

CHOLESTEROL: A POSSIBLE MEDIATOR OF APOE RISK FOR ALZHEIMER'S
DISEASE

by

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for the Alzheimer's Disease Neuroimaging Initiative*

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ABSTRACT

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Under the Supervision of Professor Ira Driscoll

Despite the well-established link between the $\epsilon 4$ allele of the apolipoprotein E (APOE) gene and AD, the underlying mechanisms that mediate the risk of developing AD remain elusive. Literature on the role of APOE in cholesterol metabolism suggests that blood cholesterol may be a key factor in the development of AD pathology. Current study aims to investigate whether total cholesterol differs by APOE status and whether this relationship is predictive of AD diagnosis and its biomarkers. Baseline total cholesterol, APOE status, AD diagnosis, global cognitive function, brain A β , plasma A $\beta 40$ and A $\beta 42$, and cerebrospinal fluid (CSF) A β , tau, and phosphorylated tau were collected between 2004 and 2019 from a sample of 3,099 older adults in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Higher total cholesterol was associated with AD incidence, higher A β in the brain, lower CSF A β , lower plasma A $\beta 40$ and A $\beta 42$, poorer cognitive function, and the APOE $\epsilon 4$ allele. Survival analysis showed that $\epsilon 4$ carriers had highest hazard rates of AD, but that cholesterol did not alter time to AD diagnosis. The findings suggest that total cholesterol in later life is predictive of AD and related biomarkers, but further research is needed to elucidate how blood cholesterol may relate to APOE risk for AD.

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INTRODUCTION

Alzheimer's disease (AD) is the most prevalent form of dementia and currently the sixth leading cause of death in the United States.¹ This neurodegenerative disease is characterized by progressive cognitive decline, hallmarked by memory loss, alterations in behavior and personality, and impaired daily functioning.¹⁻³ An estimated 5.8 million Americans currently have AD, and one in three older adults die with AD or another form of dementia.¹ With the increasing population of older adults, the number of individuals affected by Alzheimer's disease is projected to rise exponentially.¹

Despite decades of research aimed at developing effective medications to treat AD, the only medications currently available are for symptom management rather than significant disease modification or reversal.^{4,5} While research efforts have slowly been uncovering potential methods for earlier diagnosis, such as neuroimaging,^{4,7} analysis of biomarkers in cerebrospinal fluid,^{4,7} and neuropsychological evaluation,⁸ this approach does not guarantee disease reversal or delay of disease progression given the current treatment options.^{4,7} Furthermore, Alzheimer's pathology begins years prior to symptom presentation,^{4,7} suggesting that these earlier diagnostic approaches are perhaps not early enough.

With the currently incurable nature of this disease and the increasing rate of AD incidence, a shift in focus from disease reversal to disease prevention may prove more successful in combating AD. Recent findings suggest that vascular risk factors may be crucially linked to AD pathology, and that some of the AD-related pathology may be preventable.^{5,7,9-13} Further investigation into modifiable lifestyle factors related to vascular health, such as our food choices, may reveal that the development of this devastating disease is not entirely outside of our control, but perhaps preventable through simple behavioral changes. A potentially promising focus using

this research approach targets APOE, the best known genetic risk factor for AD, and its interaction with cholesterol as a possible modifiable mechanism by which APOE confers risk.³

Neuropathology of Alzheimer's Disease

Amyloid plaques and neurofibrillary tangles are the neuropathological hallmarks of AD.^{2,3,14,15} Amyloid pathology seems to occur first in the brain, which then increases tau production and initiates tau pathology, in turn further enhancing β -amyloid toxicity through a pathological feedback loop.^{16,17} Amyloid pathology involves an imbalance between the production and clearance of β -amyloid ($A\beta$) peptides in the brain, which leads to an accumulation of $A\beta$ plaques between neurons.^{2,3,14,15} Cleavage of amyloid precursor protein (APP) leads to the formation of $A\beta$ peptide, which consists of 40 to 43 amino acids.^{3,14,15} $A\beta$ plaques have a central core of a 4-kD protein consisting of a beta-pleated sheet configuration which is arranged radially.¹⁵ Abnormally formed neuronal processes, either dendrites or axons, surround the core.¹⁵ Buildup of $A\beta$ plaques injures synapses and blocks transmission of information between neurons, such that continued accumulation leads to increasing interference with neuronal signaling, ultimately resulting in neurodegeneration, neuroinflammation, and impairments in cognitive function.³

Neurofibrillary tangles are abnormal fibrous formations which form in the cytoplasm of the cell body of pyramidal neurons, typically surrounding the nucleus and extending toward apical dendrites.¹⁵ These growths consist of hyperphosphorylated tau, a microtubule-associated protein involved with intracellular transport.^{3,14,15} Neurofibrillary tangles occur in pairs and form a winding helix structure, roughly 10 nanometers in diameter.¹⁵ Neurofibrillary tangles are typically distributed heavily in CA1 and subicular hippocampal regions, layer II of the entorhinal

cortex, amygdala, and layers II, V, and VI of the neocortex.¹⁵ The distribution of neurofibrillary tangles tends to correlate with dementia severity and duration, although their presence may not reflect AD in the absence of amyloid pathology.¹⁵

The neuropathology underlying AD can manifest with a variety of cognitive, behavioral, and psychological symptoms. Primary symptoms include memory loss, impaired judgment, a tendency for forgetfulness and wandering, difficulty with language, reading, writing, and mental arithmetic, disorganized thoughts, and changes in personality and mood such as becoming aggressive, withdrawn, or anxious.¹⁸ Individuals may fail to recognize family and friends, struggle to cope with novel situations and environments, and have difficulty accomplishing daily tasks such as managing finances, getting dressed, and preparing meals.¹⁸ More severe psychological symptoms can include impulsive behavior as well as delusions, hallucinations, and paranoia.¹⁸ Physical changes may also arise, particularly in later stages of AD, such as trouble swallowing, weight loss, skin infections, and loss of bowel and bladder control.¹⁸ Cognitive impairments, particularly in the domains of executive function, attention, visuospatial ability, episodic memory, semantic memory, working memory, perceptual speed, word finding ability, and instrumental activities of daily living, are all common in patients with AD.¹⁹⁻²¹

Apolipoprotein E, A β Clearance, and Cholesterol

Apolipoprotein E (ApoE) is a multifunctional protein produced in the brain by astrocytes and microglia, and produced in the periphery by the liver and macrophages.³ ApoE is a major cholesterol carrier - monitors cholesterol metabolism in the brain and blood, and responds to stress and injury in the brain to aid in neuronal repair.^{3,22-25} This protein also contributes to lipid homeostasis throughout the body by regulating lipid transport between cells and tissues.³ In the

brain, ApoE is involved in the clearance of A β , which is a crucial step in preventing A β accumulation.^{3,22} ApoE does this by binding to soluble A β deposits and facilitating uptake by neurons and glia through various surface receptors, such as low-density lipoprotein receptor-related protein 1 (LRP1), low-density lipoprotein receptor (LDLR), and heparan sulphate proteoglycan (HSPG).^{3,22} Alternative methods of A β clearance facilitated by ApoE include drainage of deposits into interstitial fluid, drainage through the blood-brain barrier, and degradation via neprilysin and insulin-degrading enzyme (IDE).³

The APOE gene which codes for ApoE is the best known genetic risk factor for AD; the ϵ 4 allele confers highest risk of AD, the ϵ 2 allele seems to act as a protective factor against AD, and the ϵ 3 allele appears neutral.^{3,26} APOE ϵ 2, ϵ 3, and ϵ 4 alleles code for specific isoforms of ApoE (E2, E3, and E4, respectively), which in turn dictate its expression and function.²³ The ApoE isoforms differ by amino acid changes at positions 112 and 158.^{23,27} The E3 isoform encoded by the ϵ 3 allele is the most common isoform of ApoE, containing a cysteine at position 112 and an arginine at position 158.^{23,17} The E2 isoform contains a cysteine at both positions 112 and 158, while E4 contains arginine at positions 112 and 158.^{23,27}

The replacement of arginine with cysteine in the E2 isoform changes the positive ion potential of the receptor-binding domain of ApoE.^{23,24} This decreases the affinity of E2 Apolipoprotein E for the LDL receptor to approximately 1% of the binding capacity of the E3 isoform, resulting in a substantially greater amount of circulating E2 protein.²³ The replacement of cysteine with arginine in the E4 isoform, in contrast, contributes to a significantly lower amount of E4 protein than the E3 isoform due to a greater binding affinity of ApoE.^{23,24,27-30} This variation in ApoE levels across APOE genotype presumably influences the ability of ApoE to engage in clearance of A β accumulation in the brain.^{3,23,29} Greater levels of A β deposits are

associated with the $\epsilon 4$ allele, indicating that fewer available ApoE leads to reduced clearance of A β and therefore greater A β buildup in the brain.^{3,23,29}

Each APOE allele also seems to correspond to differing blood cholesterol levels due to varying ability to uptake and regulate cholesterol based on ApoE availability.²³ APOE $\epsilon 2$ allele carriers tend to have lower LDL and total cholesterol levels.^{3,23,28,29,31} This is likely due to more available ApoE and a low binding affinity of the E2 isoform for the ApoE receptor, which leads to lower levels of exogenous cholesterol entering the liver via the ApoE receptor-mediated pathway and therefore greater uptake of LDL cholesterol in cells.^{3,23,28-30} Cholesterol that enters the liver seems more likely to be eliminated from the body rather than transported into the blood in $\epsilon 2$ carriers.^{28,30} Conversely, those with the $\epsilon 4$ allele tend to have higher LDL and total cholesterol levels.^{3,23,28-31} The lower levels of ApoE and higher binding affinity of the E4 protein for the ApoE receptor are associated with rapid internalization of cholesterol in the liver, after which very low-density lipoprotein (VLDL) particles tend to be released into the bloodstream, contributing to elevated LDL and total blood cholesterol levels.^{3,23,28-30} ApoE $\epsilon 3$ is associated with slightly less ApoE than $\epsilon 2$, and cholesterol levels somewhere between those of $\epsilon 2$ and $\epsilon 4$ carriers.^{3,23,28,31} APOE genetic risk for AD may therefore be due to differences in ApoE availability and binding affinity, which influence cholesterol metabolism, blood cholesterol levels, and variation in ability to engage in A β clearance.

APOE Genotype, Dietary Cholesterol, Blood Cholesterol, and Alzheimer's Disease

Consumption of cholesterol, saturated fat, and trans fat seem to relate strongly to blood cholesterol levels. Lower cholesterol and saturated and trans fat consumption are associated with lower blood cholesterol levels, while higher consumption is associated with higher blood

cholesterol levels.^{28,29,32-49} However, there is some debate regarding the exact nature of this relationship due to conflicting findings. For instance, egg and meat consumption are not consistently associated with elevated blood cholesterol,^{50,55} and one explanation offered by the literature for varied findings is individual variation in responsiveness to cholesterol consumption.⁵⁰⁻⁵⁵ Additionally, several of the studies reporting conflicting findings have potential methodological problems, such as analysis of consumption of certain high-cholesterol foods without taking into account overall cholesterol intake,^{50-53,55} assuming that lack of statistical significance of blood cholesterol changes in response to dietary changes implies lack of clinical significance,⁵⁴ and examining cholesterol levels immediately after eating specific foods without accounting for long-term effects of wholistic dietary patterns.⁵⁴ Much of this debate also comes from the analysis of cholesterol in the context of risk for cardiovascular disease rather than AD.^{50-52,55} In this context, the ratio of LDL to HDL cholesterol is debated as being more clinically meaningful than simply the values of LDL, HDL, and total cholesterol levels.^{50-52,55} APOE genotype is also not consistently included in these studies.^{35,37-42,45,46,48-55} Given that each APOE allele is associated with notable differences in ability to monitor cholesterol metabolism as discussed previously, cholesterol consumption may affect blood cholesterol levels differently depending on APOE genotype.

In a sample of 563 blood donors (419 males and 144 females, aged 18-59), Boerwinkle and Utermann (1988) investigated the mechanism by which each APOE allele may influence cholesterol metabolism, based on prior findings suggesting that the $\epsilon 2$ allele lowers total blood cholesterol and the $\epsilon 4$ allele raises total blood cholesterol levels.²⁸ The authors found that $\epsilon 2/2$ carriers had the lowest total cholesterol levels on average, followed by $\epsilon 2/3$ carriers, $\epsilon 3/3$ carriers, and $\epsilon 3/4$ and $\epsilon 4/4$ carriers.²⁸ Furthermore, $\epsilon 2/2$ carriers had highest levels of ApoE,

followed by $\epsilon 2/3$ carriers, $\epsilon 3/3$ carriers, $\epsilon 3/4$ carriers, and $\epsilon 4/4$ carriers.²⁸ They also calculated the overall effect of each APOE allele on total cholesterol and ApoE levels in the blood using computational formulas established in prior work by Falconer, Sing, Davignon, Boerwinkle, Visvikis, Welsh, Steinmetz, and Hanash.²⁸ The authors defined the average effect of an allele as “the expected phenotypic deviation, from the population mean, of an individual carrying that allele.”²⁸ It was determined that the $\epsilon 2$ allele raised ApoE levels by 0.95 mg/dl, while the $\epsilon 4$ allele lowered ApoE levels by 0.19 mg/dl.²⁸ The effect of each APOE allele on total cholesterol levels seems to be the opposite of their effect on ApoE – the $\epsilon 2$ allele lowers cholesterol by 14.2 mg/dl, the $\epsilon 3$ allele has no effect on cholesterol, and the $\epsilon 4$ allele raises the level of cholesterol by 7.09 mg/dl.²⁸

Based on their results,²⁸ Boerwinkle and Utermann proposed that the $\epsilon 2$ allele lowers LDL and total cholesterol due to the low binding affinity of this allele for the ApoE receptor, resulting in less exogenous cholesterol (referring to cholesterol not produced by the body, such as cholesterol consumed from food) from entering the liver via the ApoE receptor-mediated pathway.²⁸ The $\epsilon 4$ allele, in contrast, is proposed to increase LDL and total cholesterol because of its higher binding affinity for the ApoE receptor and the presumed greater likelihood of internalization of cholesterol into the liver.²⁸ Based on this theory, diets low in cholesterol may reduce blood cholesterol levels, particularly for those with the $\epsilon 4$ allele who are potentially more susceptible to the deleterious effect of dietary cholesterol on blood cholesterol levels.²⁸

Clinical studies support this model; switching between a high- and low-cholesterol diet leads to notable, consistent changes in LDL and total cholesterol as a function of APOE genotype.⁵⁶⁻⁵⁸ For instance, in a sample of 29 healthy males aged 46-57, a relationship was found between dietary and blood cholesterol based on APOE genotype.⁵⁷ Participants first consumed a

low fat, low cholesterol (210 mg/day) diet for six weeks, followed by a low fat, high cholesterol (880 mg/day) diet for five weeks.⁵⁷ High cholesterol consumption was associated with significant increases in total and LDL blood cholesterol compared to baseline levels in $\epsilon 3$ and $\epsilon 4$ carriers, but not in $\epsilon 2$ carriers.⁵⁷ Following the high cholesterol diet, total and LDL cholesterol increased nonsignificantly in $\epsilon 2$ carriers by 6% and 7%, and significantly in $\epsilon 3$ and $\epsilon 4$ carriers by 9% and 11%, and 14% and 18%, respectively.⁵⁷ These findings suggest that APOE $\epsilon 3$ and especially $\epsilon 4$ carriers are more prone to elevated blood cholesterol levels compared to $\epsilon 2$ carriers as a consequence of cholesterol consumption.⁵⁷

Lopez-Miranda and colleagues (1994) studied whether switching from a high- to low-cholesterol diet can lead to improvements in blood cholesterol levels.⁵⁸ A total of 128 participants (83 males, 47 females) with $\epsilon 3/2$, $\epsilon 3/3$, or $\epsilon 3/4$ APOE genotypes were first placed on a high-fat, high-cholesterol diet modeled after the average American diet for 4-24 weeks, with 39% of calories from fat, 15% of calories from saturated fat, and 306-510 mg of cholesterol per day.⁵⁸ This was followed by 4-24 weeks on a low-fat, low-cholesterol diet consisting of 26% of calories from fat, 8% of calories from saturated fat, and 178-230 mg of daily cholesterol.⁵⁸ The intervention resulted in significant decreases in total, LDL, and HDL cholesterol in all participants, with longer duration of low-fat, low-cholesterol diet corresponding to greater cholesterol reduction.⁵⁸ Upon stratification by APOE genotype, male $\epsilon 4$, $\epsilon 3$, and $\epsilon 2$ carriers showed a decrease in total cholesterol by 43, 29, and 30 mg/dl, respectively.⁵⁸ Female $\epsilon 4$ and $\epsilon 3$ carriers had a decrease in total cholesterol by 16 and 19 mg/dl, respectively.⁵⁸ Female $\epsilon 2$ carriers had a decreased in total cholesterol by 10 mg/dl, but this decrease did not reach statistical significance.⁵⁸ Male $\epsilon 4$ carriers had higher LDL cholesterol levels than male $\epsilon 3$ carriers in the high-fat, high-cholesterol diet phase.⁵⁸ Male $\epsilon 4$ carriers also displayed greater decrease in LDL

cholesterol (-41 mg/dl, 23% change) than $\epsilon 3$ (-21 mg/dl, 14% change) and $\epsilon 2$ carriers (-23 mg/dl, 16% change) after the low-fat, low-cholesterol diet intervention.⁵⁸ Females exhibited a similar pattern, with $\epsilon 4$ carriers showing decrease in LDL cholesterol by 20 mg/dl (11% change) compared to a 11 mg/dl (7% change) decrease in $\epsilon 3$ carriers.⁵⁸ However, the changes in females did not maintain statistical significance.⁵⁸

The literature suggests that while blood cholesterol seems to be influenced by dietary cholesterol in most individuals regardless of APOE genotype, $\epsilon 4$ allele carriers tend to show greatest sensitivity to cholesterol consumption, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.^{57,58} The interaction between APOE and cholesterol may provide a glimpse into the mechanism by which APOE confers risk for AD. Perhaps differential risk for developing AD according to APOE genotype is modulated by cholesterol intake, which in turn influences blood cholesterol levels. Those with at least one $\epsilon 4$ allele who consume little to no dietary cholesterol may therefore not be at higher AD risk compared to their $\epsilon 2$ and $\epsilon 3$ counterparts. Furthermore, perhaps anyone, regardless of APOE genotype, is at greater risk of AD if they have high blood cholesterol.

