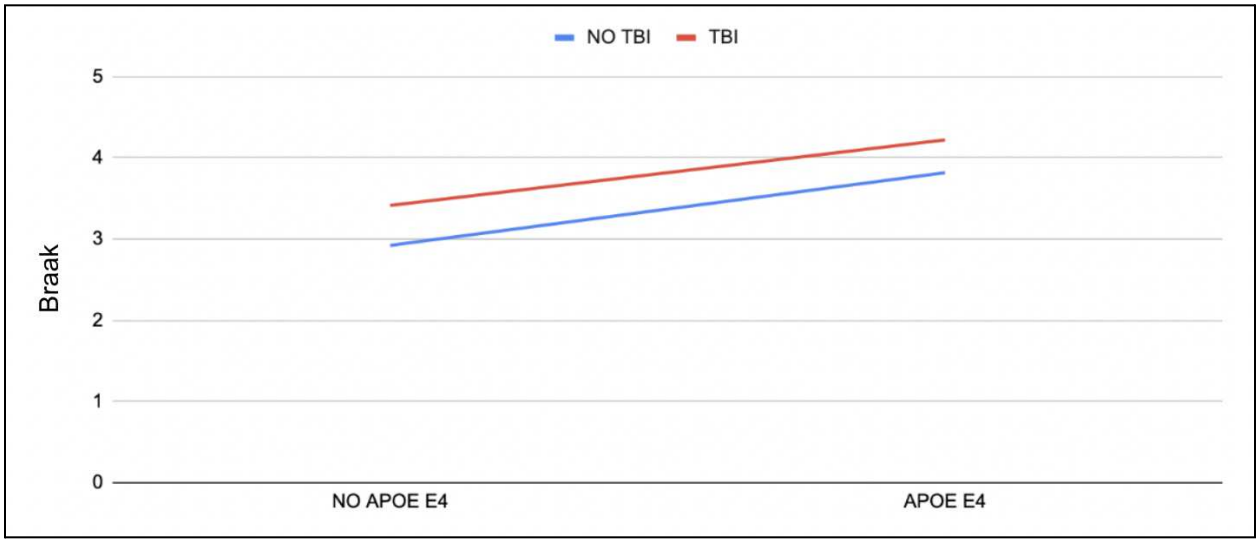

Figure 18. *2 x 2 factorial design graph using two variables: APOE ε4 presence/absence and TBI presence/absence on Braak scores.*

As seen in Figure 18, the main effect of the APOE ε4 allele on Braak scores was statistically significant [$F(1, 1) = 374.587,\ p = 0.03286$)]. The main effect of TBI was not statistically significant [$F(1, 1) = 103.733,\ p = 0.06229$]. However, an interactive effect was not present, as seen by the non-intersecting trend lines.

