Title Page

**Relationship between Cognition and Statin Use by Genotype at *APOE* and Three Proinflammatory Cytokine Loci in the Long Life Family Study**

by

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Abstract

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**ABSTRACT**

In the United States, greater than one-third of adults over the age of 40 years are using cholesterol lowering medication, and 90% of these medications are statins. The relationship between statin use and cognition is unclear: some studies report that statin use is associated with cognitive impairment, whereas other studies report that statins may have a protective effect against cognitive decline. Because cardiovascular disease and Alzheimer’s Disease (AD) are major public health issues, knowledge of the effects of statin use on AD is critical. In this study, I investigated the relationship between measures of cognition and statin use, as well as the interaction effect of statin use and genotype at *APOE* and three proinflammatory cytokine loci (*IL1B, IL6,* and *TNF⍺*), by analyzing data from the Long Life Family Study (LLFS) comprising of 1691 probands and their siblings (mean age=90yo), their 2439 offspring (mean age=61yo) and 808 spouses of the offspring (mean age=61yo). Cognitive outcome was measured by two quantitative and four verbal cognitive traits, as well as the Mini-Mental State Exam (MMSE) (in the proband generation only). Each generation was examined separately. Statin use was 16% among probands and 11% among offspring. As expected, mean cognitive scores for all measures were lower among probands than among offspring. Before adjusting for covariates, the study results showed significantly higher scores in probands taking statins for 3/7 cognitive traits (p≤0.005) and lower scores in offspring taking statins for 3/6 cognitive traits (p<0.02). After adjustment for age, sex, and education, statin use was only associated with cognitive measures digit forward in probands/siblings (p=0.038) and vegetable recall in offspring/spouses (p=0.001). Few significant genetic effects on cognition were seen, except *IL6*-174 in the probands/siblings and *TNFɑ*-308 SNP in the offspring/spouses with digit backward (p≤0.05), and *IL6*-174 and *IL1B*-31 by statin use interaction with animal recall in the probands/siblings (p<0.05). The above indicate additional studies on individuals with early stages of AD are needed. Because *APOE* is a significant risk factor in both cardiovascular disease and AD, additional pharmacological and pharmacogenetic studies of the effects of statins, particularly among *APOE* *ε4/ε4* homozygotes, are warranted.

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Preface

I would like to thank my advisor, Dr. Candace Kammerer for her mentorship and support throughout this project. I would also like to thank my essay reader, Dr. Allison Kuipers, for her feedback to help improve this essay and Dr. Susanne Gollin for her advice during the early stages of this essay. I also thank Dr. Ryan Minster for providing me with the data from the Long Life Family Study.

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# Introduction

Pharmacogenomics, the field that studies how an individual’s genotype may affect his/her response to specific drugs, has been gaining momentum over the last decade. Pharmacogenomics is a component of precision medicine, in which medical interventions and treatments are tailored to the individual’s known genetic variants and environmental factors. Genetic variation exists between individuals as well as between ethnic groups. This variation may affect whether an individual has a beneficial, adverse, or no response to a specific medication. Understanding how genetic variations affect the pathogenesis of a disease may inform the way disease is treated. Furthermore, understanding how genetics influences drug response may reduce the trial and error in finding the most effective drug [1]. The Food and Drug Administration (FDA) maintains a list of over 200 drugs with approved pharmacogenomic biomarker labels to help determine drug safety and effectiveness. This list is based on varying levels of evidence for genomic effect on clinical or adverse drug response, dosing, drug targets, and mechanism of drug action; approximately one-third of the drugs on this list have robust evidence and have a label either recommending or requiring genetic testing to determine prescription or dose [2]. Approximately one-third to one-half of the U.S. adult population takes prescription medication, thus pharmacogenomics knowledge might significantly impact the ability of healthcare professionals to prescribe drugs more effectively, as well as reduce the frequency of adverse reactions [3].