Observational studies support the idea that consumption of cholesterol may influence genetic risk for AD conferred by APOE. Populations such as the Indians, Africans, South Asians, Chinese, and Malaysians eating their traditional, non-Westernized diets have very low rates of AD, regardless of APOE genotype.^{28,59} Furthermore, Nigerian blacks reportedly have the highest observed frequency of APOE $\epsilon 4$ allele, yet they have some of the lowest rates of AD as well as unusually low levels of LDL and total cholesterol compared to age-matched individuals from populations consuming Western diets.⁶⁰⁻⁶³ While factors such as less access to healthcare, underdiagnosis of AD, and higher mortality rates early in life may influence the cross-cultural findings, the low-cholesterol diets customarily consumed by these non-Western populations have

been implicated as the primary factor underlying the markedly low rates of AD.^{28,60-63} Conversely, rates of AD are expected to rise as these countries continue to develop and incorporate dietary patterns more similar to those consumed by Westernized regions.^{56,59}

Cholesterol and Alzheimer's Disease: Findings in Nonhuman Animals

Nonhuman animal research suggests that A β accumulation may be associated with high dietary and blood cholesterol, which could shed some light on the link between cholesterol, APOE, and AD risk. Refolo et al. (2000) used double transgenic PSAPP mice, produced through crossing hemizygous APP transgenic mice (from the Tg2576 line with human APP Swe transgene) with homozygous PS1 mice (from the H8.9 line with human PS1M146L transgene).⁶³ ⁶⁶ These double transgenic PSAPP mice develop amyloid deposits by 10-12 weeks of age, and this amyloidosis is used to simulate development of AD in humans.⁶³ To investigate the influence of cholesterol consumption on amyloidosis in this model, 16 male mice aged 5 weeks were split into two dietary groups.⁶³ Nine mice were fed a high-cholesterol diet (5% of calories from cholesterol, 10% from fat, 2.0% sodium cholate, and 5.2 kcal/gram).⁶³ The remaining seven mice were fed a low-cholesterol diet (0.005% of calories from cholesterol, 10% from fat, and 3.6 kcal/gram).⁶³

After seven weeks, the high-cholesterol group had significantly higher total cholesterol levels in plasma and the central nervous system (CNS) than the group fed the low-cholesterol diet (plasma: 201.66 ± 21.5 , 99.852 ± 9.00 mg/dl, respectively; CNS: 16.68 ± 0.490 , 14.76 ± 0.387 mg/dl, respectively).⁶³ Levels of A β in the brain were also significantly different between the two groups, with 347.3 ± 41.30 versus 171.1 ± 23.98 pmol/g of tissue in the high-cholesterol and low-cholesterol groups, respectively.⁶³ Furthermore, a positive correlation was

found between plasma and both CNS cholesterol and A β accumulation ($r = 0.918$, $r = 0.787$, respectively).⁶³ Further analysis of the levels of APP holoprotein, sAPP α , β -CTFs, and PS1 to investigate the underlying mechanism revealed that hypercholesterolemia significantly increased β -CTFs and decreased sAPP α levels, leading to altered APP processing and increased amyloidogenic processing.⁶³ The authors proposed that minimizing cholesterol intake may modulate risk of developing AD.⁶³

Refolo et al., (2001) then assessed the role of cholesterol-lowering medication on A β accumulation in the same transgenic model of amyloidosis.⁶⁴ Fifteen mice (9 males, 6 females) aged 8 weeks were fed the same low-cholesterol diet used in the prior study.⁵⁴ Eight mice (5 males, 3 females) were given the cholesterol-lowering drug BM15.766 (4-(2-[1-(4-chlorocinnamyl) piperazine-4-yl]ethyl]benzoic acid), which inhibits 7-dehydrocholesterol- Δ 7-reductase (the enzyme which catalyzes the last step of cholesterol biosynthesis).⁶⁴ This drug is also permeable to the blood-brain barrier, and therefore potentially capable of reducing CNS cholesterol.⁶⁴ After 5 weeks of treatment, administration of BM15.766 lowered plasma cholesterol threefold compared to the control group, and it significantly reduced CNS cholesterol.⁶⁴ Furthermore, BM15.766 reduced levels of A β 1-40 and A β 1-42 twofold compared to the control group.⁶⁴ Regarding the effects of decreased cholesterol on APP processing in the CNS, drug treatment significantly increased sAPP α and decreased both β -CTFs and APP holoprotein levels.⁶⁴ The authors concluded that hypocholesterolemia due to drug treatment caused decreased amyloidogenic processing of APP in the brain of these mice.⁶⁴

Another study by these authors investigated the influence of Lipitor (atorvastatin), an FDA-approved medication commonly prescribed to lower cholesterol in humans, on A β production.⁶⁵ Using the same transgenic mouse model of Alzheimer's amyloidosis as previously

reported, the authors used 16 mice aged 8 weeks, 10 of which received 30mg/kg of Lipitor per day and 6 of which received a vehicle (Strawberry Kool-Aid) for a total of 8 weeks.⁶⁵ Those given Lipitor exhibited a 59% reduction in total cholesterol, but no change in brain cholesterol given the poor blood-brain barrier permeability of this drug.⁶⁵ Despite the lack of reduction in brain cholesterol, the treatment group still showed a 2.5-fold reduction in A β 40 and a twofold reduction in A β 42.⁶⁵ A significant positive correlation between plasma cholesterol and accumulation of A β 40 and A β 42 was also reported ($r = 0.831$ and $r = 0.803$, respectively).⁶⁵

Further investigation of ApoE content and processing in all mice used in the three studies previously described provide additional insight into the underlying mechanisms at play.⁶⁶ Those fed a high-cholesterol diet overexpressed ApoE in the liver and brain through enhanced mRNA transcription as a result of elevated plasma cholesterol levels.⁶⁶ This, in turn, is thought to have caused greater A β production and accumulation compared to mice on the low cholesterol diet.⁶⁶ Similarly, mice treated with BM15.766 exhibited 70% lower levels of ApoE in the liver and brain compared to the control group.⁶⁶ The mice treated with Lipitor exhibited lower ApoE levels in the brain, and lower blood cholesterol and brain amyloidosis without alterations in brain cholesterol levels.⁶⁶ Lipitor seems to exert its effects through changes in ApoE-mediated deposition and clearance of A β , rather than through reduction of A β production.⁶⁶ This suggests that use of statins may delay the onset of amyloidosis and AD through more effective deposition and clearance of A β . Furthermore, reduction in cholesterol consumption may prevent the need for medication to stave off AD by preventing elevated A β production and accumulation (and thus AD neuropathology) in the first place.

More recent work by Moser and Pike (2017) sheds light on how a diet high in saturated fat and sugar may influence genetic risk for AD.⁶⁷ Three-month-old male EFAD transgenic mice,

who were heterozygous for 5xFAD transgenes and homozygous for either human APOE3 or APOE4, were randomly assigned to either the control group fed 10% fat and 7% sucrose or the Western diet group fed 45% fat and 17% sucrose for 12 weeks (n = 7-11 per group).⁶⁷ The control diet resulted in less than 1% increase in body weight in all mice, regardless of genotype.⁶⁷ The Western diet led to significant increases in body weight compared to the control diet by $39 \pm 7.7\%$ in E3FAD mice, and $24 \pm 7.21\%$ in E4FAD mice. These genotypical differences in body weight in the Western diet group did not reach statistical significance.⁶⁷ There were no significant differences in plasma cholesterol or triglycerides regardless of diet group or genotype. There was a significant interaction between APOE genotype and diet, with E4FAD mice fed the high-fat, high-sugar diet exhibiting significantly greater A β deposition compared to controls and E3FAD mice fed the same diet.⁶⁷ E4FAD mice fed the Western diet also had more glial cells and a higher ratio of reactive versus resting glia compared to the other mice.⁶⁷ These findings indicate a gene-environment interaction, in that APOE ϵ 4 carriers may be more susceptible to the influence of diet on AD pathology.⁶⁷

Cholesterol and Alzheimer's Disease: Findings from Humans

Research suggests that AD patients tend to have considerably higher total and LDL cholesterol, as well as lower HDL (“good”) cholesterol levels compared to healthy controls.^{29,56,60-62,68-70} High levels of blood cholesterol are also associated with impairments in global cognition, as well as higher risk of developing AD.⁷¹⁻⁷⁷ For example, Kuo and colleagues (1998) analyzed the brains and blood samples of 64 AD patients and 36 healthy controls to investigate the relationship between cardiovascular risk factors, including APOE genotype, blood cholesterol, ApoE levels, and A β levels in gray matter.⁷¹ Levels of A β N-40 and N-42 were

significantly higher in AD patients, particularly in APOE ϵ 4/4 carriers, compared to the control group.⁷¹ Individuals diagnosed with AD also had significantly higher LDL cholesterol than controls, as well as lower ratios of HDL to VLDL plus LDL cholesterol.⁷¹ Furthermore, significant positive associations between LDL and total cholesterol with A β N-42 were reported in AD patients.⁷¹

Solomon and colleagues (2009) assessed the relationship between blood cholesterol levels and dementia risk in a sample of 9,844 members of the Kaiser Permanente Medical Care Program of Northern California over a period of three decades.⁷⁰ Participants 40-45 years of age at baseline were recruited between 1964 and 1973, during which comprehensive health evaluation was performed including collection of blood samples for total serum cholesterol measurement.⁷⁰ Follow-up assessment was conducted from 1994 through 2007, including recording of dementia diagnosis based on medical records from Kaiser Permanente medical centers.⁷⁰ A total of 469 participants were diagnosed with AD and 127 had vascular dementia.⁷⁰ Total cholesterol at midlife was positively associated with both AD and vascular dementia in late life, with even moderately elevated cholesterol (≥ 220 mg/dl) significantly increasing risk of developing AD three decades later.⁷⁰ This suggests that maintaining low total cholesterol, particularly in midlife, may be crucial for prevention of AD and other forms of dementia later in life.

Ylilauri and colleagues (2017) report results inconsistent with the literature.⁷⁸ In a sample of 2,497 males living in Finland who were dementia-free at baseline, the authors investigated the association between dietary cholesterol and egg consumption (a high-cholesterol food) with blood cholesterol and incident dementia and AD.⁷⁸ Dietary intake was assessed at baseline using a four-day food record.⁷⁸ To track health outcomes, cases of incident dementia and AD diagnosis

were identified through the year 2014 using three national health registers.⁷⁸ To assess the association between cholesterol intake and risk for dementia, participants were divided into four groups based on daily cholesterol intake: less than 331 mg/day, 331-387 mg/day, 388-458 mg/day, and greater than 458 mg/day.⁷⁸ Cholesterol intake was associated with higher total, LDL, and HDL cholesterol and lower triglycerides.⁷⁸ However, neither cholesterol nor egg intake were associated with risk for dementia or AD.⁷⁸ Four years after baseline assessment, the authors also assessed cognitive function in 480 men from the original sample on a battery of standardized neuropsychological tests.⁷⁸ They found that higher egg consumption was associated with better verbal fluency, short term memory, and executive function, as assessed by the Verbal Fluency Test and Trail Making Test.⁷⁸ An additional assessment of APOE genotype in a subset of 1,259 males from the original sample failed to show that APOE ϵ 4 phenotype (defined as those with ϵ 4/4 or ϵ 3/4) modified the relationships between cholesterol or egg consumption and risk for dementia or AD.⁷⁸

However, several methodological problems regarding the design of the study conducted by Ylilauri et al., (2017) should be noted. Dietary intake was assessed only at baseline, so the findings depended not only on the assumption that participants provided accurate accounts of dietary intake at baseline, but also that participants continued eating as reported at baseline for the next four years. Furthermore, the ranges used to divide the participants into four groups based on cholesterol consumption are potentially problematic. Based on decades of prior research, the National Institutes of Health (NIH) established that no more than 200mg of cholesterol should be consumed per day.⁷⁹ Furthermore, studies have found that consuming more than 300 mg per day of cholesterol is too high for optimal health and associated with risk of cardiovascular disease and all-cause mortality in addition to elevated blood cholesterol.^{53,58,80}

Thus, based on these guidelines, Ylilauri et al., (2017) may not have found an association between cholesterol intake and dementia risk due to improper group comparisons, as the ranges of cholesterol consumption for all groups in this study would be considered high according to NIH guidelines and prior research findings.^{53,58,79,80}

Findings from the Chicago Health and Aging Project (Morris et al., 2003) may provide additional insight into these inconsistent findings regarding dietary cholesterol.³³ Dietary pattern was assessed using a food-frequency questionnaire at baseline in 815 participants aged 65 years and older.³³ All individuals were cognitively normal at baseline, and clinical evaluation for AD was performed again at a mean of 3.9 years following baseline assessment.³³ Saturated and trans fat consumption was significantly associated with AD.³³ However, dietary cholesterol was not significantly associated with AD.³³ The authors emphasized prior findings relating saturated and trans fat consumption to blood cholesterol levels, where saturated and trans fat intake tend to increase LDL cholesterol levels potentially more than dietary cholesterol does.³³ Thus, perhaps consumption of saturated and trans fat should be considered in addition to cholesterol intake as an important risk factor for elevated blood cholesterol, particularly when theorizing potential mechanisms underlying the risk for AD in humans.

Several additional studies suggest that dietary and blood cholesterol may not be associated with AD, or that elevated LDL cholesterol may even promote better cognitive function in some cases.⁸¹⁻⁸³ These inconsistent findings in human studies suggest a critical need for further research using strong, evidence-based research methods with the aim of reaching a better understanding of the relationship between diet, blood cholesterol, APOE genotype, and risk for AD. It should also be noted that human research supporting the link between dietary and blood cholesterol with AD may not be due to the same underlying mechanisms as those

established in mice, as several differences have been identified in the type and size of lipid proteins between mice and humans.^{84,85} These lipoproteomic differences may in turn lead to differences in lipoprotein metabolism between the species.^{84,85} Additionally, given that rodents do not develop AD pathology in the absence of experimental manipulation, further investigation using human subjects is crucial to gain an accurate understanding of the relationship between genetic risk and dietary modification of cholesterol and its contribution to the etiology of AD in humans. The existing literature suggests that the relationship between APOE and A β accumulation may be potentially not only modulated but also modifiable by dietary cholesterol intake.^{77,86}

Proposed Study

Despite the accumulating evidence supporting the potential link between cholesterol, APOE genotype, and risk for Alzheimer's disease, the specific mechanism by which APOE confers AD risk remains elusive due to conflicting reports in the literature.^{81,82,96,87,88} It is likely that the discrepant findings are largely due to methodological inconsistencies between studies, emphasizing the need for a more comprehensive investigation. The objective of the current study is to investigate blood cholesterol as a potential mechanism through which APOE genotype confers risk for AD using longitudinal data in a large sample of individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI). ADNI is a widely accessible database including many variables with the purpose of helping researchers further explore factors related to AD, including demographics, neuroimaging data, and clinical biomarkers that are not otherwise easily accessible. This provides a collection of prospective data on thousands of participants, which is a convenient starting point to begin exploring the proposed theory.

In addition to diagnosis of dementia due to AD, ADNI includes diagnosis of Mild Cognitive Impairment (MCI) due to AD and MCI due to other etiology. Although MCI does not always lead to AD, MCI is considered a prodrome of dementia.⁸⁹ Thus, participants diagnosed with MCI due to AD were also included in this study as a separate group from AD, with the expectation that their biomarker values would be somewhere between those of cognitively normal participants and participants diagnosed with AD. The following research aims and hypotheses were explored to replicate prior findings and address the existing gaps in literature.

Aim 1: To investigate the relationship between total blood cholesterol and both diagnostic summary (AD, MCI, and cognitively normal) and related biomarkers.

Hypotheses:

- a) Total blood cholesterol at baseline is expected to be positively associated with AD and MCI diagnosis. Those who are diagnosed with AD are expected to have highest total cholesterol levels, followed by those diagnosed with MCI, and finally those considered cognitively normal.
- b) Total blood cholesterol at baseline is expected to be positively associated with cerebrospinal fluid (CSF) levels of A β , tau, and phosphorylated tau.
- c) Total blood cholesterol at baseline is expected to be positively associated with *in vivo* levels of A β in the brain, assessed using a composite standard uptake value ratio (SUVR) measured by Positron Emission Tomography (PET) scan. (See *Method* for details).
- d) Total blood cholesterol at baseline is expected to be positively associated with plasma A β 40 and A β 42.

- e) Total blood cholesterol at baseline is expected to be negatively associated with global cognitive function as assessed using the Alzheimer's Disease Assessment Scale – Cognitive (ADASCOG), Mini-Mental State Exam (MMSE), Clinical Dementia Rating scale (CDR), and Montreal Cognitive Assessment (MoCA).

Aim 2: To investigate the relationship between APOE genotype and diagnostic summary (AD, MCI, and cognitively normal) and related biomarkers.

Hypotheses:

- a) APOE genotype is expected to be associated with diagnosis of AD and MCI. APOE $\epsilon 4$ carriers are expected to exhibit higher prevalence of AD and MCI, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.
- b) APOE genotype is expected to be associated with CSF levels of A β , tau, and phosphorylated tau. APOE $\epsilon 4$ carriers are expected to have highest levels of A β , tau, and phosphorylated tau, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.
- c) APOE genotype is expected to be associated with *in vivo* levels of A β in the brain measured by PET. APOE $\epsilon 4$ carriers are expected to have highest levels of A β , followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.
- d) APOE genotype is expected to be associated with plasma A $\beta 40$ and A $\beta 42$. APOE $\epsilon 4$ carriers are expected to have highest levels of A $\beta 40$ and A $\beta 42$, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.
- e) APOE genotype is expected to be associated with global cognitive function assessed using ADASCOG, MMSE, CDR, and MoCA. APOE $\epsilon 2$ carriers are expected to exhibit better scores, followed by $\epsilon 3$ carriers, and finally $\epsilon 4$ carriers.

Aim 3: To investigate the relationship between APOE genotype and blood cholesterol.

Hypothesis: Total blood cholesterol at baseline is expected to be associated with APOE genotype. APOE ϵ 4-allele carriers are expected to have highest levels, followed by ϵ 3 carriers, and finally ϵ 2 carriers.

Aim 4: To investigate the incidence of AD using both APOE genotype and blood cholesterol as predictors.

Hypothesis: APOE ϵ 4-allele carriers with higher total cholesterol are expected to have higher incidence of AD, followed by ϵ 3 and ϵ 2 carriers with high cholesterol, and finally individuals with lower total cholesterol, regardless of APOE genotype.