ii) APOE ε4 presence/absence & TBI occurrence on CERAD scores

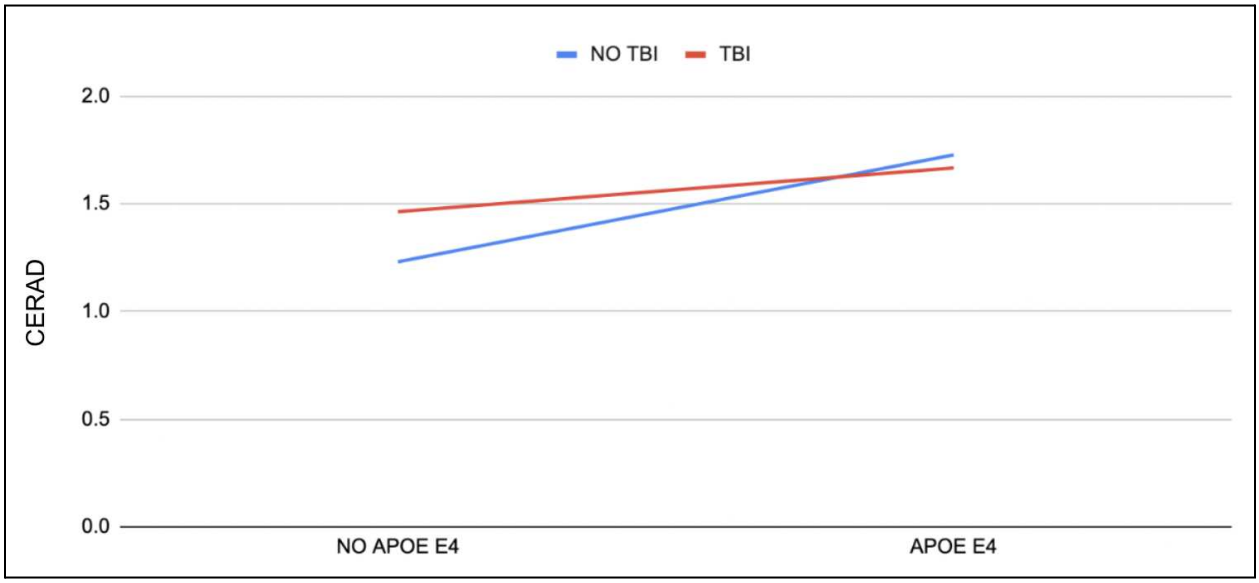

Figure 19. *2 x 2 factorial design graph using two variables: APOE ε4 presence/absence & TBI occurrence on CERAD scores*

As seen in Figure 19, the main effect of the APOE ε4 allele on CERAD scores was not significant [$F(1, 1) = 5.695,\ p = 0.253$]. The main effect of TBI on CERAD scores was also not significant [$F(1, 1) = 0.345,\ p = 0.662$)]. However, an interactive effect exists, as evidenced by the intersecting line trend lines.

iii) APOE ε4 presence/absence & TBI occurrence on NIA Reagan scores

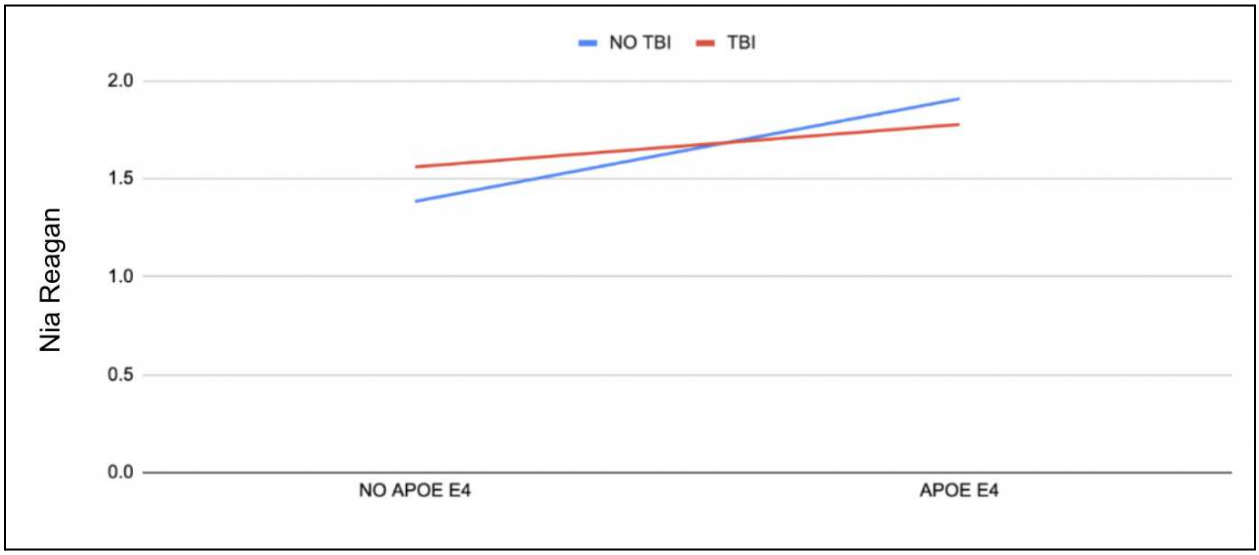

Figure 20. *2 x 2 factorial design graph using two variables: APOE ε4 presence/absence and TBI presence/absence on NIA Reagan scores*