One drug that illustrates the potential of pharmacogenomic utilization is statins, a class of drug that is widely prescribed to treat high cholesterol. Although statin use has contributed to a reduction in cardiovascular events over the past several decades, recent evidence indicates that the use of certain statins may be related to myopathy, especially among individuals with a specific genotype at the *SLCOB1* locus. Consequently, the FDA developed recommendations on dosage for simvastatin as well as the Clinical Pharmacogenomics Implementation Consortium (CPIC) guidelines on the gene-drug interaction between simvastatin and the *SLCO1B1* genotype [4, 5].Warnings regarding other potential adverse events related to statin use, such as increased blood sugar levels and neurocognitive dysfunction, have been recently issued by the FDA as well [6]. However, the evidence for these adverse events remains inconclusive, thus warranting further investigation.

With regard to how statins affect cognition, several researchers have reported that statin use is associated with cognitive impairment, which is reversed once the drug is stopped, whereas others report that statin use is associated with decreased risk of dementia. In a literature review by Schultz *et al.* (2018), six studies and the FDA safety literature review with sample sizes ranging from 60 to 4867 participants found evidence supporting cognitive impairment with statins use, whereas six studies (with sample sizes ranging from 548 to 56,655 participants) reported no cognitive impairment with use [7].A handful of studies report that higher IL-6 levels are associated with cognitive decline and that statins may reduce IL-6 levels [8, 9]. However, little research has been done to connect these two concepts, namely that statins may improve cognition and reduce the risk of dementia by reducing IL-6 levels in the blood. Furthermore, research on the possible mechanism by which specific genes affect statin-induced neurocognitive events is scarce. Determining the effect of statins on neurocognition will allow better guidelines to be developed for the use of statins.

In an effort to fill the gaps in the literature, I explored the impact of statin use on cognitive function. I hypothesized that the use of statins is associated with cognitive function and may affect the risk of dementia; furthermore, this possible effect may vary by genotypes at three proinflammatory cytokine loci and/or *APOE*. If an association is found between statin use and cognition, further research of the mechanism of action may provide insights into new applications of statins. Furthermore, this knowledge would enable development of new and clear guidelines for statin use and facilitate optimization of statin drug therapy.

# Background

## Dementia

### Dementias and Aging

As individual lifespan increases worldwide, the prevalence of chronic and degenerative diseases will increase substantially. As of 2016, 15% of the U.S. population was over the age of 65, and this number is expected to continue to rise [10]. Dementias, primarily Alzheimer’s Disease (AD), are the fifth leading cause of death among individuals over the age of 65 in the U.S., affecting nearly 6 million people [10]. The time from onset of symptoms to death can be years to decades and most of this time the individual is dependent on a caregiver, placing a high emotional and financial burden on family members and a high economic burden on the country [11]. The cost to the healthcare system in the U.S. is astronomical, with nearly $130 million of the total cost of care being paid for by Medicare [10]. Healthcare is one of the largest drivers of the national deficit in the U.S.; thus the increasing economic burden of AD due to the aging U.S. population will likely further strain the healthcare system and increase the national deficit [12].

Innate inflammatory activation, such as elevated proinflammatory cytokines, may be prevalent among elderly individuals and may contribute to the cognitive decline associated with aging [13].Cribbs and colleagues compared the expression of genes involved in microglial activation and immune response in the brain among 22 younger individuals (age 20-59 years), 33 elderly individuals (age 60 to 99 years), and 26 AD individuals (age 74 to 95 years). They used DNA microarray to conduct mRNA expression profiling and measure differences in the expression of several proinflammatory cytokine genes in aging or AD; they then used qPCR to validate these age and AD related changes. They reported that the upregulation in genes involved in immune response was higher in the elderly compared to the younger individuals, with 64-86% upregulated with increasing age (p-value <0.01) [14]. This result suggests that the change in immune system activation typically seen in AD brains may begin with normal aging, accumulate over time, and eventually reach a threshold that leads to the onset of AD.