METHODS

Participants

Data from 3,099 individuals from the United States and Canada is available through the Alzheimer's Disease Neuroimaging Initiative (ADNI).⁹⁰ All participants were between ages 53 and 91 at baseline.⁹⁰ Individuals recruited for any phase of ADNI were used, including ADNI 1, ADNI GO, ADNI 2, and ADNI 3, which began in 2004, 2009, 2011, and 2016, respectively.⁹⁰

Materials

Blood samples taken at screening provide APOE genotype, total blood cholesterol, and plasma A β 40 and A β 42.⁹⁰ The Concurrent Medications Log was used to collect medication usage, from which use of statins is retrieved.⁹⁰⁻⁹² Levels of A β , tau, and phosphorylated tau in CSF were collected via lumbar puncture.⁹² Brain levels of A β were assessed with PET scan using

Florbetapir F-18 (AV-45) and by calculating the average standard uptake value ratio (SUVR) in cortical gray matter regions of interest.⁹¹⁻⁹⁴ The composite SUVR consists of frontal, lateral temporal, lateral parietal, and anterior/posterior cingulate cortical regions, using the whole cerebellum as a reference region.⁹¹⁻⁹⁴

ADNI assessed cognitive function across multiple domains using a battery of neuropsychological tests, including assessment of global cognitive function using the Alzheimer's Disease Assessment Scale – Cognitive, Mini-Mental State Exam, Clinical Dementia Rating scale, and Montreal Cognitive Assessment.^{92,95} Physical and neurological exams of all participants are also completed by physicians to assess for neurological symptoms and any changes in health status.⁹² A full list of tests and procedures has been previously published.⁹⁶

Procedure

Participants were recruited between 2004 and 2019 at 57 sites across the United States and Canada.⁹⁰ Upon an initial screening session, all participants provided demographic and medication information and also underwent screening labs, neurological examination, and assessment of vital signs.⁹⁰ Further examination at a later “baseline” appointment involved cognitive assessment, blood samples, collection of medication information, lumbar puncture for willing participants, and completion of a diagnostic summary.⁹⁰

Diagnosis at each visit was classified as one of multiple groups, of which the present study is focusing on Cognitively Normal (CN; control group), MCI due to AD, and dementia due to AD. Diagnosis is based on cognitive function and health status criteria established and previously published by ADNI.⁹⁰⁻⁹² Further reference to these diagnostic groups will be referred to as CN, MCI, and AD, respectively.

Those classified as CN were free of abnormal memory complaints, exhibited normal memory function based on scoring above cutoffs adjusted for education on the Logical Memory II subscale (Delayed Paragraph Recall) from the Wechsler Memory Scale - Revised, scored 24 to 30 on the MMSE, score 0 on the CDR (with a Memory Box score of 0), and showed an “absence of significant impairment in cognitive function or activities of daily living.”⁹² Those diagnosed with MCI presented with a memory complaint verified by a study partner, displayed abnormal memory function based on performance on Logical Memory II (Delayed Paragraph Recall), scored 24 to 30 on MMSE, score 0.5 on the CDR with a Memory Box score of at least 0.5, and exhibited preserved general cognitive and functional performance to not warrant a diagnosis of AD at time of visit.⁹² Finally, those diagnosed with AD had a memory complaint verified by a study partner, had abnormal memory function based on performance on Logical Memory II subscale (Delayed Paragraph Recall), scored 20 to 26 on MMSE, scored 0.5 or 1.0 on CDR, and met criteria for probable AD based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA).⁹² Ongoing follow-up of all participants includes continued cognitive and neurological assessments, diagnostic summary, PET amyloid imaging, and collection of CSF, plasma, and medication information.⁹⁰

Statistical Analysis

Aim 1: To investigate the relationship between total blood cholesterol and both diagnostic summary (AD, MCI, and CN) and related biomarkers.

- a) Total blood cholesterol at baseline is expected to be positively associated with AD and MCI diagnosis. Those diagnosed with AD are expected to have highest total*

cholesterol levels, followed by those diagnosed with MCI, and finally those considered CN.

A generalized linear mixed model (GLMM) was used to analyze the association between baseline levels of total cholesterol with diagnostic summary (n = 1,763) at screening, baseline, and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. Sex and age were included as covariates in this model.

b) Total blood cholesterol at baseline is expected to be positively associated with CSF levels of A β , tau, and phosphorylated tau.

Three separate GLMMs were used to analyze the associations between baseline total cholesterol and CSF levels of A β , tau, and phosphorylated tau (n = 1,141) at baseline and months 6, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, 108, and 120. Sex and age were included as covariates in these models.

c) Total blood cholesterol at baseline is expected to be positively associated with levels of A β in the brain measured by PET scan.

GLMM was used to analyze the association between baseline total cholesterol and levels of A β in the brain measured by AV-45 composite SUVR via PET scan (n = 977) at baseline and months 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. Sex and age were included as covariates in this model.

d) Total blood cholesterol at baseline is expected to be positively associated with plasma A β 40 and A β 42.

Two separate GLMMs were used to analyze the associations between baseline total cholesterol and plasma levels of A β 40 and A β 42 (n = 716) at baseline and months 6, 12, 18, 24, 36, and 48. Sex and age were included as covariates in these models.

e) Total blood cholesterol at baseline is expected to be negatively associated with global cognitive function as assessed using ADASCOG, MMSE, CDR, and MoCA.

GLMM was used to analyze the association between baseline total cholesterol and total score on ADASCOG (n = 1,597) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the associations between baseline total cholesterol and total score on MMSE (n = 1,892) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the associations between baseline total cholesterol and global score on CDR (n = 1,879) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the associations between baseline total cholesterol and total score on MoCA (n = 1,087) at baseline and months 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. Sex and age were included as covariates in these models.

Aim 2: To investigate the relationship between APOE genotype and diagnostic summary (AD, MCI, and CN) and related biomarkers.

a) APOE genotype is expected to be associated with diagnosis of AD and MCI.

APOE $\epsilon 4$ carriers are expected to exhibit higher prevalence of AD and MCI, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.

GLMM was used to analyze the relationship between APOE genotype and diagnosis (n = 2,048) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. Sex, age, and use of cholesterol-lowering medication were included as covariates in this model.

b) APOE genotype is expected to be associated with CSF levels of $A\beta$, tau, and phosphorylated tau. APOE $\epsilon 4$ carriers are expected to have highest levels of $A\beta$, tau, and phosphorylated tau, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers.

Three separate GLMMs were used to analyze the relationships between APOE genotype and CSF levels of A β , tau, and phosphorylated tau (n = 1,255) at baseline and months 6, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, 108, and 120. Sex, age, and use of cholesterol-lowering medication were included as covariates in this model.

- c) APOE genotype is expected to be associated with in vivo levels of A β in the brain measured by PET. APOE ϵ 4 carriers are expected to have highest levels of A β , followed by ϵ 3 and finally ϵ 2 carriers.*

GLMM was used to analyze the association between APOE genotype and total level of A β in the brain measured by AV-45 composite SUVR via PET scan (n = 1,137) at baseline and months 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. Sex, age, and use of cholesterol-lowering medication were included as covariates in this model.

- d) APOE genotype is expected to be associated with plasma A β 40 and A β 42. APOE ϵ 4 carriers are expected to have highest levels of A β 40 and A β 42, followed by ϵ 3 and finally ϵ 2 carriers.*

Two separate GLMMs were used to analyze the relationship between APOE genotype and plasma levels of A β 40 and A β 42 (n = 732) at baseline and months 6, 12, 18, 24, 36, and 48. Sex, age, and use of cholesterol-lowering medication were included as covariates in these models.

- e) APOE genotype is expected to be associated with global cognitive function assessed using ADASCOG, MMSE, CDR, and MoCA. APOE ϵ 2 carriers are expected to exhibit better scores, followed by ϵ 3 carriers, and finally ϵ 4 carriers.*

GLMM was used to analyze the relationship between APOE genotype and total score on ADASCOG (n = 1,841) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between APOE genotype and

total score on MMSE (n = 2,048) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between APOE genotype and global score on CDR (n = 2,048) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between APOE genotype and total score on MoCA (n = 1,316) at baseline and months 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. Sex, age, and use of cholesterol-lowering medication were included as covariates in these models.

Follow-Up Analysis for Aims 1 and 2

a) CSF Biomarkers and Diagnosis

GLMM was used to analyze the relationship between diagnosis and CSF levels of A β (n = 1,255) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, and 120. GLMM was used to analyze the relationship between diagnosis and CSF levels of tau (n = 1,255) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, and 120. GLMM was used to analyze the relationship between diagnosis and CSF levels of phosphorylated tau (n = 1,255) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, and 120. Sex and age were also included as covariates in these models.

b) Plasma Biomarkers and Diagnosis

GLMM was used to analyze the relationship between diagnosis and plasma levels of A β 40 (n = 732) at baseline and months 6, 12, 18, 24, 36, and 48. GLMM was used to analyze the relationship between diagnosis and plasma levels of A β 42 (n = 732) at baseline and months 6, 12, 18, 24, 36, and 48. Sex and age were also included as covariates in these models.

c) Brain A β and Diagnosis

GLMM was used to analyze the relationship between diagnosis and levels of A β in the brain (n = 1,210) at baseline and months 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. Sex and age were also included as covariates in this model.

d) Global Cognitive Function and Diagnosis

GLMM was used to analyze the relationship between diagnosis and ADASCOG score (n = 2,124) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between diagnosis and MMSE score (n = 2,541) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between diagnosis and MoCA score (n = 1,597) at baseline and months 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. GLMM was used to analyze the relationship between diagnosis and CDR score (n = 2,531) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156. Sex and age were also included as covariates in these models.

Aim 3: To investigate the relationship between APOE genotype and blood cholesterol.

Total blood cholesterol at baseline is expected to be associated with APOE genotype.

APOE ϵ 4-allele carriers are expected to have highest levels, followed by ϵ 3 carriers, and finally ϵ 2 carriers.

Two generalized linear models (GLMs) were used to analyze the association between baseline total cholesterol and APOE genotype (n = 1,586). The first used the three-group APOE categorization, while the second used the six-group APOE categorization. Two additional GLMs were then performed to include sex, age, and cholesterol-lowering medication as covariates in the relationship between baseline total cholesterol and APOE status.

Aim 4: To investigate the incidence of AD using both APOE genotype and blood cholesterol as predictors.

APOE $\epsilon 4$ -allele carriers with high total cholesterol are expected to have higher incidence of AD, followed by $\epsilon 3$ and $\epsilon 2$ carriers with high cholesterol, and finally individuals with lower total cholesterol, regardless of APOE genotype.

Cox Proportional Hazards model was used to assess the hazard ratios for incidence of AD over time using APOE genotype and total cholesterol as predictors ($n = 1,021$) at baseline and months 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, and 156.

Analyses involving APOE genotype (Aims 2, 3, and 4) were performed twice, first by stratifying APOE genotype into six separate genotypes ($\epsilon 2/2$, $\epsilon 2/3$, $\epsilon 2/4$, $\epsilon 3/3$, $\epsilon 3/4$, $\epsilon 4/4$). APOE genotypes were then stratified into three separate groups based on differential cholesterol metabolism found in previous studies. APOE $\epsilon 2$ carriers are denoted by $\epsilon 2/2$, $\epsilon 2/3$, and $\epsilon 2/4$ genotype, $\epsilon 3$ carriers include those with $\epsilon 3/3$ genotype, and $\epsilon 4$ carriers consist of participants with $\epsilon 3/4$ and $\epsilon 4/4$ genotype.⁶⁰

Use of statins or other cholesterol-lowering medication may lower blood cholesterol,⁹⁷ potentially interfering with risk for AD. This medication was therefore used as a covariate in analyses involving APOE genotype and total blood cholesterol, as well as APOE genotype and AD incidence and its biomarkers, respectively.

Data Preparation and Statistical Procedure

ADNI data was accessed via the “ADNIMERGE” package in the statistical software RStudio. Formation of dataframes for each test was done using RStudio. A total of 24 separate dataframes were created with relevant variables for each separate test in Aims 1 through 4 –

including eight dataframes for Aim 1, eight for Aim 2, seven for follow-up GLMMs related to Aims 1 and 2, one for Aim 3, and one for Aim 4.

Aims 1 and 2: Prior to running each GLMM, distributions of the dependent variable of each model were determined in RStudio. SAS was used to execute each GLMM in Aims 1 and 2. Despite checking distributions in RStudio prior to running GLMMs, multiple GLMMs were performed for each model using all possible distributions available pertaining to each dependent variable (depending on whether the dependent variable was discrete or continuous) to ensure that the correct distribution was selected. Akaike's Information Criterion (AIC) values and residual plots were also used to assess model fit and check for overdispersion. The model with the lowest AIC value and smallest standardized deviance residual was chosen for each GLMM.

Aim 3: Two ANOVAs were initially performed in SAS for each genotype grouping prior to including additional covariates in ANCOVA models. Homogeneity of variances using Levene's test and normality of residuals were assessed for both ANOVAs to ensure that the assumptions were met. The assumption of homogeneity of variances was not met, possibly due to unequal sizes of genotype groups. Thus, generalized linear models were used instead of ANOVA and ANCOVA to handle these differences between groups.

Aim 4: Survival analysis using Cox Proportional Hazards to model time to diagnosis of AD was additionally completed in SAS. Time to diagnosis was first assessed using only baseline total cholesterol and APOE status as predictors. Another Cox Proportional Hazards was then performed using cholesterol-lowering medication, sex, and age as predictors. A final model was tested to check for an interaction between total cholesterol and APOE status, after which the best-fitting model was selected based on fit statistics, including -2 Log Likelihood, Akaike Information Criterion, and Schwarz Bayesian Information Criterion.

Diagnostic tests were conducted to check assumptions of this model. The log of the negative log of survival probability was plotted using each categorical predictor (APOE genotype, sex, and use of cholesterol-lowering medication) to check that the assumption of proportional hazards was met. Martingale residuals were plotted to assess functional form of continuous predictors by checking observed versus expected number of events for total blood cholesterol and age. Each model was also repeated without identified outliers to assess their potential influence on the results.

Given the *a priori* hypotheses, significance was set at $p < 0.05$.

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org.

RESULTS

Demographics

Sample demographics at baseline based on APOE status and total cholesterol are provided in *Table 1* and *Table 2*. Approximately half of this sample is male (52%), with mean baseline age of 72.49 (SD = 7.56; range = 53–91). This sample is predominantly white (91%) and non-Hispanic (96%). Most participants have at least a high school education (97%), and

many participants completed graduate-level education (43%) compared to college (23%), some college (19%), and high school (11%) only. The majority of this sample was married at enrollment (75%) and retired (76%). One third of participants were considered cognitively normal at enrollment (33%), 14% were diagnosed with dementia due to AD, 2% had dementia due to other etiology, 35% were diagnosed with MCI due to AD, and 2% presented with MCI due to other etiology. A total of 12% of participants were current smokers at enrollment, while 16% were former smokers. A total of 1,411 (46%) have cardiovascular disease, of which 744 (53%) were prescribed cholesterol-lowering medication. Very few have a history of alcohol (3%) and drug (1%) abuse, respectively, and 25% have been diagnosed with a psychiatric disorder.

Demographics by APOE status and baseline total cholesterol are presented in *Table 1* and *Table 2*. Missing values for participants who provided APOE status include: 2 for age, 6 for race, 15 for ethnicity, 3 for education, 8 for marital status, 30 for employment status, 2 for smoking, 2 for CVD, and 2 for psychiatric history. Missing values for participants who provided baseline total cholesterol include: 68 for race, 72 for ethnicity, 65 for education, 12 for marital status, 33 for employment status, and 8 for smoking, CVD, alcohol abuse, drug abuse, and psychiatric history. Of the 2,044 participants who were genotyped for APOE status, 9.6% were $\epsilon 2$ carriers, 46.5% were $\epsilon 3$ carriers, and 43.8% were $\epsilon 4$ carriers. The estimated global frequency of each APOE allele is approximately 7-8% for the $\epsilon 2$ allele, 78-79% for the $\epsilon 3$ allele, and 14% for the $\epsilon 4$ allele.^{3,98} Thus, despite the smaller number of $\epsilon 2$ carriers compared to $\epsilon 3$ and $\epsilon 4$ carriers in this sample, the amount of $\epsilon 2$ carriers is similar to the estimated frequency of the allele and is therefore a representative sample of the population. However, the number of $\epsilon 4$ carriers is unexpectedly higher than the percentage observed in the general population.

Characteristic	APOE Status			p-value
	ε2 carriers N (%)	ε3 carriers N (%)	ε4 carriers N (%)	
Sex				
Male	101 (51.27)	505 (53.1)	488 (54.46)	< 0.0001
Female	96 (48.73)	446 (46.9)	408 (45.54)	
Age at baseline				
53-59	1 (0.51)	36 (3.79)	38 (4.24)	< 0.0001
60-69	50 (25.38)	238 (25.03)	277 (30.92)	
70-79	104 (52.79)	456 (47.95)	452 (50.45)	
80-89	40 (20.30)	216 (22.71)	128 (14.29)	
90-91	2 (1.02)	4 (0.42)	0 (0)	
Race				
American Indian or Alaskan Native	0 (0)	3 (0.32)	1 (0.11)	0.15
Asian	3 (1.52)	21 (2.21)	9 (1)	
Black or African American	15 (7.61)	31 (3.26)	35 (3.91)	
Native Hawaiian or Other Pacific Islander	0 (0)	0 (0)	2 (0.22)	
White	177 (89.85)	882 (92.74)	835 (93.19)	
More than one race	2 (1.02)	12 (1.26)	10 (1.12)	
Ethnicity				
Hispanic or Latino	2 (1.02)	36 (3.79)	29 (3.24)	0.14
Not Hispanic or Latino	193 (97.97)	911 (95.79)	858 (95.76)	
Education				
Less than HS graduation	6 (3.05)	31 (3.26)	31 (3.46)	0.01
HS graduation	21 (10.66)	88 (9.25)	126 (14.06)	
Some college	39 (19.8)	176 (18.51)	168 (18.75)	
College graduation	46 (23.35)	221 (23.24)	226 (25.22)	
Graduate education	85 (43.15)	434 (45.64)	343 (38.28)	
Marital Status				
Divorced	19 (9.64)	95 (9.99)	68 (7.59)	0.42
Married	141 (71.57)	694 (72.98)	709 (79.13)	
Never Married	8 (4.06)	38 (4)	26 (2.9)	
Widowed	27 (13.71)	120 (12.62)	91 (10.12)	
Employment Status				
Employed	46 (23.35)	176 (18.82)	158 (17.63)	0.15
Retired	146 (74.11)	762 (80.13)	726 (81.03)	
Diagnosis				
Cognitively Normal	101 (51.27)	436 (45.85)	219 (24.36)	< 0.0001
Dementia due to AD	19 (9.64)	107 (11.25)	232 (25.81)	
Dementia due to Other Etiology	0 (0)	0 (0)	0 (0)	
MCI due to AD	74 (37.56)	391 (41.11)	431 (48.1)	
MCI due to Other Etiology	3 (1.52)	17 (1.79)	14 (1.56)	
Smoking				
Current	33 (16.75)	154 (16.19)	166 (18.46)	0.55
Former	38 (19.29)	155 (16.3)	140 (15.63)	
Never	126 (64)	642 (67.51)	588 (65.63)	
CVD				
Yes	121 (61.42)	541 (58.74)	558 (62.28)	0.05
No	76 (38.58)	410 (43.11)	336 (37.37)	
Alcohol Abuse				
Yes	6 (3.05)	31 (3.26)	38 (4.24)	0.47
No	190 (97.46)	917 (96.42)	853 (95.2)	
Drug Abuse				
Yes	0 (0)	5 (0.53)	8 (0.89)	0.30
No	196 (99.49)	943 (99.16)	883 (98.55)	
Psychiatric History				
Yes	52 (26.4)	263 (27.66)	281 (31.26)	0.14
No	145 (73.6)	688 (72.34)	613 (68.42)	

Table 1. Baseline sample characteristics by APOE status, which is available for N = 2,044 participants. Number of participants in each APOE group (N (% of sample)): ε2: 197 (9.64%); ε3: 951 (46.53%); ε4: 896 (43.84%). *HS* = high school; *CVD* = cardiovascular disease.