As seen in Figure 20, the main effect of the APOE ε4 allele on NIA Reagan was not statistically significant [$F(1, 1) = 5.808,\ p = 0.2504$)]. The main effect of TBI on NIA Reagan was also not statistically significant [$F(1, 1) = 0.0214,\ p = 0.9076$)]. However, an interaction could be observed as evidenced by the intersecting line trend lines.

f) Other Factors

i) Education Years on NIA Reagan Scores

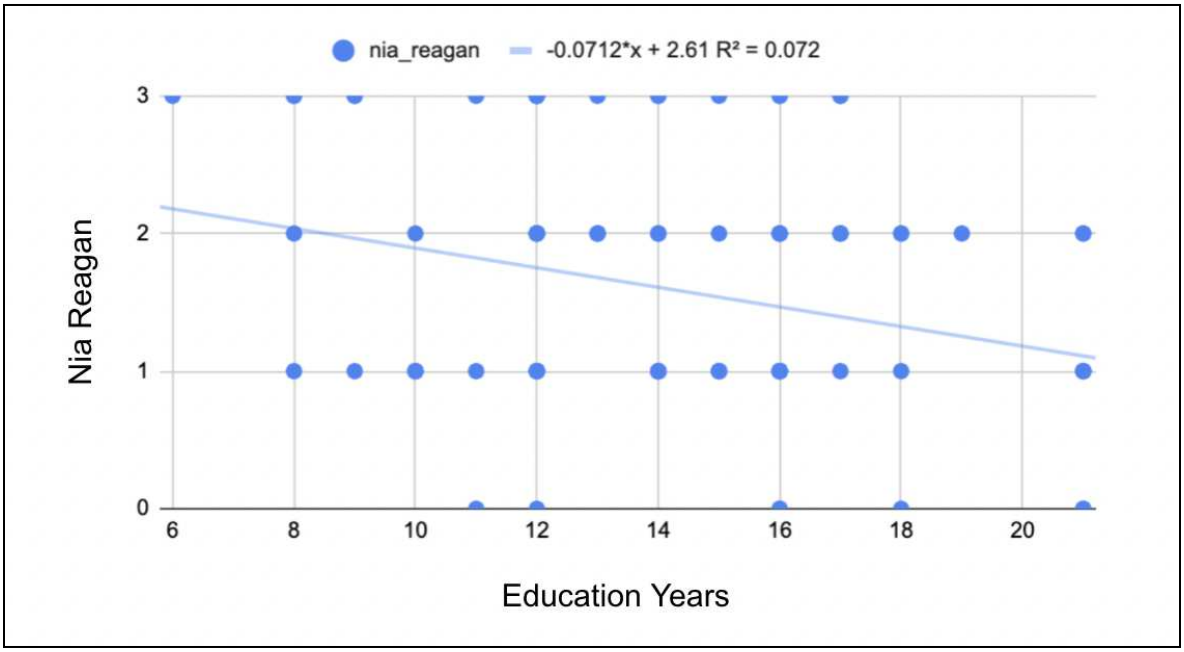

Figure 21. *Scatter Plot for Education Years vs. NIA Reagan Scores. Points are representative of patients in the data set with regards to these two variables.*

From Figure 21, the graph produced a line of best-fit between education years and NIA Reagan scores of $y = -0.712x + 2.61$, and the Pearson's Correlation Coefficient (R^2) was -0.072, showing a weak negative association.