### Pathology of Alzheimer’s Disease

AD is a complex, multifactorial neurodegenerative disease with a genetic component. It is the most common type of dementia and currently affects over five million Americans, a number that is projected to increase by nearly three-fold over the next few decades [15]. The two main pathological hallmarks of AD are deposition of amyloid-β plaques in the extracellular space of the brain and the intracellular accumulation of tau neurofibrillary tangles.

The amyloid-β plaques are formed as a result of abnormal cleavage of the amyloid precursor protein (APP) by the β- and *𝛾*-secretases. About 90% of APP cleavage occurs through the nonamyloidogenic pathway, where ⍺- and *𝛾*-secretases cleave a soluble benign peptide 40 amino acids in length. However, the other 10% of APP cleavage occurs through the amyloidogenic pathway, where β- and *𝛾*-secretases cleave a larger, hydrophobic peptide 42 amino acids in length, which accumulates and forms insoluble plaques [16, 17].

The tau neurofibrillary tangles are a result of unstable microtubules. Microtubules are important structural components of axons and thus neuronal transportation. The tau protein binds to the microtubules to provide stability and support. However, in AD the tau protein is hyperphosphorylated and cannot bind the microtubules. As a result, the tau proteins aggregate and form tangles within neurons. Cognitive impairment occurs because the unstable microtubules collapse, destroying the axon’s transport and communication capability, and leading to neuronal death [17, 18].

While the genetics of AD, and all dementias, is complex and still being investigated, variants at several loci have been associated with susceptibility to AD. *APOE* is the primary gene found to be a heritable risk factor for AD. Three other genes, *APP, PSEN1,* and *TFF1,* have also been implicated in familial AD [17]. More recently, however, several researchers have reported that variation in loci involved in the immune response, including *IL1, IL4, IL6, IL10, TNF⍺,* and TGFβ, may also play a role in the pathogenesis of AD. These loci include those expressed by the microglia as well as those involved in expression of proinflammatory cytokines [19, 20].

## Dementias and Neuroinflammation

Research in the last decade has shown evidence that neuroinflammation may be part of the pathophysiology of dementia, especially AD. A handful of studies have shown that amyloid-β plaques and tau neurofibrillary tangles, the neuropathological hallmarks of AD, may activate microglia. Microglia are the resident macrophages, or immune cells, of the central nervous system (CNS) and, under normal circumstances, play a role in surveillance and homeostasis of the CNS. However, under abnormal circumstances such as injury or infection they activate the complement system to secrete pro-inflammatory cytokines and chemokines [20]. The presence of an immune response, specifically astrocytes and microglia, surrounding amyloid-β plaques has been reported in AD patients [21]. This immune response has been seen in very early stages of clinical AD and is thought to occur very early on in the pathogenesis of AD [22]. Researchers hypothesize that the inflammatory response of microglia may contribute to the formation of amyloid-β plaques and the subsequent neuronal damage and death. This appears to occur through a cyclic process where, in the early stages of AD, microglia may play a protective role by assisting in the clearance of amyloid-β. As AD advances and cognitive decline grows more severe, microglia are unable to efficiently clear the amyloid-β. Amyloid-β aggregates, which causes the microglia to increase secretion of proinflammatory cytokines and eventually function abnormally. When the microglia are unable to function properly, the CNS surveillance that is crucial for neuronal development and function is reduced, resulting in neuronal death [23].