Characteristic	Baseline Total Cholesterol		p-value
	Below 150 mg/dL N (%)	Above 150 mg/dL N (%)	
Sex			
Male	181 (83.03)	790 (51.13)	< 0.0001
Female	37 (16.97)	755 (48.87)	
Age at baseline			
53-59	5 (2.29)	70 (4.53)	< 0.0001
60-69	31 (14.22)	393 (25.44)	
70-79	126 (57.8)	776 (50.23)	
80-89	56 (25.69)	302 (19.55)	
90-91	0 (0)	4 (0.26)	
Race			
American Indian or Alaskan Native	0 (0)	2 (0.13)	0.13
Asian	7 (3.21)	22 (1.42)	
Black or African American	13 (5.96)	73 (4.72)	
Native Hawaiian or Other Pacific Islander	1 (0.46)	1 (0.06)	
White	192 (88.07)	1,369 (88.61)	
More than one race	3 (1.38)	12 (0.78)	
Ethnicity			
Hispanic or Latino	2 (0.92)	55 (3.56)	0.03
Not Hispanic or Latino	215 (98.62)	1,419 (91.84)	
Education			
Less than HS graduation	7 (3.21)	57 (3.69)	0.33
HS graduation	35 (16.06)	181 (11.72)	
Some college	39 (17.89)	291 (18.83)	
College graduation	54 (24.77)	355 (22.98)	
Graduate education	82 (37.61)	597 (38.64)	
Marital Status			
Divorced	14 (6.42)	146 (9.45)	0.51
Married	176 (80.73)	1,135 (73.46)	
Never Married	5 (2.29)	56 (3.62)	
Widowed	22 (10.09)	197 (12.75)	
Employment Status			
Employed	31 (14.22)	294 (19.03)	0.07
Retired	186 (85.32)	1,219 (78.9)	
Diagnosis, missing			
Cognitively Normal	65 (29.82)	434 (28.09)	1
Dementia due to AD	42 (19.27)	291 (18.83)	
Dementia due to Other Etiology	0 (0)	1 (0.06)	
MCI due to AD	104 (47.71)	786 (50.87)	
MCI due to Other Etiology	7 (3.21)	33 (2.14)	
Smoking			
Current	44 (20.18)	300 (19.42)	0.79
Former	39 (17.89)	306 (19.81)	
Never	133 (61.01)	933 (60.39)	
CVD			
Yes	179 (82.11)	994 (64.34)	< 0.0001
No	37 (16.97)	545 (35.28)	
Alcohol Abuse			
Yes	6 (2.75)	78 (5.05)	0.14
No	210 (96.33)	1,461 (94.56)	
Drug Abuse			
Yes	0 (0)	17 (1.1)	0.12
No	216 (99.08)	1,522 (98.51)	
Psychiatric History			
Yes	67 (30.73)	535 (34.63)	0.28
No	149 (68.35)	1,004 (64.98)	

Table 2. Baseline sample characteristics by baseline total cholesterol, which is available for N = 1,763 participants. Government and medical guidelines tend to recommend total cholesterol value of 200 mg/dL or below as healthy.⁹⁹⁻¹⁰¹ However, more stringent criteria based on both experimental and clinical research suggest that a total cholesterol

below 150 mg/dL is optimal and necessary for prevention and reversal of atherosclerosis and heart disease.¹⁰²⁻¹⁰⁷ Thus, participants were divided into two groups by cholesterol using this cutoff of 150 mg/dL. Number of participants in each cholesterol group (N (% of sample)): below 150 mg/dL: 218 (12.37%); 150 mg/dL or above: 1,545 (87.63%). *HS* = high school; *CVD* = cardiovascular disease.

Aim 1: Modeling Diagnosis and Biomarkers Over Time by Baseline Total Cholesterol using GLMM

a) Diagnosis and Baseline Total Cholesterol

Participants diagnosed with AD had highest levels of total cholesterol (M (SD) = 197.58 (39.7) mg/dL), followed by those classified as CN (M (SD) = 194.52 (39.45) mg/dL), and finally those diagnosed with MCI (M (SD) = 193.04 (38.65) mg/dL; $F(1, 7833) = 22.37, p < 0.01$) (see *Table 3*). Males had higher rates of MCI and AD than females ($F(1, 1758) = 40.45, p < 0.01$). Age was not associated with diagnosis ($F(1, 1758) = 2.52, p = 0.1$). Later visit was associated with more cases of CN, followed by AD, and finally MCI ($F(1, 7833) = 10.08, p < 0.01$).

b) CSF Biomarkers and Baseline Total Cholesterol

Higher baseline total blood cholesterol was associated with lower levels of CSF A β ($F(1, 1095) = 23.86, p < 0.01$). Females (M (SD) = 977.9 (461.68) pg/mL) had higher levels of CSF A β than males (M (SD) = 919.9 (457.6) pg/mL; $F(1, 1136) = 18.78, p < 0.01$). CSF A β was not significantly associated with visit ($F(1, 1095) = 0.3, p = 0.6$). Older individuals had lower levels of CSF A β ($F(1, 1136) = 11.05, p < 0.01$) compared to younger individuals.

Baseline total cholesterol was not significantly associated with CSF levels of tau ($F(1, 1095) = 0.01, p = 0.9$). Higher levels of CSF tau were associated with later visit ($F(1, 1095) = 4.5, p = 0.03$), suggesting that CSF tau increased over time in this sample. Females (M (SD) = 362.89 (613.17) pg/mL) had higher levels than males (M (SD) = 293.12 (326.16) pg/mL; $F(1, 1136) = 37.00, p < 0.01$). Older individuals had higher levels of tau ($F(1, 1136) = 23.16, p < 0.01$) compared to younger individuals.

Baseline total cholesterol was not significantly associated with CSF levels of phosphorylated tau ($F(1, 1094) = 0.52, p = 0.47$). CSF phosphorylated tau was not associated with visit ($F(1, 1094) = 2.00, p = 0.2$). Females (M (SD) = 30.58 (16.68) pg/mL) had higher levels of CSF phosphorylated tau than males (M (SD) = 27.25 (13.13) pg/mL; $F(1, 1136) = 20.27, p < 0.01$). Older individuals had higher levels of phosphorylated tau compared to younger individuals ($F(1, 1136) = 19.2, p < 0.01$).

c) *Brain A β and Baseline Total Cholesterol*

Higher total blood cholesterol at baseline was associated with higher levels of A β in the brain ($F(1, 1261) = 4.18, p = 0.04$). Later visits were associated with lower A β ($F(1, 1261) = 6.85, p < 0.01$). Older individuals had higher levels of A β ($F(1, 973) = 55.74, p < 0.01$). There were no sex differences in brain A β ($F(1, 973) = 0.25, p = 0.61$).

d) *Plasma Biomarkers and Baseline Total Cholesterol*

Higher baseline total cholesterol was associated with lower plasma levels of A β 40 ($F(1, 1651) = 13.86, p < 0.01$). Later visits were associated with higher levels of plasma A β 40 ($F(1, 1651) = 75.87, p < 0.01$). Females had lower levels of A β 40 on average (M (SD) = 157.79 (47.28) pg/mL) than males (M (SD) = 166.83 (46.12) pg/mL; $F(1, 706) = 6.29, p = 0.012$). Older individuals had higher levels of A β 40 on average ($F(1, 706) = 49.65, p < 0.01$) compared to younger individuals.

Higher baseline total cholesterol was associated with lower levels of plasma A β 42 ($F(1, 1660) = 13.49, p < 0.01$). Later visit was associated with higher levels of plasma A β 42 ($F(1, 1660) = 74.27, p < 0.01$). Females had lower levels on average (M (SD) = 38.17 (10.64) pg/mL) compared to males (M (SD) = 40.41 (11.25) pg/mL; $F(1, 706) = 9.19, p < 0.01$). Older

participants had higher levels of A β 42 compared to younger participants ($F(1, 706) = 60.99, p < 0.01$).

e) Global Cognitive Function and Baseline Total Cholesterol

Those with higher baseline total cholesterol exhibited higher ADASCOG score, indicative of poorer cognitive function ($F(1, 7335) = 9.87, p < 0.01$). ADASCOG score was not significantly associated with visit ($F(1, 7335) = 1.81, p = 0.18$). Females performed better on the ADASCOG (M (SD) = 11.34 (9.09)) than males (M (SD) = 11.74 (8.18); $F(1, 1586) = 48.41, p < 0.01$). Older individuals performed worse compared to younger individuals ($F(1, 1586) = 137.83, p < 0.01$).

Those with higher baseline total cholesterol scored lower on the MMSE, indicative of poorer cognitive function ($F(1, 7402) = 18.61, p < 0.01$). Later visit was associated with poorer performance on the MMSE ($F(1, 7402) = 20.38, p < 0.01$). Sex was not significantly associated with MMSE score ($F(1, 1881) = 0.33, p = 0.57$). Older individuals performed worse on the MMSE compared to younger individuals ($F(1, 1881) = 53.61, p < 0.01$).

Total blood cholesterol at baseline was not significantly associated with MoCA score ($F(1, 116) = 3.02, p = 0.09$). Later visit was associated with lower MoCA score, which reflects poorer cognitive function ($F(1, 116) = 9.81, p < 0.01$). Sex was not significantly associated with MoCA score ($F(1, 319) = 21.83, p = 0.46$). Older individuals performed worse on the MoCA compared to younger individuals ($F(1, 319) = 21.83, p < 0.01$).

Those with higher baseline total cholesterol exhibited higher CDR scores and therefore poorer cognitive function ($F(1, 7488) = 17.33, p < 0.01$). Later visit was associated with worse performance on CDR ($F(1, 7488) = 64.42, p < 0.01$). Males (M (SD) = 0.48 (0.45)) performed

worse on average compared to females ($M (SD) = 0.46 (0.51)$; $F(1, 1866) = 8.99, p < 0.01$).

Older participants also performed worse than younger participants ($F(1, 1866) = 9.71, p < 0.01$).

Model	Predictor	Estimate	Num DF	Den DF	t-value	F-value
Diagnosis x TBC	TBC	0.001	1	7833	4.73 ^d	22.37 ^d
	Sex		1	1758		40.45 ^d
	F	-0.05			-6.36 ^d	
	M	0				
	Age	0.001	1	1758	1.59	2.52
CSF A β x TBC	Visit	-0.004	1	7833	-3.18 ^b	10.08 ^b
	TBC	-0.001	1	1095	-4.88 ^d	23.86 ^d
	Sex		1	1136		18.78 ^d
	F	0.09			4.33 ^d	
	M	0				
CSF tau x TBC	Age	-0.005	1	1136	-3.32 ^c	11.05 ^c
	Visit	0.002	1	1095	0.55	0.30
	TBC	-0.0000	1	1095	-00.9	0.01
	Sex		1	1136		37.00 ^d
	F	0.13			6.08 ^d	
CSF ptau x TBC	M	0				
	Age	0.01	1	1136	4.81 ^d	23.16 ^d
	Visit	0.01	1	1095	2.12 ^a	4.50 ^a
	TBC	0.0002	1	1094	0.72	0.52
	Sex		1	1136		20.27 ^d
Brain A β x TBC	F	0.096			4.50 ^d	
	M	0				
	Age	0.01	1	1136	4.38 ^d	19.20 ^d
	Visit	0.005	1	1094	1.41	2.00
	TBC	0.0002	1	1261	2.04 ^a	4.18 ^a
Plasma A β 40 x TBC	Sex		1	973		0.25
	F	0.005			0.50	
	M	0				
	Age	0.005	1	973	7.47 ^d	55.74 ^d
	Visit	-0.003	1	1261	-2.62 ^b	6.85 ^b
Plasma A β 42 x TBC	TBC	-0.001	1	1651	-3.72 ^c	13.86 ^c
	Sex		1	706		6.29 ^a
	F	-0.032			-2.51 ^a	
	M	0				
	Age	0.006	1	706	7.05 ^d	49.65 ^d
ADASCOG x TBC	Visit	0.03	1	1651	8.71 ^d	75.87 ^d
	TBC	-0.01	1	1660	-3.67 ^c	13.49 ^c
	Sex		1	706		9.19 ^b
	F	-0.037			-3.03 ^b	
	M	0				
MMSE x TBC	Age	0.01	1	706	7.81 ^d	60.99 ^d
	Visit	0.03	1	1660	8.62 ^d	74.27 ^d
	TBC	0.001	1	7335	3.14 ^b	9.87 ^b
	Sex		1	1586		48.41 ^d
	F	-0.12			-6.96 ^d	
MMSE x TBC	M	0				
	Age	0.01	1	1586	11.74 ^d	137.83 ^d
	Visit	0.003	1	7335	1.34	1.81
	TBC	-0.0002	1	7402	-4.31 ^d	18.61 ^d
	Sex		1	1881		0.33
MMSE x TBC	F	0.002			0.57	
	M	0				
	Age	-0.002	1	1881	-7.32 ^d	53.61 ^d

MoCA x TBC	Visit	-0.002	1	7402	-4.51 ^d	20.38 ^d
	TBC	0.001	1	116	1.74	3.02
	Sex		1	319		0.54
	F	-0.02			-0.74	
	M	0				
CDR x TBC	Age	-0.01	1	319	-4.67 ^d	21.83 ^d
	Visit	-0.01	1	116	-3.13 ^b	9.81 ^b
	TBC	0.001	1	7488	4.16 ^d	17.33 ^d
	Sex		1	1866		8.99 ^b
	F	-0.07			-3.00 ^b	
	M	0				
	Age	0.005	1	1866	3.12 ^b	9.71 ^b
	Visit	0.02	1	7488	8.03 ^d	64.42 ^d

Table 3. Aim 1 results: Modeling diagnosis and biomarkers over time using APOE (three-group categorization). TBC = total blood cholesterol at baseline. *Ptau* = phosphorylated tau. ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$

Aim 2: Modeling Diagnosis and Biomarkers Over Time by APOE Genotype using GLMM

Analyses using Three-Group APOE Categorization ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ carriers)

a) *Diagnosis and APOE Genotype*

APOE genotype was significantly associated with diagnosis ($F(3, 1939) = 777.97, p < 0.01$) (see Table 4). APOE $\epsilon 4$ carriers had highest rates of AD, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers. APOE $\epsilon 4$ carriers had highest rates of MCI, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers. APOE $\epsilon 2$ carriers had highest rates of CN, followed by $\epsilon 3$ carriers, and finally $\epsilon 4$ carriers. Those using cholesterol-lowering medication had lower rates of AD and higher rates of CN and MCI ($F(1, 838) = 6.71, p < 0.01$).

b) *CSF Biomarkers and APOE Genotype*

APOE $\epsilon 2$ carriers had highest levels of CSF A β (M (SD) = 1237.64 (444.23) pg/mL), followed by $\epsilon 3$ carriers (M (SD) = 1131.82 (431.78) pg/mL), and finally $\epsilon 4$ carriers (M (SD) = 679.21 (320.43) pg/mL; $F(2, 645) = 769.64, p < 0.01$). Cholesterol-lowering medication was not significantly associated with CSF A β ($F(1, 99) = 0.01, p = 0.93$).

APOE $\epsilon 4$ carriers had highest levels of CSF tau (M (SD) = 340.99 (319.94) pg/mL), followed by $\epsilon 3$ carriers (M (SD) = 286 (408.15) pg/mL), and finally $\epsilon 2$ carriers (M (SD) =

252.45 (120.98) pg/mL; $F(2, 645) = 161.11, p < 0.01$). Cholesterol-lowering medication was not significantly associated with CSF tau ($F(1, 99) = 0.66, p = 0.42$).

APOE $\epsilon 4$ carriers had highest levels of CSF phosphorylated tau (M (SD) = 32.67 (14.33) pg/mL), followed by $\epsilon 3$ carriers (M (SD) = 24.87 (12.14) pg/mL), and finally $\epsilon 2$ carriers (M (SD) = 23.06 (12.84) pg/mL; $F(2, 645) = 201.85, p < 0.01$). Cholesterol-lowering medication was not significantly associated with CSF phosphorylated tau ($F(1, 99) = 0.98, p = 0.32$).

c) Brain A β and APOE Genotype

APOE $\epsilon 4$ carriers had highest levels of A β in the brain (M (SD) = 1.51 (0.29)), followed by $\epsilon 3$ carriers (M (SD) = 1.31 (0.28)), and finally $\epsilon 2$ carriers (M (SD) = 1.29 (0.23); $F(3, 788) = 315.83, p < 0.01$). Cholesterol-lowering medication was not associated with brain A β ($F(1, 247) = 0.09, p = 0.76$).

d) Plasma Biomarkers and APOE Genotype

APOE $\epsilon 3$ carriers had highest levels of plasma A $\beta 40$ (M (SD) = 167.89 (47.90) pg/mL), followed by $\epsilon 2$ carriers (M (SD) = 164.07 (42.11) pg/mL), and finally $\epsilon 4$ carriers (M (SD) = 162.54 (46.97) pg/mL; $F(2, 573) = 3.29, p = 0.04$). Cholesterol-lowering medication was not associated with A $\beta 40$ ($F(1, 72) = 0.01, p = 0.93$).

APOE $\epsilon 3$ carriers had highest levels of plasma A $\beta 42$ (M (SD) = 41.94 (11.21) pg/mL), followed by $\epsilon 4$ carriers (M (SD) = 37.95 (10.3) pg/mL), and finally $\epsilon 2$ carriers (M (SD) = 37.79 (10.51) pg/mL; $F(2, 573) = 66.58, p < 0.01$). Cholesterol-lowering medication was not significantly associated with A $\beta 42$ ($F(1, 73) = 0.00, p = 0.97$).

e) Global Cognitive Function and APOE Genotype

APOE $\epsilon 4$ carriers performed worse on the ADASCOG on average (M (SD) = 14.61 (10.58)), followed by $\epsilon 3$ carriers (M (SD) = 9.94 (7.3)), and finally $\epsilon 2$ carriers (M (SD) = 8.98

(6.57); $F(3, 1615) = 557.21, p < 0.01$). Use of cholesterol-lowering medication was associated with better performance (M (SD) = 11.38 (8.68)) than nonuse of medication (M (SD) = 12.01 (9.27); $F(1, 475) = 6.12, p = 0.01$).