ii) Age on NIA Reagan Scores

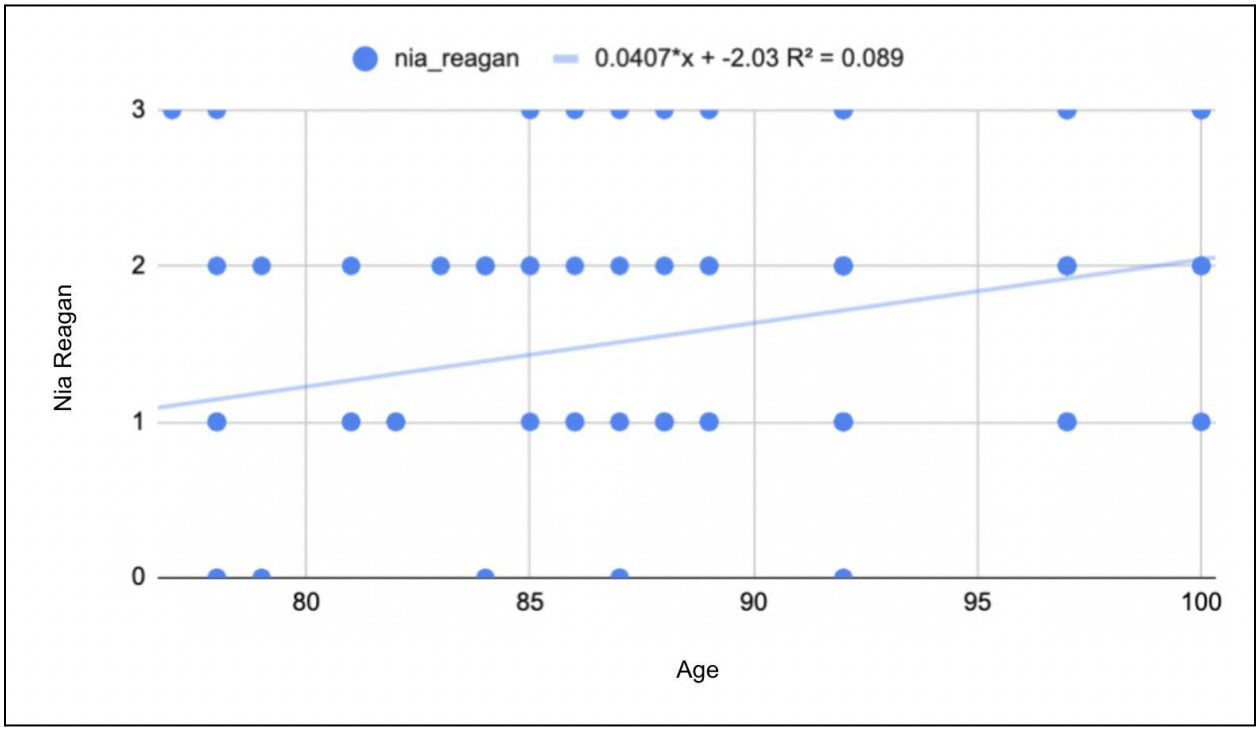

Figure 22. *Scatter Plot for Age vs. NIA Reagan Scores. Points are representative of patients in the data set with regards to these two variables.*

From Figure 22, the graph produced a line of best fit between age and NIA Reagan scores of $y = -0.0407x - 2.03$, and the Pearson's Coefficient (R^2) was 0.089, showing a weak positive association.