## Genetic Involvement in Alzheimer’s Disease

### *APOE*

Apolipoprotein E (ApoE) is a protein responsible for the regulation of plasma cholesterol in the body and the transportation of cholesterol in the brain. In contrast to plasma cholesterol, which is primarily made in the liver, cholesterol in the brain is synthesized by astrocytes and microglia. Brain cholesterol is a main component of neurons and thus plays a critical role in the development and maintenance of neuronal function [24]. ApoE is encoded by the *APOE* gene which has three clinically relevant alleles: *ε2, ε3*, and *ε4*. The *ε3* allele is the most common, such that approximately three-quarters of the global population carry at least one *ε3* allele. The *ε2* is prevalent in approximately 8% of the global population and is thought to have a protective effect against AD. Inheritance of the *ε4* allele, which is prevalent in approximately 15% of the global population, predisposes an individual to increased risk for both cardiovascular disease and late-onset AD [24, 25]. Individuals who are homozygous for the *ε4* allele have an earlier age of onset of AD and have a higher frequency of AD.

These three alleles produce three protein isoforms, ApoE2, ApoE3, and ApoE4, caused by changes at amino acid position 112 or 158. The E2 isoform has cystine residues at positions 112 and 158, the E3 isoform has a cysteine residue at position 112 and arginine residue at position 158, and the E4 isoform has arginine residues at positions 112 and 158 [24, 26]. These differing amino acid sequences alter the structure and function of ApoE; in particular, they affect the ability of ApoE to transport and clear cholesterol in the brain [26]. The E4 isoform results in inefficient cholesterol regulation in the brain with aging and predisposes individuals to neuronal damage, reduced synaptic transmission, and neurodegeneration, consequently leading to impaired cognitive function. The mechanism by which the E4 isoform contributes to neurodegeneration is hypothesized to be a combination of inefficient cholesterol transportation and amyloid-β deposition. Cholesterol is necessary for neuronal development and function. The ApoE4 isoform is associated with inefficient transportation of cholesterol from astrocytes to neurons, leading to neuronal impairment and death. Additionally, the E4 isoform has been shown to increase β-secretase APP cleavage, leading to an increased production of the amyloid-β peptide [27].

While the *ε4* allele is a known risk factor for AD, the effect of the *ε4* allele on individuals without AD or dementia is not as clear. Several studies have shown that there may be an increased risk of cognitive decline associated with the *ε4* allele in non-demented individuals over the age of 65 as well [24]. One study by Bretsky *et al.* (2003) evaluated 965 participants of the MacArthur Successful Aging Study, a prospective study in which individuals aged 70-79 were selected on the basis of health status and high cognitive and physical functioning between 1987-1989. Bretsky *et al.* evaluated and compared performance of 227 ε4 carriers and 738 noncarriers on six cognitive measures at baseline, 3-year follow-up, and 7-year follow up. Results showed a significant association between cognitive decline and the *ε4* allele on two cognitive measures (naming and spatial ability) at the 3-year evaluation and 4 cognitive measures (memory, naming, figures, and similarity) at the 7-year evaluation [28]. These findings suggest that the *ε4* allele may, to a lesser extent than with AD, also be associated with cognitive decline in non-demented older adults.

Results from numerous studies since the late 1990s using either animal models or clinical and observational studies in humans have supported the hypothesis that cholesterol-lowering medications, such as statins, may reduce the risk of AD. The clinical and observational studies examined the effect of long-term statin use and *APOE* status on cognitive decline and dementia risk [29-33]. One study by Rockwood *et al.* (2007) investigated the risk of cognitive impairment in 347 non-demented individuals from the Canadian Study of Health and Aging with the use lipid-lowering agents alone and by *APOE* status. They found that statins, but not other lipid-lowering medications, significantly reduced the risk for cognitive decline; however, these results did not differ by *APOE ε4* status [31]. Another study by Geifman *et al.* (2017) used data from 18 previously reported clinical and observational studies to create a large integrated dataset of 4574 participants. They conducted an analysis of the effect of statin use alone, as well as the effect of statin use by *APOE* status on cognitive impairment in AD patients. Overall, the results showed a trend of less cognitive impairment in AD individuals homozygous for the *ε4* allele and taking statins compared to those not taking statins [29]. Although this design has its limitations, the benefit of pooling multiple study cohorts is that a larger sample size increases the ability to identify smaller subgroups, such as *APOE* ε4/ε4 homozygotes, who may have a different response to treatment.