APOE $\epsilon 4$ carriers performed worse on the MMSE (M (SD) = 25.76 (4.46)), followed by $\epsilon 3$ carriers (M (SD) = 27.55 (3.16)), and finally $\epsilon 2$ carriers (M (SD) = 27.85 (2.73); $F(3, 2035) = 752.02, p < 0.01$). Use of cholesterol-lowering medication was associated with better performance on average (M (SD) = 27.10 (3.47)) compared to nonuse (M (SD) = 26.82 (3.86); $F(1, 1067) = 10.72, p < 0.01$).

APOE $\epsilon 4$ carriers performed worse on the MoCA (M (SD) = 22.87 (4.99)), followed by $\epsilon 2$ carriers (M (SD) = 23.23 (3.77)), and finally $\epsilon 3$ carriers (M (SD) = 23.49 (4.63); $F(3, 469) = 23.52, p < 0.01$). Cholesterol-lowering medication was not associated with MoCA score ($F(1, 184) = 0.27, p = 0.60$).

APOE $\epsilon 4$ carriers performed worse on the CDR (M (SD) = 0.58 (0.55)), followed by $\epsilon 3$ carriers (M (SD) = 0.35 (0.38)), and finally $\epsilon 2$ carriers (M (SD) = 0.32 (0.43); $F(3, 2035) = 827.07, p < 0.01$). Use of cholesterol-lowering medication was associated with better performance on average (M (SD) = 0.42 (0.45)) compared to nonuse (M (SD) = 0.45 (0.48); $F(1, 1070) = 9.12, p < 0.01$).

Model	Predictor	Estimate	Num DF	Den DF	t-value	F-value
Diagnosis x APOE	APOE		3	1939		777.97 ^d
	NA	-0.69			-6.79 ^d	
	$\epsilon 2$	-0.29			-32.34 ^d	
	$\epsilon 3$	-0.23			-43.36 ^d	
	$\epsilon 4$	0				
Sex			1	1939		362.66 ^d
	F	-0.10			-19.04 ^d	
	M	0				
Age		0.004	1	1939	10.31 ^d	106.35 ^d
Visit		0.003	1	24516	6.79 ^d	46.07 ^d
Chol Med			1	838		6.71 ^b
	No	0.03			2.59 ^b	
	Yes	0				

CSF A β x APOE	APOE		2	645		769.64 ^d
	ϵ 2	0.64			23.54 ^d	
	ϵ 3	0.57			37.09 ^d	
	ϵ 4	0				
	Sex		1	645		37.99 ^d
	F	0.09			6.16 ^d	
	M	0				
	Age	-0.01	1	645	-7.60 ^d	57.72 ^d
	Visit	-0.001	1	2522	-0.65	0.42
	Chol Med		1	99		0.01
No	-0.003			-0.09		
Yes	0					
CSF tau x APOE	APOE		2	645		161.11 ^d
	ϵ 2	-0.24			-11.35 ^d	
	ϵ 3	-0.24			-16.72 ^d	
	ϵ 4	0				
	Sex		1	645		23.54 ^d
	F	0.07			4.85 ^d	
	M	0				
	Age	0.005	1	645	4.77 ^d	22.75 ^d
	Visit	0.004	1	2524	2.74 ^b	7.52 ^b
	Chol Med		1	99		0.66
No	-0.03			-0.81		
Yes	0					
CSF ptau x APOE	APOE		2	645		201.85 ^d
	ϵ 2	-0.36			-12.72 ^d	
	ϵ 3	-0.30			-18.70 ^d	
	ϵ 4	0				
	Sex		1	645		7.30 ^b
	F	0.04			2.70 ^b	
	M	0				
	Age	0.003	1	645	2.86 ^b	8.17 ^b
	Visit	0.002	1	2522	1.28	1.63
	Chol Med		1	99		0.98
No	-0.04			-0.99		
Yes	0					
Brain A β x APOE	APOE		3	788		315.83 ^d
	NA	-0.15			-1.38	
	ϵ 2	-0.17			-20.18 ^d	
	ϵ 3	-0.15			-29.28 ^d	
	ϵ 4	0				
	Sex		1	788		1.50
	F	-0.01			-1.23	
	M	0				
	Age	0.005	1	788	13.90 ^d	193.31 ^d
	Visit	0.002	1	5890	4.35 ^d	18.92 ^d
Chol Med		1	247		0.09	
No	-0.003			-0.30		
Yes	0					
Plasma A β 40 x APOE	APOE		2	573		3.29 ^a
	ϵ 2	0.01			0.27	
	ϵ 3	0.03			2.52 ^a	
	ϵ 4	0				
	Sex		1	573		17.96 ^d
	F	-0.05			-4.24 ^d	
	M	0				
	Age	0.01	1	573	9.36 ^d	87.60 ^d
	Visit	0.02	1	2166	7.93 ^d	62.81 ^d
	Chol Med		1	72		0.01
No	0.003			0.09		
Yes	0					

Plasma A β 42 x APOE	APOE		2	573		66.58 ^d
	ϵ 2	-0.04			-2.03 ^a	
	ϵ 3	0.11			10.27 ^d	
	ϵ 4	0				
	Sex		1	573		5.85 ^a
	F	-0.02			-2.42 ^a	
	M	0				
	Age	0.01	1	573	6.69 ^d	44.79 ^d
	Visit	0.02	1	2169	7.88 ^d	62.13 ^d
	Chol Med		1	73		0.00
No	0.001			0.04		
Yes	0					
ADASCOG x APOE	APOE		3	1615		557.21 ^d
	NA	-0.62			-1.54	
	ϵ 2	-0.51			-29.40 ^d	
	ϵ 3	-0.40			-37.00 ^d	
	ϵ 4	0				
	Sex		1	1615		86.25 ^d
	F	-0.10			-9.29 ^d	
	M	0				
	Age	0.01	1	1615	16.31 ^d	266.14 ^d
	Visit	0.02	1	17573	17.32 ^d	299.94 ^d
Chol Med		1	475		6.12 ^a	
No	0.06			2.47 ^a		
Yes	0					
MMSE x APOE	APOE		3	2035		752.02 ^d
	NA	0.10			3.88 ^c	
	ϵ 2	0.08			31.46 ^d	
	ϵ 3	0.07			44.27 ^d	
	ϵ 4	0				
	Sex		1	2035		2.63
	F	0.003			1.62	
	M	0				
	Age	-0.002	1	2035	-17.08 ^d	291.81 ^d
	Visit	-0.003	1	31266	-23.03 ^d	530.36 ^d
Chol Med		1	1067		10.72 ^b	
No	-0.01			-3.27 ^b		
Yes	0					
MoCA x APOE	APOE		3	469		23.52 ^d
	NA	0.08			0.75	
	ϵ 2	0.04			3.91 ^c	
	ϵ 3	0.06			8.38 ^d	
	ϵ 4	0				
	Sex		1	469		5.74 ^a
	F	0.01			2.40 ^a	
	M	0				
	Age	-0.01	1	469	-14.37 ^d	206.47 ^d
	Visit	-0.01	1	3506	-15.02 ^d	225.55 ^d
Chol Med		1	184		0.27	
No	-0.01			-0.52		
Yes	0					
CDR x APOE	APOE		3	2035		827.07 ^d
	NA	-20.97			-0.00	
	ϵ 2	-0.63			-30.49 ^d	
	ϵ 3	-0.53			-45.99 ^d	
	ϵ 4	0				
	Sex		1	2035		115.76 ^d
	F	-0.12			-10.76 ^d	
	M	0				
	Age	0.01	1	2035	7.70 ^d	59.31 ^d
	Visit	0.02	1	31810	28.91 ^d	835.77 ^d

Chol Med		1	1070		9.12 ^b
No	0.08			3.02 ^b	
Yes	0				

Table 4. Aim 2 results: Modeling diagnosis and biomarkers over time using APOE (three-group categorization). *Ptau* = phosphorylated tau. *Chol Med* = cholesterol-lowering medication.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$

Analyses using Six-Group APOE Categorization ($\epsilon 2/2$, $\epsilon 2/3$, $\epsilon 2/4$, $\epsilon 3/3$, $\epsilon 3/4$, and $\epsilon 4/4$ carriers)

a) *Diagnosis and APOE Genotype*

APOE was significantly associated with diagnosis ($F(6, 1936) = 526.57, p < 0.01$) (see Table 5). APOE $\epsilon 4/4$ carriers had highest rates of AD, followed by $\epsilon 3/4$ carriers, $\epsilon 2/4$ carriers, $\epsilon 3/3$ carriers, and $\epsilon 2/3$ carriers. No $\epsilon 2/2$ carriers had a diagnosis of AD. APOE $\epsilon 3/4$ carriers had highest rates of MCI, followed by $\epsilon 4/4$ carriers, $\epsilon 2/4$ carriers, $\epsilon 3/3$ carriers, $\epsilon 2/3$ carriers, and finally $\epsilon 2/2$ carriers. APOE $\epsilon 2/2$ carriers had highest rates of CN, followed by $\epsilon 2/3$ carriers, $\epsilon 3/3$ carriers, $\epsilon 2/4$ carriers, $\epsilon 3/4$ carriers, and finally $\epsilon 4/4$ carriers. Those who did not use cholesterol-lowering medication had higher rates of AD, while those using cholesterol-lowering medication had highest rates of CN and MCI ($F(1, 838) = 7.01, p < 0.01$).

b) *CSF Biomarkers and APOE Genotype*

APOE $\epsilon 2/3$ carriers had highest levels of A β in CSF (M (SD) = 1323.01 (375.93) pg/mL), followed by $\epsilon 3/3$ carriers (M (SD) = 1131.82 (431.78) pg/mL), $\epsilon 2/4$ carriers (M (SD) = (797.76 (510.75) pg/mL), $\epsilon 3/4$ carriers (M (SD) = 729.35 (329.34) pg/mL), and finally $\epsilon 4/4$ carriers (M (SD) = 495.15 (196.00) pg/mL); $F(4,643) = 500.44, p < 0.01$). No $\epsilon 2/2$ carriers provided CSF A β in this sample.

APOE $\epsilon 4/4$ carriers had highest levels of CSF tau (M (SD) = 341.47 (135.36) pg/mL), followed by $\epsilon 3/4$ carriers (M (SD) = 340.86 (353.92) pg/mL), $\epsilon 2/4$ carriers (M (SD) = 295.24 (143.20) pg/mL), $\epsilon 3/3$ carriers (M (SD) = 286 (408.15) pg/mL), and finally $\epsilon 2/3$ carriers (M (SD)

= 243.47 (113.10) pg/mL); $F(4, 643) = 82.19, p < 0.01$). No $\epsilon 2/2$ carriers provided CSF tau in this sample.

APOE $\epsilon 4/4$ carriers had highest levels of CSF phosphorylated tau (M (SD) = 34.12 (15.40) pg/mL) followed by $\epsilon 3/4$ carriers (M (SD) = 32.27 (14.01) pg/mL), $\epsilon 2/4$ carriers (M (SD) = 29.59 (15.25) pg/mL), $\epsilon 3/3$ carriers (M (SD) = 24.87 (12.14) pg/mL), and finally $\epsilon 2/3$ carriers (M (SD) = 21.75 (11.80) pg/mL; $F(4, 643) = 105.80, p < 0.01$). No $\epsilon 2/2$ carriers provided CSF phosphorylated tau in this sample.

c) Brain A β and APOE Genotype

APOE $\epsilon 4/4$ carriers had highest levels of A β in the brain (M (SD) = 1.61 (0.31)), followed by $\epsilon 2/4$ carriers (M (SD) = 1.51 (0.27)), $\epsilon 3/4$ carriers (M (SD) = 1.49 (0.29)), $\epsilon 3/3$ carriers (M (SD) = 1.31 (0.28)), $\epsilon 2/3$ carriers (M (SD) = 1.24 (0.19)), and finally $\epsilon 2/2$ carriers (M (SD) = 1.19 (0.10)); $F(6, 785) = 199.33, p < 0.01$).

d) Plasma Biomarkers and APOE Genotype

APOE $\epsilon 2/4$ carriers had highest levels of A $\beta 40$ in plasma (M (SD) = 172.62 (50.33) pg/mL), followed by $\epsilon 3/3$ carriers (M (SD) = 167.89 (47.90) pg/mL), $\epsilon 3/4$ carriers (M (SD) = 164.07 (48.48) pg/mL), $\epsilon 2/3$ carriers (M (SD) = 161.92 (39.85) pg/mL), and finally $\epsilon 4/4$ carriers (M (SD) = 157.81 (41.61) pg/mL; $F(4, 571) = 2.95, p = 0.02$). No $\epsilon 2/2$ carriers provided plasma A $\beta 40$ in this sample.

APOE $\epsilon 3/3$ carriers had highest levels of plasma A $\beta 42$ (M (SD) = 41.94 (11.21) pg/mL), followed by $\epsilon 2/4$ carriers (M (SD) = 40.11 (9.51) pg/mL), $\epsilon 3/4$ carriers (M (SD) = 37.99 (10.68) pg/mL), $\epsilon 4/4$ carriers (M (SD) = 37.82 (9.05) pg/mL), and finally $\epsilon 2/3$ carriers (M (SD) = 37.14 (10.70) pg/mL; $F(4, 571) = 36.16, p < 0.01$). No $\epsilon 2/2$ carriers provided plasma A $\beta 42$ in this sample.

e) *Global Cognitive Function and APOE Genotype*

APOE ϵ 4/4 carriers performed worse on ADASCOG on average (M (SD) = 17.84 (10.68)), followed by ϵ 3/4 carriers (M (SD) = 13.84 (10.41)), ϵ 2/4 carriers (M (SD) = 11.93 (8.73)), ϵ 3/3 carriers (M (SD) = 9.94 (7.3)), ϵ 2/2 carriers (M (SD) = 8.35 (5.62)), and finally ϵ 2/3 carriers (M (SD) = 8.14 (5.54); $F(6, 1612) = 368.47, p < 0.01$).

APOE ϵ 4/4 carriers performed worse on the MMSE (M (SD) = 24.55 (4.74)), followed by ϵ 3/4 carriers (M (SD) = 26.06 (4.33)), ϵ 2/4 carriers (M (SD) = 26.79 (3.57)), ϵ 3/3 carriers (M (SD) = 27.55 (3.16)), ϵ 2/2 carriers (M (SD) = 27.97 (3.61)), and finally ϵ 2/3 carriers (M (SD) = 28.14 (2.34); $F(6, 2032) = 479.65, p < 0.01$).

APOE ϵ 4/4 carriers performed worse on the MoCA (M (SD) = 21.61 (5.8)), followed by ϵ 2/4 carriers (M (SD) = 21.67 (4.77)), ϵ 3/4 carriers (M (SD) = 23.12 (4.77)), ϵ 3/3 carriers (M (SD) = 23.49 (4.63)), and finally ϵ 2/3 carriers (M (SD) = 23.68 (3.29); $F(5, 467) = 31.60, p < 0.01$). No ϵ 2/2 carriers provided MoCA scores in this sample.

APOE ϵ 4/4 carriers performed worse on the CDR (M (SD) = 0.77 (0.62)), followed by ϵ 3/4 carriers (M (SD) = 0.53 (0.52)), ϵ 2/4 carriers (M (SD) = 0.52 (0.60)), ϵ 3/3 carriers (M (SD) = 0.35 (0.38)), ϵ 2/2 carriers (M (SD) = 0.27 (0.4)), and finally ϵ 2/3 carriers (M (SD) = 0.26 (0.034); $F(6, 2032) = 549.72, p < 0.01$).

Model	Predictor	Estimate	Num DF	Den DF	t-value	F-value
Diagnosis x APOE	APOE		6	1936		526.57 ^d
	NA	-0.85			-8.43 ^d	
	ϵ 2/2	-0.24			-3.55 ^c	
	ϵ 2/3	-0.51			-42.07 ^d	
	ϵ 2/4	-0.28			-15.96 ^d	
	ϵ 3/3	-0.39			-46.21 ^d	
	ϵ 3/4	-0.21			-24.06 ^d	
	ϵ 4/4	0				
	Sex		1	1936		329.91 ^d
	F	-0.091			-18.16 ^d	
	M	0				
	Age		1	1936	12.99 ^d	168.76 ^d
	Visit		1	24516	8.06 ^d	64.97 ^d

	Chol Med		1	838		7.01 ^b
	No				2.65 ^b	
	Yes					
CSF Aβ x APOE	APOE		4	643		500.44 ^d
	ε2/2	-				
	ε2/3	1.04			29.45 ^d	
	ε2/4	0.51			7.72 ^d	
	ε3/3	0.91			34.90 ^d	
	ε3/4	0.40			15.17 ^d	
	ε4/4	0				
	Sex		1	643		29.83 ^d
	F	0.08			5.46 ^d	
	M	0				
	Age	-0.01	1	643	-9.65 ^d	93.03 ^d
	Visit	-0.0003	1	2522	-0.17	0.03
	Chol Med		1	99		0.17
	No	-0.02			-0.42	
	Yes					
CSF tau x APOE	APOE		4	643		82.19 ^d
	ε2/2	-				
	ε2/3	-0.33			-9.80 ^d	
	ε2/4	-0.19			-2.98 ^b	
	ε3/3	-0.26			-10.41 ^d	
	ε3/4	-0.03			-1.07	
	ε4/4	0				
	Sex		1	643		25.20 ^d
	F	0.07			5.02 ^d	
	M	0				
	Age	0.01	1	643	4.96 ^d	24.63 ^d
	Visit	0.004	1	2524	2.61 ^b	6.83 ^b
	Chol Med		1	99		0.61
	No	-0.03			-0.78	
	Yes	0				
CSF ptau x APOE	APOE		4	643		105.80 ^d
	ε2/2	-				
	ε2/3	-0.43			-11.44 ^d	
	ε2/4	-0.15			-2.18 ^a	
	ε3/3	-0.33			-11.86 ^d	
	ε3/4	-0.04			-1.42	
	ε4/4	0				
	Sex		1	643		8.94 ^b
	F	0.05			2.99 ^b	
	M	0				
	Age	0.004	1	643	3.19 ^b	10.18 ^b
	Visit	0.002	1	2522	1.07	1.15
	Chol Med		1	99		0.89
	No	-0.04			-0.94	
	Yes	0				
Brain Aβ x APOE	APOE		6	785		199.33 ^d
	NA	-0.23			-2.11 ^a	
	ε2/2	-0.31			-4.95 ^d	
	ε2/3	-0.29			-21.66 ^d	
	ε2/4	-0.06			-3.23 ^b	
	ε3/3	-0.23			-20.54 ^d	
	ε3/4	-0.09			-7.91 ^d	
	ε4/4	0				
	Sex		1	785		0.04
	F	-0.001			-0.21	
	M	0				
	Age	0.01	1	785	15.25 ^d	232.61 ^d
	Visit	0.002	1	5890	4.56 ^d	20.76 ^d