iii) Braak on NIA Reagan Scores

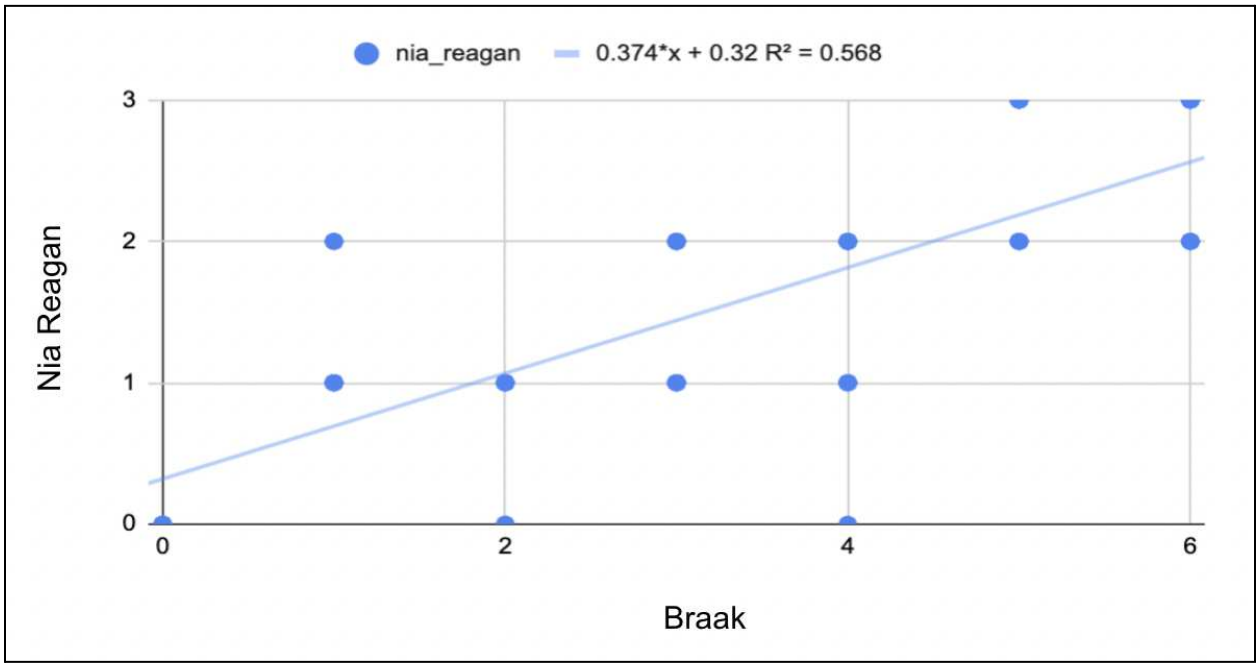

Figure 23. *Scatter Plot for Braak vs. NIA Reagan Scores. Points are representative of patients in the data set with regards to these two variables.*

From Figure 23, the graph produced a line of best fit between Braak and NIA Reagan scores of $y = 0.374x + 0.32$, and the Pearson's Coefficient (R^2) was 0.568, showing a moderately positive association.