	Chol Med		1	247		0.06
	No	-0.003			-0.25	
	Yes	0				
Plasma Aβ40 x APOE	APOE		4	571		2.95 ^a
	ε2/2	-				
	ε2/3	-0.02			-0.73	
	ε2/4	0.08			1.92	
	ε3/3	0.02			1.32	
	ε3/4	-0.01			-0.34	
	ε4/4	0				
	Sex		1	571		15.81 ^d
	F	-0.04			-3.98 ^d	
	M	0				
	Age	0.01	1	571	9.41 ^d	88.63 ^d
	Visit	0.02	1	2166	7.99 ^d	63.76 ^d
	Chol Med		1	72		0.01
	No	0.002			0.07	
	Yes	0				
Plasma Aβ42 x APOE	APOE		4	571		36.16 ^d
	ε2/2	-				
	ε2/3	-0.08			-3.67 ^c	
	ε2/4	0.01			0.25	
	ε3/3	0.08			4.59 ^d	
	ε3/4	-0.04			-2.49 ^a	
	ε4/4	0				
	Sex		1	571		4.84 ^a
	F	-0.02			-2.20 ^a	
	M	0				
	Age	0.006	1	571	7.13 ^d	50.90 ^d
	Visit	0.02	1	2169	7.99 ^d	63.83 ^d
	Chol Med		1	73		0.02
	No	0.004			0.13	
	Yes	0				
ADASCOG x APOE	APOE		6	1612		368.47 ^d
	NA	-0.93			-2.34 ^a	
	ε2/2	-0.67			-3.63 ^c	
	ε2/3	-0.93			-36.50 ^d	
	ε2/4	-0.53			-14.37 ^d	
	ε3/3	-0.73			-36.57 ^d	
	ε3/4	-0.39			-19.34 ^d	
	ε4/4	0				
	Sex		1	1612		73.55 ^d
	F	-0.09			-8.58 ^d	
	M	0				
	Age	0.01	1	1612	18.65 ^d	347.95 ^d
	Visit	0.02	1	17573	18.69 ^d	349.40 ^d
	Chol Med		1	475		7.22 ^b
	No	0.07			2.69 ^b	
	Yes	0				
MMSE x APOE	APOE		6	2032		479.65 ^d
	NA	0.16			5.96 ^d	
	ε2/2	0.13			6.94 ^d	
	ε2/3	0.15			39.47 ^d	
	ε2/4	0.10			17.29 ^d	
	ε3/3	0.13			43.50 ^d	
	ε3/4	0.07			22.91 ^d	
	ε4/4	0				
	Sex		1	2032		0.47
	F	0.001			0.68	
	M	0				
	Age	-0.002	1	2032	-19.42 ^d	377.11 ^d

	Visit	-0.003	1	31266	-24.17 ^d	584.12 ^d
	Chol Med		1	1067		11.56 ^c
	No	-0.01			-3.40 ^c	
	Yes	0				
MoCA x	APOE		5	467		31.60 ^d
APOE	NA	0.17			1.61	
	ε2/2	-				
	ε2/3	0.16			9.43 ^d	
	ε2/4	0.03			1.49	
	ε3/3	0.15			10.41 ^d	
	ε3/4	0.11			7.26 ^d	
	ε4/4	0				
	Sex		1	467		6.16 ^a
	F	0.02			2.48 ^a	
	M	0				
	Age	-0.01	1	467	-15.42 ^d	237.80 ^d
	Visit	-0.01	1	3506	-15.65 ^d	244.80 ^d
	Chol Med		1	184		0.38
	No	-0.01			-0.61	
	Yes	0				
CDR x APOE	APOE		6	2032		549.72 ^d
	NA	-21.27			-0.00	
	ε2/2	-0.92			-5.81 ^d	
	ε2/3	-1.13			-40.67 ^d	
	ε2/4	-0.43			-11.57 ^d	
	ε3/3	-0.84			-47.20 ^d	
	ε3/4	-0.40			-22.44 ^d	
	ε4/4	0				
	Sex		1	2032		93.81 ^d
	F	-0.11			-9.69 ^d	
	M	0				
	Age	0.01	1	2032	10.87 ^d	118.22 ^d
	Visit	0.02	1	31810	29.43 ^d	866.07 ^d
	Chol Med		1	1070		10.02 ^b
	No	0.08			3.17 ^b	
	Yes	0				

Table 5. Aim 2 results: Modeling diagnosis and biomarkers over time using APOE (six-group categorization). APOE using six-group categorization as a function diagnosis and related biomarkers. *Ptau* = phosphorylated tau. *Chol Med* = cholesterol-lowering medication.
^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$

Follow-Up Analyses for Aims 1 and 2: Further Exploration of the Relationship between Diagnosis and Biomarkers using GLMM

Additional analyses were conducted between diagnosis and each biomarker to better understand the results in Aims 1 and 2 between total cholesterol and APOE status with each biomarker (see Table 6).

a) CSF Biomarkers and Diagnosis

Those who were considered CN had higher levels of A β in CSF on average (M (SD) = 1188.44 (442.33) pg/mL), followed by those diagnosed with MCI (M (SD) = 951.19 (443.33) pg/mL), and finally those diagnosed with AD (M (SD) = 632.88 (297.18) pg/mL; $F(1, 1076) = 543.63, p < 0.01$). Those diagnosed with AD had higher levels of CSF tau (M (SD) = 397.56 (494.47) pg/mL), followed by those classified as CN (M (SD) = 308.65 (684.20) pg/mL), and finally those diagnosed with MCI (M (SD) = 289.96 (128.99) pg/mL; $F(1, 1076) = 17.34, p < 0.01$). Those diagnosed with AD had higher levels of phosphorylated tau (M (SD) = 36.36 (16.27) pg/mL), followed by those with MCI (M (SD) = 27.99 (14.54) pg/mL), and finally those considered CN (M (SD) = 23.58 (11.30) pg/mL; $F(1, 1075) = 264.17, p < 0.01$).

b) Brain A β and Diagnosis

Those diagnosed with AD had higher levels of A β in the brain (M (SD) = 1.57 (0.29)), followed by those with MCI (M (SD) = 1.38 (0.29)), and finally those considered CN (M (SD) = 1.31 (0.26); $F(1, 1286) = 235.95, p < 0.01$).

c) Plasma Biomarkers and Diagnosis

Diagnosis was not significantly associated with plasma levels of A β 40 ($F(1, 1630) = 0.62, p = 0.43$). Those classified as CN had highest levels of plasma A β 42 (M (SD) = 40.21 (11.18) pg/mL), followed by those with AD (M (SD) = 39.02 (9.91) pg/mL), and finally those with MCI (M (SD) = 39.18 (11.74) pg/mL; $F(1, 1640) = 4.32, p = 0.04$).

d) Global Cognitive Function and Diagnosis

Those with AD had performed worse on the ADASCOG (M (SD) = 22.08 (9.69)), followed by those with MCI (M (SD) = 10.17 (5.03)), and finally those considered CN (M (SD) = 6.10 (3.13); $F(1, 7448) = 7011.00, p < 0.01$). Those with AD performed worse on the MMSE (M (SD) = 21.46 (4.75)), followed by those with MCI (M (SD) = 27.52 (2.2)), and finally those

classified as CN (M (SD) = 29.04 (1.23); $F(1, 6595) = 6326.25, p < 0.01$). Those with AD performed worse on the MoCA (M (SD) = 15.34 (5.29)), followed by those with MCI (M (SD) = 22.31 (3.2)), and finally those considered CN (M (SD) = 25.49 (2.51); $F(1, 129) = 559.58, p < 0.01$). AD was associated with worse performance on average on the CDR (M (SD) = 1.00 (0.53)), followed by MCI (M (SD) = 0.48 (0.15)), and finally CN (M (SD) = 0.03 (0.13); $F(1, 6530) = 14452.3, p < 0.01$).

Model	Predictor	Estimate	Num DF	Den DF	t-value	F-value
Diagnosis x CSF A β	CSF A β	-0.0004	1	1076	-23.32 ^d	543.63 ^d
	Sex		1	1233		5.60 ^a
	F	-0.04			-2.37 ^a	
	M	0				
	Age	-0.002	1	1233	-2.09 ^a	4.36 ^a
Diagnosis x CSF tau	Visit	-0.003	1	1076	-1.07	1.15
	CSF tau	0.0001	1	1076	4.16 ^d	17.34 ^d
	Sex		1	1233		14.44 ^c
	F	-0.06			-3.80 ^c	
	M	0				
Diagnosis x CSF ptau	Age	-0.001	1	1233	-0.55	0.30
	Visit	-0.003	1	1076	-1.02	1.05
	CSF ptau	0.008	1	1075	16.25 ^d	264.17 ^d
	Sex		1	1233		28.02 ^d
	F	-0.08			-5.29 ^d	
Diagnosis x Brain A β	M	0				
	Age	-0.001	1	1233	-1.24	1.55
	Visit	-0.003	1	1075	-1.23	1.52
	Brain A β	0.41	1	1286	15.36 ^d	235.95 ^d
	Sex		1	1187		23.40 ^d
Diagnosis x Plasma A β 40	F	-0.08			-4.84 ^d	
	M	0				
	Age	-0.002	1	1187	-1.48	2.18
	Visit	-0.003	1	1286	-1.38	1.90
	Plasma A β 40	0.0001	1	1630	0.79	0.62
Diagnosis x Plasma A β 42	Sex		1	725		3.20
	F	-0.03			-1.79	
	M	0				
	Age	-0.002	1	725	-1.61	2.61
	Visit	-0.001	1	1630	-0.36	0.13
Diagnosis x ADASCOG	Plasma A β 42	-0.002	1	1640	-2.08 ^a	4.32 ^a
	Sex		1	725		4.29 ^a
	F	-0.04			-2.07 ^a	
	M	0				
	Age	-0.002	1	725	-1.19	1.42
Diagnosis x ADASCOG	Visit	0.001	1	1640	0.17	0.03
	ADASCOG	0.03	1	7448	83.73 ^d	7011.00 ^d
	Sex		1	2105		64.09 ^d
	F	-0.05			-8.01 ^d	
	M	0				
Diagnosis x ADASCOG	Age	-0.002	1	2105	-3.50 ^c	12.25 ^c
	Visit	-0.006	1	7448	-6.82 ^d	46.50 ^d

Diagnosis x MMSE	MMSE	-0.06	1	6595	-79.54 ^d	6326.25 ^d
	Sex		1	2492		90.36 ^d
	F	-0.06			-9.51 ^d	
	M	0				
	Age	-0.0003	1	2492	-0.63	0.40
Diagnosis x MoCA	Visit	-0.004	1	6595	-3.91 ^d	15.28 ^d
	MoCA	-0.06	1	129	-23.66 ^d	559.58 ^d
	Sex		1	732		7.60 ^b
	F	-0.06			-2.76 ^b	
	M	0				
Diagnosis x CDR	Age	0.001	1	732	0.46	0.21
	Visit	0.01	1	129	3.84 ^c	14.73 ^c
	CDR	0.70	1	6530	120.22 ^d	14452.3 ^d
	Sex		1	2463		66.13 ^d
	F	-0.05			-8.13 ^d	
Diagnosis x CDR	M	0				
	Age	-0.0003	1	2463	-0.77	0.60
	Visit	-0.005	1	6530	-6.58 ^d	43.26 ^d

Table 6. Follow-up analyses for Aims 1 and 2: Association between diagnosis and biomarkers. *Ptau* = phosphorylated tau. ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$

Aim 3: Analyzing Baseline Total Cholesterol by APOE Genotype using GLM

Generalized linear modeling (GLM) indicates baseline total cholesterol between $\epsilon 4$ and $\epsilon 3$ carriers differed significantly ($\chi = 52.07$, $p < 0.01$), but did not differ between $\epsilon 4$ and $\epsilon 2$ carriers ($\chi = 1.97$, $p = 0.16$). Least square means (SE) of baseline total cholesterol of each genotype include: $\epsilon 4$: 193.54 (0.52) mg/dL, $n = 725$; $\epsilon 3$: 188.15 (0.53) mg/dL, $n = 711$; $\epsilon 2$: 191.74 (1.17) mg/dL, $n = 150$.

Modeling baseline total cholesterol using the six-group categorization of APOE genotype revealed statistical significance between $\epsilon 4/4$ and $\epsilon 3/4$ carriers ($\chi = 13.70$, $p < 0.01$), $\epsilon 4/4$ and $\epsilon 3/3$ carriers ($\chi = 54.15$, $p < 0.01$), $\epsilon 4/4$ and $\epsilon 2/3$ carriers ($\chi = 7.49$, $p < 0.01$), and $\epsilon 4/4$ and $\epsilon 2/2$ carriers ($\chi = 45.83$, $p < 0.01$), but not between $\epsilon 4/4$ and $\epsilon 2/4$ carriers ($\chi = 0.31$, $p = 0.58$). Least square means (LSM) (SE) of baseline total cholesterol by genotype include: $\epsilon 4/4$: 197.09 (1.09) mg/dL, $n = 164$; $\epsilon 3/4$: 192.48 (0.6) mg/dL, $n = 561$; $\epsilon 3/3$: 188.15 (0.53) mg/dL, $n = 711$; $\epsilon 2/4$: 195.54 (2.56) mg/dL, $n = 32$; $\epsilon 2/3$: 192.37 (1.33) mg/dL, $n = 113$; $\epsilon 2/2$: 152.59 (6.48) mg/dL, $n = 5$.

Two additional GLMs were conducted with the addition of cholesterol-lowering medication, sex, and age as covariates to evaluate their potential influence on the relationship between baseline total cholesterol and both APOE genotype classifications. The difference in total cholesterol between APOE $\epsilon 4$ (LSM (SE) = 189.02 (0.79) mg/dL) and $\epsilon 3$ (LSM (SE) = 184.04 (0.81) mg/dL) carriers remained significant ($\chi = 48.14, p < 0.01$), and the difference between $\epsilon 4$ and $\epsilon 2$ (LSM (SE) = 186.69 (1.29) mg/dL) carriers remained statistically insignificant in this model ($\chi = 3.64, p = 0.06$). Females (LSM (SE) = 200.33 (0.86) mg/dL) had significantly higher levels of total cholesterol than males (LSM (SE) = 172.83 (0.83) mg/dL; $\chi = 1608.25, p < 0.01$). Those using cholesterol-lowering medication (LSM (SE) = 180.23 (1.41) mg/dL) had significantly lower cholesterol levels than those not using medication (LSM (SE) = 192.94 (0.45) mg/dL; $\chi = 80.34, p < 0.01$). Furthermore, older participants had lower cholesterol levels compared to younger participants ($\chi = 102.54, p < 0.01$). Least square means of each APOE group based on this GLM are depicted in *Figure 1*.

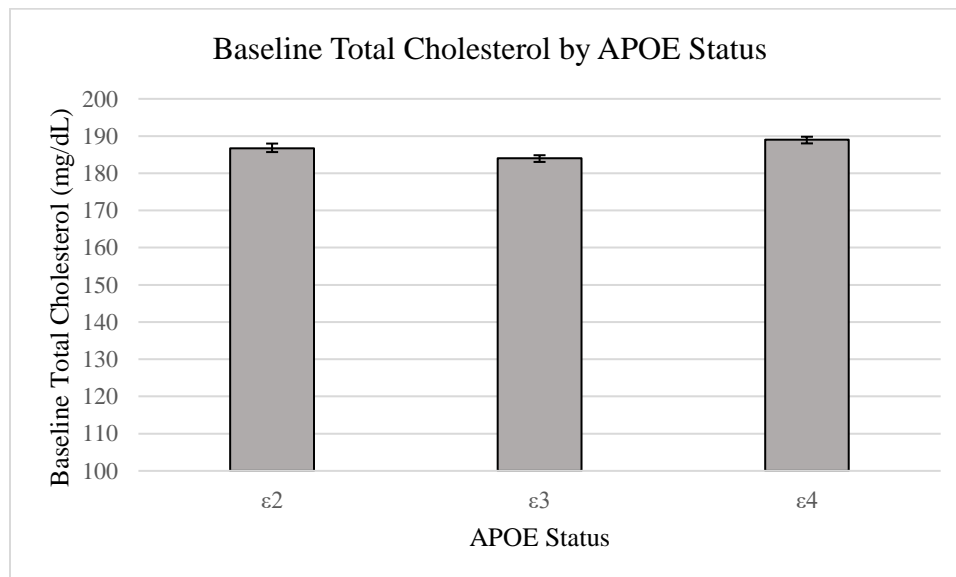


Figure 1. Baseline total cholesterol by APOE status using three-group classification. APOE $\epsilon 2$ carriers include $\epsilon 2/2$, $\epsilon 2/3$, and $\epsilon 2/4$ genotypes, $\epsilon 3$ carriers include those with $\epsilon 3/3$ genotype, and $\epsilon 4$ carriers include $\epsilon 3/4$ and $\epsilon 4/4$ genotypes. These adjusted least square means and standard errors are based on the GLM including sex, age, and cholesterol-lowering medication as covariates. The difference in total cholesterol between $\epsilon 4$ and $\epsilon 3$ carriers reached statistical significance, while the difference between $\epsilon 4$ and $\epsilon 2$ carriers did not.

Using the six-group APOE categorization with these additional covariates, $\epsilon 4/4$ carriers (LSM (SE) = 192.40 (1.21) mg/dL) had significantly higher total cholesterol levels than $\epsilon 3/4$ carriers (LSM (SE) = 188.06 (0.84) mg/dL; $\chi = 13.27, p < 0.01$), $\epsilon 3/3$ carriers (LSM (SE) = 184.03 (0.81) mg/dL; $\chi = 50.99, p < 0.001$), $\epsilon 2/3$ carriers (LSM (SE) = 187.73 (1.43) mg/dL; $\chi = 8.02, p < 0.01$), and $\epsilon 2/2$ carriers (LSM (SE) = 147 (6.12) mg/dL; $\chi = 53.97, p < 0.01$). Once again, the difference in total cholesterol between $\epsilon 4/4$ and $\epsilon 2/4$ carriers (LSM (SE) = 189.16 (2.53) mg/dL) did not meet significance ($\chi = 1.48, p = 0.22$). Females (LSM (SE) = 195.22 (1.35) mg/dL) had higher total cholesterol than males (LSM (SE) = 167.57 (1.33) mg/dL; $\chi = 1628.08, p < 0.01$). Those using cholesterol-lowering medication (LSM (SE) = 175.05 (1.75) mg/dL) had significantly lower total cholesterol than those not using medication (LSM (SE) = 187.75 (1.14) mg/dL; $\chi = 80.66, p < 0.01$). Furthermore, older participants had lower total cholesterol than younger participants ($\chi = 92.09, p < 0.01$). Least square means of each APOE group based on this GLM are presented in *Figure 2*.

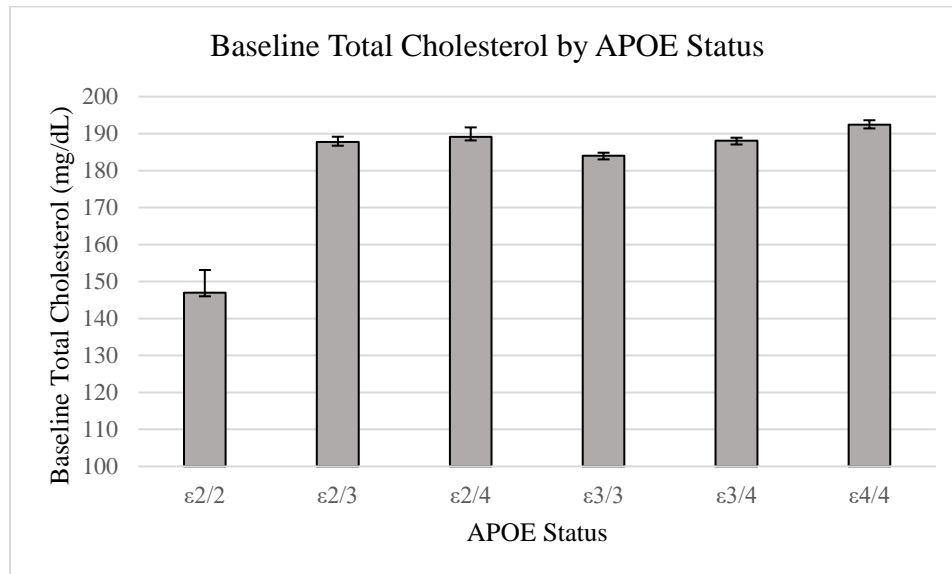


Figure 2. Baseline total cholesterol by APOE status using six-group classification. These adjusted least square means and standard errors are based on the GLM including sex, age, and cholesterol-lowering medication as covariates. The differences between $\epsilon 4$ and all genotypes except $\epsilon 2/4$ met significance.

Aim 4: Modeling Time to AD Incidence based on APOE Status and Baseline Total Cholesterol using Survival Analysis

Using the three-group APOE classification, APOE genotype had a significant effect on time to diagnosis of AD ($p < 0.01$), while total cholesterol did not ($p = 0.92$). As expected, APOE $\epsilon 4$ carriers had the highest hazard rate, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers (Table 7). Using the six-group APOE categorization, APOE genotype again had a significant effect on time to diagnosis ($p < 0.01$), while total cholesterol did not ($p = 0.85$). APOE $\epsilon 4/4$ carriers had the highest hazard rate, followed by $\epsilon 3/4$ carriers, $\epsilon 2/4$ carriers, $\epsilon 3/3$ carriers, $\epsilon 2/2$ carriers, and finally $\epsilon 2/3$ carriers.

Cox Proportional Hazards model including cholesterol-lowering medication, sex, and age as additional covariates with the three-group APOE categorization shows that APOE status once again met significance ($p < 0.01$) along with age ($p = 0.04$), while total cholesterol ($p = 0.55$), cholesterol-lowering medication ($p = 0.94$), and sex ($p = 0.15$) did not. APOE $\epsilon 4$ carriers again had the highest hazard rate, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers.

Similar results were found using the six-group APOE classification with age, cholesterol-lowering medication, and sex as covariates. APOE genotype ($p < 0.01$) and age ($p = 0.02$) again met significance, while total cholesterol ($p = 0.58$), cholesterol-lowering medication ($p = 0.93$), and sex ($p = 0.26$) did not. A slightly different trend for hazard rates were observed, where APOE $\epsilon 4/4$ carriers had highest hazard rates, followed by $\epsilon 3/4$, $\epsilon 2/4$, $\epsilon 2/2$, $\epsilon 3/3$, and finally $\epsilon 2/3$ carriers.

Two more Cox Proportional Hazards models were then performed with cholesterol-lowering medication, sex, and age, using the interaction between total cholesterol and APOE status as an additional covariate. Using the three-group APOE categorization, age met

significance ($p = 0.04$) while neither APOE status ($p = 0.73$), total cholesterol ($p = 0.35$), cholesterol-lowering medication ($p = 0.92$), sex ($p = 0.15$), nor the interaction between cholesterol and APOE genotype ($p = 0.72$) met significance. Similar results were found using the six-group APOE categorization of this model, as age met significance ($p = 0.02$), but neither APOE status ($p = 0.95$), total cholesterol ($p = 0.28$), cholesterol-lowering medication ($p = 0.81$), sex ($p = 0.24$), nor the interaction between cholesterol and APOE genotype ($p = 0.81$) did.

Model	Predictor	Estimate	Chi-Square	Hazard Ratio
AD incidence using only APOE (3 groups) and TBC	APOE		118.99 ^d	
	ε2	-1.11	39.71 ^d	0.33
	ε3	-0.88	96.64 ^d	0.41
	ε4	0		
	TBC	0.0001	0.01	1.00
AD incidence using only APOE (6 groups) and TBC	APOE		142.78 ^d	
	ε2/2	-1.54	2.35	0.21
	ε2/3	-1.83	53.72 ^d	0.16
	ε2/4	-0.59	4.54 ^a	0.56
	ε3/3	-1.23	99.22 ^d	0.29
	ε3/4	-0.44	15.03 ^c	0.64
	ε4/4	0		
	TBC	0.0002	0.04	1.00
AD incidence using all predictors (APOE: 3 groups)	APOE		55.84 ^d	
	ε2	-1.06	19.31 ^d	0.35
	ε3	-0.84	44.92 ^d	0.43
	ε4	0		
	TBC	0.001	0.36	1.00
	Chol Med		0.01	
	No	0.02	0.01	1.02
	Yes	0		
	Sex		2.10	
	F	-0.18	2.10	0.83
	M	0		
Age	0.02	4.09 ^a	1.02	
AD incidence using all predictors (APOE: 6 groups)	APOE		76.04 ^d	
	ε2/2	-0.98	0.93	0.38
	ε2/3	-1.94	31.53 ^d	0.14
	ε2/4	-0.58	2.28	0.56
	ε3/3	-1.30	58.19 ^d	0.27
	ε3/4	-0.57	13.75 ^b	0.57
	ε4/4	0		
	TBC	0.001	0.31	1.00
	Chol Med		0.01	
	No	0.02		1.02
	Yes	0		
	Sex		1.25	
	F	-0.14	1.25	0.87
	M	0		
	Age	0.02	5.52 ^a	1.02

Table 7. Aim 4 results: Time to AD incidence based on APOE status and baseline total cholesterol. TBC = total blood cholesterol at baseline. Chol Med = cholesterol-lowering medication. ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$

Model fit statistics of each of these models were compared to select the best-fitting model for predicting time to diagnosis of AD. The models including APOE status, total cholesterol, cholesterol-lowering medication, sex, and age produced the lowest -2 Log Likelihood value, Akaike Information Criterion, and Schwarz Bayesian Information Criterion. The model using the six-group APOE classification (-2 Log Likelihood = 3608.838; AIC = 3624.838; SBC = 3658.920) had slightly lower values for these fit statistics compared to the three-group APOE classification (-2 Log Likelihood = 3626.998; AIC = 3638.998; SBC = 3661.719). Survival functions for these models stratified by APOE status are shown in *Figure 3*.

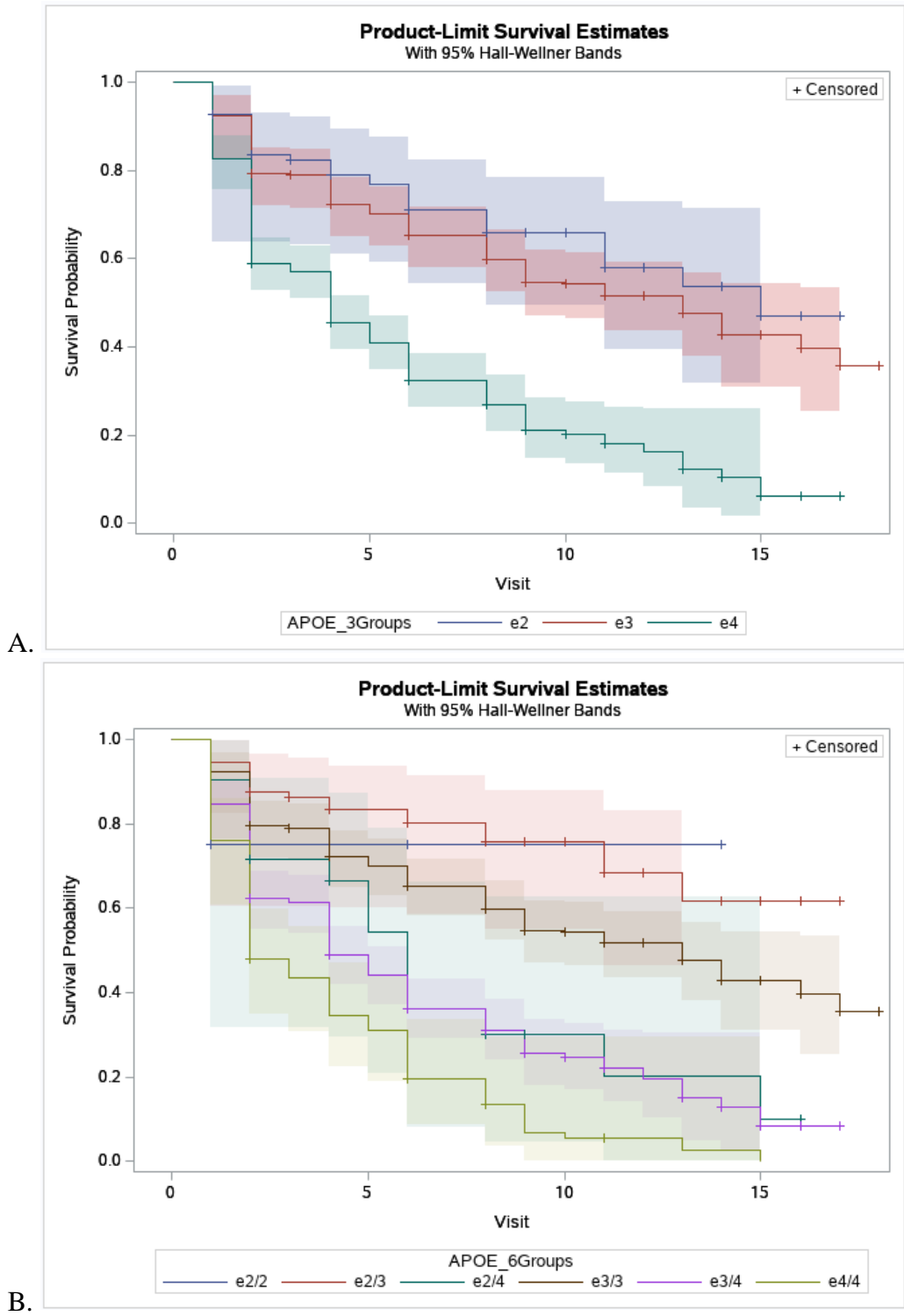


Figure 3. Survival curves showing time to diagnosis based on APOE status, using the three-group (A) and six-group (B) categorizations. Censored participants remained cognitively normal throughout follow-up, while non-censored participants were diagnosed with dementia due to AD. Visit refers to number of months after baseline, where month 0 is diagnosis at baseline appointment.

A key assumption of the Cox Proportional Hazards model is that the hazards are proportional, which can be verified for discrete variables by plotting the log of negative log of survival rates by each predictor and assessing whether the lines cross. While the assumption of proportional hazards is met by sex and APOE status using three groups, this assumption is violated by cholesterol-lowering medication and APOE when using all six possible genotypes. Regarding the continuous predictors, the observed residuals for age and total cholesterol are not larger than predicted under the proposed model, based on cumulative martingale sums and a supremum test of the null hypothesis. These diagnostic tests suggest that age and total cholesterol satisfy the assumption of proportional hazards.

Several outliers were identified—two for total cholesterol, and one for age. However, repeating the analyses for each genotype grouping without these three outliers did not lead to meaningful changes in the significance levels of the predictors, so these outliers were not excluded.

DISCUSSION

The main goal of this study was to investigate the relationship between baseline total cholesterol and APOE status, AD incidence, and related biomarkers. As predicted, a number of associations were found in support of total cholesterol mediating APOE risk for AD. While there were some inconsistencies in findings, particularly between results of Aims 1-3 versus Aim 4 which I discuss in more detail below, the majority of this study's results support the notion that maintaining low total cholesterol through older adulthood may be protective against the development of AD pathology.

Aims 1 and 2: AD Incidence and Biomarkers, Baseline Total Cholesterol, and APOE Status

Diagnosis

As hypothesized, those with AD had highest baseline levels of total cholesterol. Literature suggests that high LDL and total cholesterol, particularly at midlife,^{70,75,77} is indicative of AD onset in later life,^{71,73} and that individuals with AD have higher LDL and total cholesterol than age-matched controls.⁷³ Our results expand on the state of the literature regarding the pathology of AD, suggesting that total cholesterol in later life may be a key aspect of AD pathology. Elevated total cholesterol in middle-aged and older individuals may be a useful clinical indicator, suggesting importance of initiating cholesterol-lowering lifestyle changes early. Lowering cholesterol levels could potentially translate to lower probability of developing AD, or at least put a stop to underlying amyloid pathology which may have already begun despite the absence of noticeable symptoms.

Contrary to what was hypothesized, the CN group had higher total cholesterol than those diagnosed with MCI. This conflicts with the general state of the literature reporting that individuals with MCI had higher total cholesterol than normal controls.^{108,109} However, Toro and colleagues (2014) found that despite higher baseline total cholesterol in individuals with MCI and AD compared to healthy controls, those with MCI and AD exhibited a decline in total cholesterol over 14 years of follow-up, compared to stable levels in the control group.¹⁰⁹ This parallels earlier findings by Solomon et al., (2007), where high midlife total cholesterol signified risk for more severe cognitive impairment later in life, yet total cholesterol decreased from midlife to later life in those with greatest cognitive impairment.¹¹⁰ The onset of MCI- and AD-related pathology could involve a decline in total cholesterol over time due to biological mechanisms not yet fully understood.^{109,110} Although the present study did not assess cholesterol

after baseline, perhaps the lower levels observed in participants with MCI support the notion that total cholesterol may decline in early stages of dementia, once the underlying disease pathology has been initiated. This would be consistent with findings by Notkola et al. (1998), where high cholesterol predicted dementia 30 years later, but cholesterol declined prior to dementia onset.⁸⁶

Oxysterols may be related to fluctuations in total cholesterol across the stages of AD. For blood cholesterol to influence AD pathology in the brain, cholesterol presumably must cross the blood-brain barrier (BBB). While it was initially believed that the BBB does not allow cholesterol in the periphery to enter the brain and that all cholesterol present in the brain is produced locally,¹¹¹⁻¹¹⁶ more recent studies suggest that cholesterol can cross the BBB via oxidation.¹¹¹⁻¹²⁰ Several oxysterols, which are oxidized derivatives of cholesterol, have been found to cross the BBB.¹¹⁴ Those most implicated in AD pathology include 24-hydroxycholesterol (24-OH) and 27-hydroxycholesterol (27-OH).^{117,118} 24-OH seems to be produced primarily in the brain and transports excess cholesterol into the periphery.^{117,118} 24-OH may also have a protective role by preventing A β accumulation through modification of APP.^{117,118} 27-OH, in contrast, may come from the periphery through consumption of cholesterol-containing foods which have already been oxidized, through various storage and heating treatments for example, or through oxidation of excess cholesterol in the blood.^{16,17,121} Unlike 24-OH, 27-OH seems to promote A β production and accumulation.¹¹⁸

Higher levels of 24-OH have been reported in the periphery and CNS during early stages of AD, which has been interpreted as a compensatory mechanism in response to accumulating amyloid pathology.^{117,119,120} Conversely, lower levels of 24-OH have been documented in later stages of AD.^{117,119,120} 27-OH tends to be higher in the CNS and lower in plasma throughout all stages of AD.^{117-120,122,123} Presumably, by the time clinical symptoms become evident, excess 27-

OH produced in the periphery has already entered the brain and initiated a cascade of events leading to AD pathology through impairment of 24-OH production and instigation of amyloid and tau deposition.^{117-120,122,123} Perhaps varying levels of oxysterols across the stages of AD correspond to total cholesterol in the periphery, where blood cholesterol that is initially high prior to onset of clinically diagnosable symptoms then drops during early stages of AD (MCI) – as cholesterol is transported into the brain from the periphery via oxidation. A balance between 24-OH and 27-OH in the brain may be necessary for keeping AD pathology at bay, a process which may benefit from reducing blood cholesterol levels via diet.

As hypothesized, $\epsilon 4$ carriers had highest rates of AD and MCI, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers. More specifically, $\epsilon 4/4$ carriers had highest rates of AD, followed by $\epsilon 3/4$, $\epsilon 2/4$, $\epsilon 3/3$, $\epsilon 2/3$, and finally $\epsilon 2/2$ carriers. The same trend emerged for rates of MCI, with the exception that $\epsilon 3/4$ carriers had higher rates than $\epsilon 4/4$ carriers in this group. These rates of AD and MCI prevalence in relation to APOE genotype are consistent with the literature which reports a greater likelihood for those with at least one copy of the $\epsilon 4$ allele to develop MCI or AD, while $\epsilon 2$ seems protective.^{3,23,26,29}

CSF A β , Tau, and Phosphorylated Tau

In contrast to what was hypothesized, higher total cholesterol was associated with lower CSF levels of A β . This is consistent with differences in CSF levels of A β by genotype and diagnosis, as $\epsilon 4$ carriers had lowest levels, followed by $\epsilon 3$ carriers, and finally $\epsilon 2$ carriers. More specifically, APOE $\epsilon 4/4$ carriers had lowest levels, followed by $\epsilon 3/4$, $\epsilon 2/4$, $\epsilon 3/3$, and $\epsilon 2/3$ carriers. Additionally, participants diagnosed with AD had lowest levels of A β , followed by those with MCI, and finally those considered CN. Lower levels of A β in CSF may actually be indicative of AD pathology. Prior studies have reported that CSF A β_{42} levels inversely

correlated with brain A β levels, and that CSF A β 42 is lower over time in AD patients compared to healthy controls and those with MCI.¹²⁴⁻¹²⁶ This finding has been proposed to be reflective of the severity of pathologic A β plaques in the brain.¹²⁴⁻¹²⁶ Our results support the general state of the literature, suggesting that lower CSF A β may be diagnostic of AD pathology.