iv) CERAD on NIA Reagan Scores

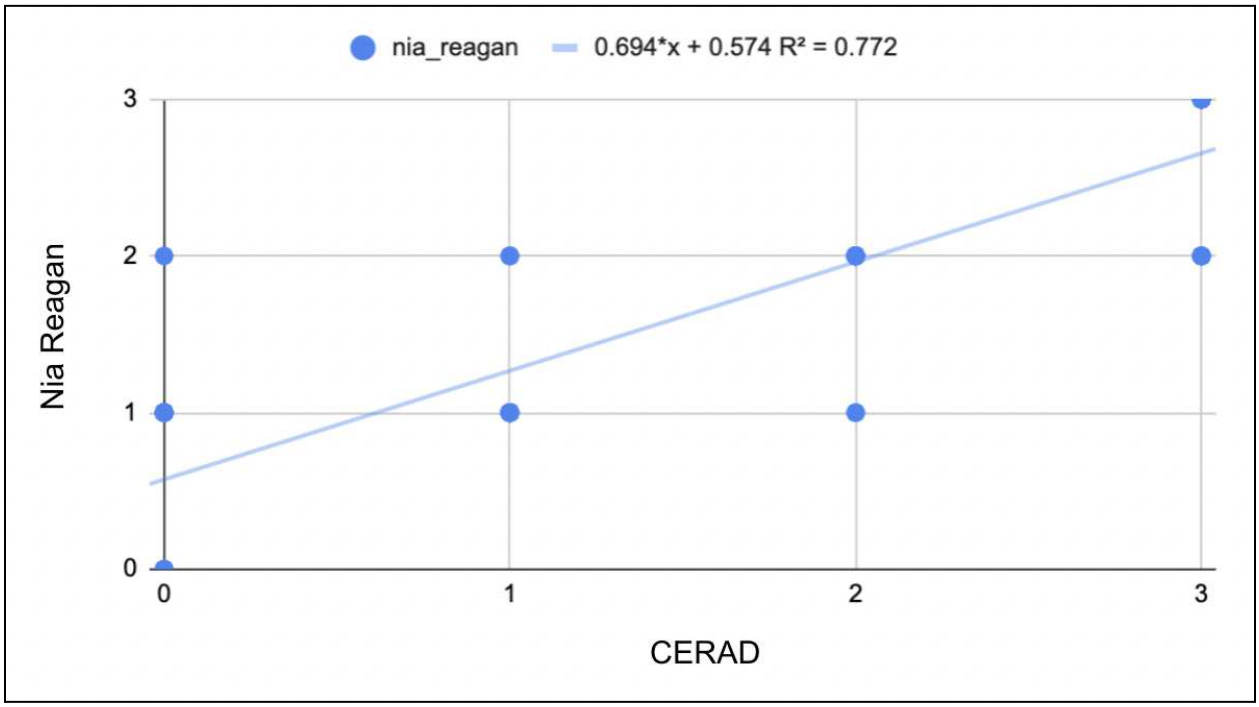

Figure 24. *Scatter Plot for CERAD vs. NIA Reagan Scores. Points are representative of patients in the data set with regards to these two variables.*

From Figure 24, the graph produced a line of best fit between CERAD and NIA Reagan scores of $y = 0.694 + 0.574$, and the Pearson's Coefficient (R^2) was 0.772, showing a strong positive association.

Discussion

For this paper, the main interest and purpose of the investigation were to observe the combined effects of TBI and APOE ε4 genotype on risk for dementia and AD through their neural biomarkers. The main findings were tested based on the neural biomarker metrics and found that the APOE ε4 allele influenced non-AD dementia and AD. On the other hand, TBIs played a less significant role, influencing mostly non-AD dementia. It could be deduced that these two variables interacted to a certain extent in increasing the likelihood of AD diagnosis, with TBIs playing a more considerable role in NFT formation and the same for APOE ε4 in producing plaques. One unexpected finding was that the greatest duration of unconsciousness following TBI did not produce the worst outcomes, as the specific time frame between 10 seconds and 5 minutes did so.

First, it should be noted the APOE ε4 genotype confers an increased risk for non-AD dementia diagnosis, as seen in the chi-squared test. As for AD diagnosis, there is an increase in average neural biomarker scores (Braak and CERAD) for those with the APOE ε4 allele vs. those that do not have the allele; however, the differences were not statistically significant, as shown by the bar graphs comparing the four possible combinations of TBI and genotype. This can be corroborated by the stacked bar chart in Figure 17, as there was significantly greater "possible AD" and "probable AD" diagnoses for those with the allele compared to those without the allele. Lastly, the 2 x 2 factorial design graphs showed the presence of the APOE ε4 allele significantly affected Braak scores. Although it is not certain, APOE ε4 seems to play a significant role in this relationship, and it may be due to apolipoprotein's role in lipid metabolism, as evidenced by MIT researchers Dr. Li-Huei Tsi and Dr. Susan Linquist. The team

used pluripotent stem cells (from skin cells of those carrying the APOE ε3 or APOE ε4 allele), which later differentiated into brain cells known as astrocytes. APOE ε4 astrocytes showed a considerable buildup of neutral lipids and cholesterol compared to APOE ε3. The APOE ε4 astrocytes also amassed droplets of triglyceride, which had more unsaturated fatty acid chains than normal. With lipid homeostasis being disrupted, essential processes such as trafficking (intracellular/vesicular) are compromised (Trafton, 2021). Adding on, apolipoprotein is strongly associated with senile plaques and may potentially cause cerebral amyloid angiopathy (CAA). Noticeably amongst 50-59 year-olds, Aβ deposition in the form of senile plaques was around 40.7% for APOE ε4 carriers while a mere 8.2% for non-carriers (Liu et al., 2013). With the brain being enriched with lipids, any form of imbalance or disruption in its homeostasis may contribute to neurodegeneration, whether in the form of AD or non-AD dementia (Kao et al., 2020).

The role of TBI on dementia and AD is at least two-fold. First, a history of TBI increases the risk for non-AD dementia but not AD. Intriguingly, TBIs confer a greater influence on non-AD dementia, as there was a statistically significant difference in the 100% bar chart comparing the diagnoses for those with and without TBIs. This may result from TBIs contributing to NFTPD (neurofibrillary tangle predominant dementia) because the shearing forces from the multitude of rotational movements during a TBI release tau protein to form NFTs. NFTPDs are associated with later onset and limited cognitive impairment as well as being limited to allosteric and limbic regions of the brain, as evidenced by its high density in those particular regions and the limited progression to the neocortex. Due to the varying symptoms of NFTPDs and AD stemming from the specific biomarker production, TBIs may not be a reliable

risk factor for predicting AD diagnosis. These results support Umeå University's findings, which found that the risk of dementia diagnosis increased by four to six times for those with a TBI (Nordström & Nordström, 2018). Another interesting observation was that TBIs only seemed to have conferred an effect when an individual did not have the APOE ε4 allele. Elaborating upon this point, in Figure 15, the average CERAD score for the conditions: "YES APOE ε4 / NO TBI" and "YES APOE ε4 / YES TBI" were nearly identical. In contrast, the average CERAD score for the conditions: "NO APOE ε4 / NO TBI" and "NO APOE ε4 / YES TBI" showed a marked increase in the average score for those with TBI. This pattern held for the NIA Reagan scores. These results can be extrapolated to align with Guo et al.'s MIRAGE study from 2006, which found that the influence of head injury on the risk for AD was greater when the individual lacked the APOE ε4 allele compared to when they had either one or two of the alleles. However, Braak scores increased with a TBI or APOE ε4 presence, meaning that the two risk factors play a similar role in developing NFTs. As a result, it may be hypothesized that CERAD scores are more closely linked to NIA Reagan scores (predicting the likelihood of AD), meaning that plaques play a more significant role in AD diagnosis than NFTs. In fact, this can be corroborated by Figures 23 and 24, where CERAD scores were more strongly associated with NIA Reagan than Braak scores, as seen by the higher correlation coefficient.

The four-bar graphs for the Braak score from Figure 14 also verify the combinatory roles of both genes and the environment on AD when comparing the conditions: "NO APOE ε4 / NO TBI" and "YES APOE ε4 / YES TBI," as these were the only two groups which had a statistical significance. This indicates a combinatory effect for the two risk factors on AD diagnosis as both risk factors were required to produce a statistically significant difference in NFT formation from

the possible combinations. Having solely the APOE ε4 allele or solely the TBI incident was not enough to produce statistically significant differences in those individuals without any of the aforementioned risk factors. Additionally, the interactive effects of these risk factors could be seen for CERAD and NIA Reagan scores from the 2 x 2 factorial results. This is thoroughly corroborated by Mayeux et al.'s findings, where it was stated that TBIs increase the development of AD; however, it is only through a synergistic relationship between APOE ε4.

Next, there were intriguing results based on the duration of unconsciousness during a TBI event. While the presence of TBI alone did not increase the risk for AD and its neural biomarkers, when observing the duration of unconsciousness for the TBIs, the effects were such that the worst outcomes (in terms of pathology) were observed when participants experienced 5 minutes or less of unconsciousness, specifically between 10 seconds and 5 minutes. Modern literature would suggest that the greater the length of the unconsciousness, the more severe the TBI and hence a more extensive network of NFTs. However, it should be noted that this could have been confounded by participant variability within the time duration groups through variables such as gender, age, and years of education. For instance, it should be noted that the 10-second to 5-minute duration groups had considerably the lowest average age at which the first TBI occurred at 34.3 years, whereas the less than 10-second group had an average age of 50.8 and the greater than the five-minute group has an average of 35.2 years. Therefore, it can be assumed that the younger the age of TBI, the greater the likelihood neural biomarkers have a chance of developing. Supporting this hypothesis, a study found that TBIs in early to mid-life had a 2-4 times greater likelihood of developing dementia later on (Neurol, 2013). Furthermore, the number of years of education for the 10-second to the 5-minute group was 15.2 years, while

the less than the 10-second group was 14.0 years, and the greater than the 5-minute group was 13.3 years. Thus, this demonstrated that the amount of formal education one receives in their lifetime does not affect biomarker formation after a TBI simply because the more educated individuals had similar levels of neuritic plaques and NFTs. However, how they recover may be a different story and can be further investigated in the future as greater cognitive reserves developed through learning may influence recovery (Johns Hopkins Medicine, 2014). This can be supported by Figure 21, as those with a greater education tended to be less likely to develop AD. Corroborating these findings, the 10-second to the 5-minute group had the highest average age at which the data was collected at 91.4 years, while the less than 10-second group and greater than the 5-minute group had an average age of 88.9 and 88.6 years on average, respectively. With dementia generally being an age-dependent illness, dementia rates increase exponentially after 90 years (James & Schneider, 2010). So much so that the APOE ε4 allele risk factor seems to diminish with an increase in age (Bullain & Corrada, 2013). As a result, this could partly explain why NIA Reagan scores were seemingly inexplicably high for this particular duration of unconsciousness.