Contrary to what was hypothesized, there was no association between total cholesterol and levels of CSF tau despite the fact that ϵ 4 carriers had highest levels of tau, followed by ϵ 3 and finally ϵ 2 carriers. Based on all six genotype groups, ϵ 4/4 carriers had highest levels, followed by ϵ 3/4, ϵ 2/4, ϵ 3/3, and ϵ 2/3 carriers. Those diagnosed with AD also had higher levels of CSF tau, followed by those considered CN, and finally those with MCI.

Total cholesterol was also not associated with CSF phosphorylated tau. However, APOE ϵ 4 carriers had highest levels, followed by ϵ 2 and finally ϵ 3 carriers. More specifically, ϵ 4/4 carriers had highest levels, followed by ϵ 3/4, ϵ 2/4, ϵ 3/3, and finally ϵ 2/3 carriers. Those diagnosed with AD had higher levels of CSF phosphorylated tau, followed by those with MCI, and finally the CN group.

These differences in CSF levels of tau and phosphorylated tau between genotype and diagnostic group are consistent with prior findings.¹²⁴⁻¹²⁶ The lack of association between total cholesterol and CSF tau and phosphorylated tau could suggest that total cholesterol levels do not influence this aspect of AD pathology. However, this lack of significance could also be indicative of more elusive pathological changes associated with AD. For instance, Niemantsverdriet et al. (2017) reported that the first A β plaques occur at least 10 years, but possibly 20-30 years, prior to initial presentation of AD symptoms.¹²⁵ These early changes are detectable in CSF A β levels for early diagnosis.¹²⁵ CSF tau biomarkers, in comparison, change later in the pathophysiological process compared to CSF A β .¹²⁵ High cholesterol seems to be

predictive of onset of amyloid pathology underlying AD, including lower CSF A β and initial buildup of A β in the brain. Cholesterol may then begin to decrease during early stages of AD, which could precede increases in CSF tau and phosphorylated tau.¹²⁵ These changes in cholesterol and CSF A β , tau, and phosphorylated tau at different times throughout AD stages may account for the association between cholesterol and A β , but seeming lack of association between cholesterol and tau or phosphorylated tau. The timing of collection of cholesterol and CSF biomarkers in relation to stage of AD may therefore be an important factor of consideration in future studies to better elucidate these pathological changes.

Brain A β

As hypothesized, higher baseline total cholesterol was associated with higher levels of A β in the brain. Furthermore, as predicted, this trend is consistent with genotype and diagnosis, as ϵ 4 carriers and those with AD also had highest levels of A β , followed by ϵ 3 carriers and those with MCI, and finally ϵ 2 carriers and participants considered CN. When looking at all possible genotypes, ϵ 4/4 carriers had highest levels of A β , followed by ϵ 2/4, ϵ 3/4, ϵ 3/3, ϵ 2/3, and ϵ 2/2 carriers. High total cholesterol may therefore be a risk factor for A β accumulation, an essential component to AD neuropathology.^{3,23,29} These results also replicate prior findings regarding the relative genetic risk for amyloid pathology based on APOE status.^{3,23,29}

Plasma A β 40 and A β 42

Contrary to predictions, higher baseline total cholesterol was associated with lower levels of plasma A β 40 and A β 42. APOE ϵ 4 carriers had lowest levels of A β 40, followed by ϵ 2 and finally ϵ 3 carriers. Among the six genotype groups, ϵ 4/4 carriers had lowest levels of A β 40,

followed by $\epsilon 2/3$, $\epsilon 3/4$, $\epsilon 3/3$, and finally $\epsilon 2/4$ carriers. APOE $\epsilon 4$ carriers had lowest levels of A β 42, followed by $\epsilon 2$ and finally $\epsilon 3$ carriers. APOE $\epsilon 2/3$ carriers had lowest levels of plasma A β 42, followed by $\epsilon 4/4$, $\epsilon 3/4$, $\epsilon 2/4$, and finally $\epsilon 3/3$ carriers. Diagnosis was not associated with plasma A β 40, but those with MCI had lowest levels of A β 42, followed by AD and finally the CN group.

The literature suggests lower A β 42 and A β 42/A β 40 ratio in AD patients, which is also associated with higher A β load in the brain.¹²⁷ However, Lui et al. (2010) proposed that further research must be done on plasma biomarkers to determine whether they have a strong enough diagnostic value for AD.¹²⁷ Palmqvist et al., (2019) reported that plasma A β 40 and A β 42 declined as A β load in the brain increased, similarly to CSF A β 40 and A β 42.¹²⁸ Thus, our findings that higher cholesterol is associated with the plasma biomarkers in this sample are in line with the existing literature. The lack of relationship between diagnosis and plasma A β 40 in our sample may be affected by the time of plasma collection in relation to the course of AD, as plasma biomarkers in AD seem to decline over time similarly to CSF biomarkers.^{127,128} Furthermore, differences in A β 42, with the MCI group being the lowest and the CN group being the highest, are consistent with the declining levels of plasma biomarkers across stages of AD reported previously.^{127,128}

Global Cognitive Function

As predicted, higher total cholesterol was associated with lower global cognitive function based on participants' scores on the ADASCOG, MMSE, and CDR. However, there was no significant association between total cholesterol and MoCA. The relationship between APOE genotype and global cognitive function based on ADASCOG, MMSE, and CDR scores was also

consistent with predictions, as poorest cognitive function was associated with $\epsilon 4$ carriers, followed by $\epsilon 3$ and finally $\epsilon 2$ carriers. On the MoCA, $\epsilon 4$ carriers had poorest performance followed by $\epsilon 2$ carriers, and finally $\epsilon 3$ carriers. On the ADASCOG, MMSE, and CDR, APOE $\epsilon 4/4$ carriers performed poorly, followed by $\epsilon 3/4$, $\epsilon 2/4$, $\epsilon 3/3$, $\epsilon 2/2$, and finally $\epsilon 2/3$ carriers. On the MoCA, $\epsilon 4/4$ carriers performed worse, followed by $\epsilon 2/4$, $\epsilon 3/4$, $\epsilon 3/3$, and finally $\epsilon 2/3$ carriers. Additionally, those diagnosed with AD performed worse on all four assessments, followed by the MCI group, and finally the CN group.

The reported differences in global cognitive function based on genotype and diagnosis are generally consistent with existing literature.^{3,129-132} Poorer performance on ADASCOG, MMSE, and CDR of those with higher total cholesterol replicates prior findings^{72,74,76} and is indicative of the importance of maintaining healthy total cholesterol to potentially prevent AD-related cognitive decline.

Aim 3: Differences in Total Cholesterol by APOE Status

Total cholesterol levels were only different between APOE $\epsilon 4$ and $\epsilon 3$ carriers. APOE $\epsilon 2$ carriers had higher cholesterol than $\epsilon 3$ carriers. However, comparing all six genotypes, $\epsilon 4/4$ carriers had highest levels, followed by $\epsilon 2/4$, $\epsilon 3/4$, $\epsilon 2/3$, $\epsilon 3/3$, and finally $\epsilon 2/2$ carriers. Differences between $\epsilon 4/4$ carriers and all other genotypes except $\epsilon 2/4$ carriers met statistical significance. Using the six-group APOE categorization may therefore be most clinically meaningful, given that more informative differences in total cholesterol were revealed compared to the three-group categorization.

Our findings are largely consistent with the predicted differences in cholesterol by APOE status based on prior literature.^{3,23,28-31} However, the higher cholesterol of $\epsilon 2/3$ compared to $\epsilon 3/3$

carriers in this sample may show that APOE genotype alone does not determine blood cholesterol, in spite of differences between genotypes in ability to metabolize cholesterol. These somewhat unexpected trends in total cholesterol between APOE groups signify the importance of controlling for other factors which have been found to influence blood cholesterol, such as diet and exercise.^{28,29,32-49,56-63,133}

The difference in total cholesterol between females and males is further informative, as the notably higher levels in females could relate to the greater rates of AD observed in females compared to males.^{134,135} Sex differences in cholesterol have previously been examined, with females exhibiting higher cholesterol than males across all races.¹³⁶ This trend parallels sex differences in AD risk, as females also have higher prevalence of AD regardless of race.¹³⁷⁻¹³⁹ Further research into differential cholesterol metabolism or potential confounding factors may help elucidate the reason for the observed sex differences.

Aim 4: Time to AD Incidence Based on APOE Status and Baseline Total Cholesterol

Modeling time to diagnosis in this sample using Cox Proportional Hazards suggests that APOE status and age were the only significant predictors of time to AD onset. Baseline total cholesterol, cholesterol-lowering medication, and sex were not predictive of time to diagnosis. The lack of predictive value of baseline total cholesterol in this model may suggest that total cholesterol in later life is not a clinically meaningful predictor of AD. Perhaps total cholesterol during midlife (ages 40-45), rather than later life, is more predictive of AD risk.⁷⁰ However, the lack of significance of sex and cholesterol-lowering medication in predicting time to AD incidence is inconsistent with prior findings, where male sex¹⁴⁰⁻¹⁴² and use of statins seem to be protective.^{143,144} The significant associations in this study between high total cholesterol and AD

incidence, higher A β in the brain, lower CSF A β , lower plasma A β 40 and A β 42, and poorer cognitive function also contradict the lack of significance of cholesterol in this survival analysis.

Several problems revealed by diagnostic tests suggest that the Cox Proportional Hazards model may not allow for the best assessment of the influence of cholesterol, cholesterol-lowering medication, age, sex, and APOE status on time to diagnosis. Possibly the biggest indicator that this model may not be the most statistically appropriate approach is the violation of proportional hazards by cholesterol-lowering medication and APOE when using all six genotypes. Alternative survival analysis models may therefore be more informative regarding the influence of total cholesterol, age, sex, and APOE status on time to diagnosis.

Suggestions for Future Work

A future prospective study tracking both LDL and total cholesterol levels across adulthood in combination with APOE genotype, diet, oxysterols, and AD biomarkers would help elucidate how these variables interact to produce the underlying pathology of AD. Analyzing change in rate of biomarkers over time would also be a valuable addition to the literature, particularly in relation to APOE status, changes in cholesterol over time, diagnosis, and stage of AD. Further research on oxysterols is also needed to confirm the proposed role of oxidized cholesterol as a potential initiator of A β pathology in the brain.

In combination with findings from prior studies reporting a relationship between cholesterol, APOE status, oxysterols, diet, and AD, our results are consistent with the possibility that these variables may all contribute to AD pathology. A proposed mechanism integrating these factors is presented in *Figure 4*, depicting a potential pathway underlying one aspect of AD pathology. Avoiding high blood cholesterol altogether through minimal consumption of

cholesterol, saturated fat, and trans fat may be essential to prevent 27-OH from accumulating in the periphery and subsequently instigating A β pathology in the brain. The associations found in this study between the APOE ϵ 4 allele and high total cholesterol, AD incidence, cognitive function, and biomarkers in the brain, CSF, and plasma all reinforce the possibility that ϵ 4 carriers may benefit most from this dietary approach to maintaining healthy cholesterol levels. While the ADNI sample did not provide oxysterols, our significant findings regarding blood cholesterol, APOE, and AD incidence and biomarkers are potentially compatible with the mechanistic changes in oxysterols reported in prior studies. Future work should be oriented towards establishing the connection between the variables presented in this proposed mechanism.

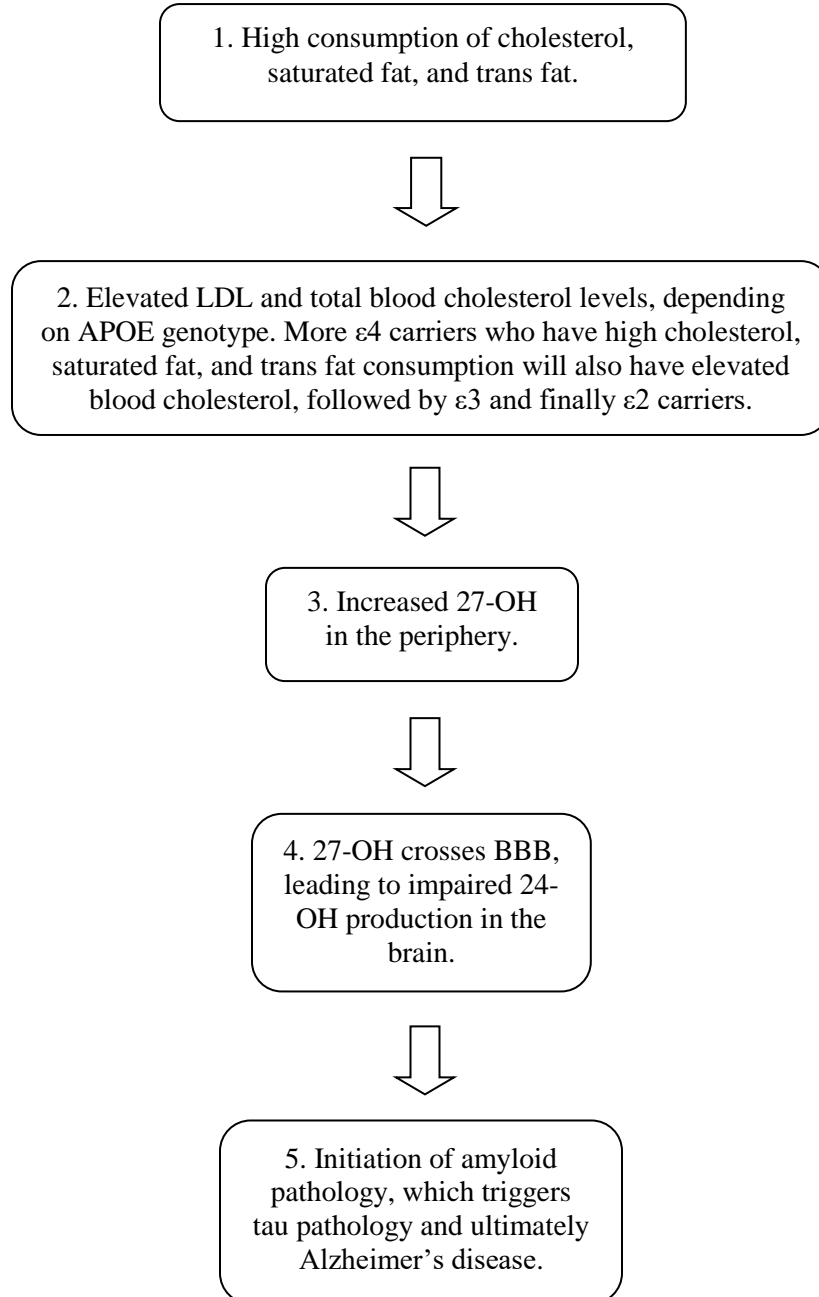


Figure 4. Proposed mechanism by which diet, blood cholesterol, APOE status, and oxysterol activity may influence risk of developing Alzheimer's disease. This chain of events is predicted to be dependent on APOE genotype between steps 1 and 2, such that $\epsilon 4$ carriers are most susceptible to changes in blood cholesterol as a result of cholesterol, saturated fat, and trans fat consumption. While this mechanism is proposed to contribute to the development of AD regardless of APOE genotype, it explains why $\epsilon 4$ carriers are expected to be at highest risk of the initiation of this chain of events given their greater vulnerability to the influence of dietary factors on blood cholesterol levels.

Study Strengths and Limitations

Some of the strengths of this study include a large sample of participants from across the U.S. and Canada. This sizeable sample included multiple biomarkers that are not otherwise readily available in many other studies, due to both cost and burden associated with data collection. Furthermore, this wealth of data was available over 15 years of follow-up, providing valuable information regarding the role of clinical and cognitive biomarkers in the development of AD and MCI. Given the growing literature on the link between vascular risk factors and AD, this study also offers a unique perspective on the critical role that cholesterol may play in the pathophysiology of AD.

The study also has several limitations. While most of this sample did not have a history of alcohol abuse, drug abuse, or psychiatric diagnosis, participants with such histories were not excluded from analyses, nor were these factors controlled for. There were also a number of missing values for multiple demographic and medical history characteristics. Furthermore, this sample is not representative of the general population in terms of APOE genotype. APOE ϵ 2 carriers made up 9.6% of this sample, which is close to the estimated global frequency of 7-8%.^{3,98} However, 46.5% of the sample consisted of ϵ 3 carriers and 43.8% were ϵ 4 carriers, compared to the estimated 78-79% and 14% for these alleles in the general population, respectively.^{3,98} This could be due to participant self-selection bias. Individuals aware of their own decline in cognitive function may have been more likely to volunteer, given ADNI's purpose of investigating AD. Family and caregivers noticing symptoms may also have encouraged these individuals to enroll. Statistically, participants exhibiting dementia-related symptoms are more likely to have an ϵ 4 allele, which could have led to this disproportionate amount of ϵ 4 carriers.

Prior findings highlight the importance of LDL cholesterol in relation to AD,^{29,61,71,73} yet only total cholesterol was available in this sample. Furthermore, cholesterol was only collected at baseline and not at follow-up appointments. Tracking change in cholesterol over time in conjunction with biomarkers and diagnostic status may provide crucial information regarding the precise nature of relationship between cholesterol levels and AD development. Additionally, the lack of information about nutritional intake of participants, particularly of foods containing saturated fat, trans fat, and cholesterol, prevented us from analyzing diet in relation to blood cholesterol, AD, and related biomarkers.

Another drawback is that many participants in this sample (46%) had cardiovascular disease at baseline. Of this group, 53% were taking cholesterol-lowering medication at baseline, and more may have been on other medications related to CVD or encouraged to engage in lifestyle changes to reduce vascular risk factors. However, while these changes may have led to lower total cholesterol following onset of CVD, perhaps AD pathology was initiated during progression of CVD when cholesterol levels were likely high. If that was the case, the lower cholesterol levels of these participants after beginning statin use or initiating lifestyle changes may be misleading our interpretation of results. This possibility emphasizes the importance of knowing cholesterol levels earlier in life, as well as a comprehensive medical history, to be able to track and properly understand the real-time progression of AD pathology in conjunction with cholesterol and other potentially relevant cardiovascular risk factors.

CONCLUSION

The rapid rise in AD incidence is becoming a major societal economic burden, in addition to the substantial impact it has on the quality of life of everyone affected.¹ Identification

of exact processes by which APOE influences the development of AD is critical for development of successful methods for prevention or treatment. The current study replicates the association between blood cholesterol and AD in a sample larger than that of most existing studies. This study also extends the literature by shedding more light on the association between cholesterol, APOE status, and AD by assessing biomarkers of AD in CSF, plasma, and the brain - rather than relying on only clinical diagnosis and cognitive function. Most importantly, the significant associations found between cholesterol and APOE status, diagnosis, global cognitive function, and A β in the brain, CSF, and plasma support prior findings regarding the impact that high cholesterol may have on risk for AD. Maintaining healthy blood cholesterol may therefore be a critical aspect of preventative care for AD, particularly for APOE ϵ 4 carriers.

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