However, caution must be observed when commenting on this matter as the data set was limited to only binary options: "yes" or "no" for the question of if the participants had the APOE ε4 allele. This meant that it could not be ascertained whether individuals who responded "yes" had one allele (carrier) or two alleles of APOE ε4, drastically affecting their risk for AD and age of onset. For instance, it was found that people who are heterozygous for the APOE ε4 genotype had a 47% frequency rate for AD and a mean age of clinical onset of 76 years. On the other hand, people homozygous for the APOE ε4 genotype had a 91% frequency rate for AD and a

mean age of clinical onset of 68 years (Neurol, 2013). Another area of uncertainty that emerges is that TBIs often go undiagnosed; however, this data set only looked into diagnosed cases of TBIs, meaning that TBIs could have played a more significant role in AD as the data analysis could not demonstrate this. Lastly, the data set could not specify the specific regions of these traumatic brain injuries, which could have drastically impacted the outcome of diagnoses as different aspects of behavior are localized to specific brain regions. Despite these deficiencies in the data, they bolster the theory put forth at the beginning of the paper as the effect of APOE ε4 may have been over-valued, whereas the impact of TBIs was possibly less appreciated, showing that TBIs may have a profound impact on AD diagnosis.

Conclusion

Based on the data, APOE ε4 seems to place greater leverage on AD diagnosis, as seen by the statistically significant differences in metrics between individuals with and without this allele. However, TBIs play an essential role in combination by contributing to producing NFTs on top of the neuritic plaques, both of which are hallmark AD traits. However, their role is less significant than the allele as they contribute more towards NFTPDs through the clumping of tau protein from shearing forces to produce NFTs, suggesting that plaques play a more significant role in AD diagnosis. Thus, APOE ε4 may outweigh the effects of TBIs simply due to the biomarker they affect. However, it should be noted that this data set could not take into account numerous factors such as undiagnosed TBIs, which understated the effect of this risk factor while also not distinguishing between APOE ε4 carriers vs. those homozygous for the allele, which possibly overstates this risk factor's effect. From the results and scientific literature, TBIs only seemed to serve an additive effect with the presence of APOE ε4, indicating a synergistic interaction of genes and the environment. This is just a microcosm of the nature vs. nurture debate, so to expand upon this work, other genes and environmental factors may be investigated for mediating this relationship. For instance, other genetic risk factors include PSEN-1 (chromosome 14) and PSEN-2 (chromosome 1), code for presenilin one and presenilin two, subunits of gamma-secretase. Thus, mutations here affect the location where gamma-secretase cuts, resulting in amyloid plaques of different sizes that can clump up better due to their insolubility (Osmosis, 2016). For environmental factors, individuals' exposure to repeated head trauma (such as contact sports athletes) may be potential subjects of interest, as the number of TBIs from this data set (ranging from 0-3) does not drastically affect AD risk but this may not be the case for insidious brain damage. Furthermore, vascular risk factors such as hypertension and

diabetes that affect cerebral blood flow may be studied to deduce their leverage on AD diagnosis. Moreover, socioeconomic factors and upbringing may also play an unrecognized role, which can have measurable impacts on variables such as diet, stress levels, and physical activity. Further inquisition will be fundamental as it will enable for greater allocation of resources to those with AD-specific risk factors so that they can be treated promptly before they find out too late. Therefore, a better understanding of the formation of NFTs and plaques and their significance in Alzheimer's diagnosis can optimize treatment mechanisms regarding the biological pathways they target. Although investigating numerous risk factors may seem overly comprehensive and time-consuming, it will be necessary to combat this pernicious disease.

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