A handful of studies using animal models have provided further evidence that the mechanism by which this response to statins occurs may be through modulation of APP activity and amyloid-β production [34, 35]. One study by Refolo *et al.* (2001) used hemizygous, amyloid-precursor protein and presenilin-1 double mutant (PSAPP) mice to determine the ability of cholesterol-lowering drugs to reduce the amyloid-β level. The results showed that both Aβ-40 and Aβ-42 levels significantly decreased with the treatment with the drug. Moreover, their results showed that lowering cholesterol levels reduced APP processing, indicating a possible mechanism by which cholesterol-lowering drugs may decrease AD risk [35]. Another study by Petanceska *et al.* (2003) demonstrated the potential role ApoE may play in AD pathology and risk. Using double transgenic PSAPP mice, they found that higher levels of ApoE were associated with increased amyloid-β levels, and that ApoE levels in mice treated with several different cholesterol-lowering drugs, including lovastatin and atorvastatin, were much lower than in mice not treated with the drugs [34]. These results indicate that statins may reduce AD risk by reducing amyloid-β accumulation in the brain, either through regulation of amyloid-β production (by affecting APP processing) or clearance.

### Proinflammatory Cytokine Gene Polymorphisms

Recently, research into the role of genes associated with the expression of proinflammatory cytokines, primarily *IL-1, IL-6, and TNF⍺*, on AD pathogenesis has increased. IL-6 is involved in the transition from innate to acquired immunity, B- and T-cell differentiation, and the recruitment and apoptosis of T-cells [36]. Researchers have assessed possible associations between several alleles at the *IL6* locus and dementia, but most focused on the *IL6*-174 G/C polymorphism. The G-allele is the common allele. The *IL6-*174 polymorphism is located in the promoter region, which affects regulation of the *IL6* gene and is thought to increase circulating plasma concentrations of IL-6 by regulating the transcription rate of the *IL6* locus [37]. The *IL1B*-31 T/C polymorphism is located in the promoter region and affects expression of *IL1B* and thus regulation of IL-1Bβ. IL-1Bβ is involved in the systemic immune system and stimulates the production of other proinflammatory cytokines such as IL-6 and IL-12 during inflammatory response [38]. TNF⍺ is a proinflammatory cytokine with varying roles, depending on the situation. When acute, TNF⍺ regulates the clearance of infectious agents, as well as upregulates adhesion molecules, sending macrophages and neutrophils to the site of infection or damage. However, prolonged exposure to TNF⍺ can be harmful [39]. *TNF⍺* is located within the major histocompatibility complex, which is largely implicated in many autoimmune and inflammatory diseases. The TNF⍺-308 G/A polymorphism is located in the promoter region and is involved in upregulation of the cytokine TNF⍺ [39].

Elevated levels of IL-6 have been implicated in the pathogenesis of several chronic and degenerative diseases. Several studies described below have found that there is an association between AD and increased circulation of these proinflammatory cytokines. Several studies in European, South American, and Asian populations have investigated the relationship between the risk of dementia, in most cases specifically AD, and allelic variants in genes associated with increased expression of cytokines IL-1, IL-6, and TNF⍺ [19]. However, much of this research has yielded conflicting results. The association between AD and *IL6* genotypes differed based on the population that was being studied, suggesting the possibility that genotype and haplotype effects on AD risk may vary by ethnicity.

For example, the studies conducted in Indian and Brazilian populations found that the *IL6*-174 C allele was associated with increased risk for AD, whereas studies in the Japanese and Italian populations found the G allele to be associated with increased risk. In contrast, other studies such as the Rotterdam Study, have reported no evidence of an effect of the *IL6-*174genotype on dementia. This study was a prospective cohort study of 7983 individuals over 55 years of age, conducted between 1990 and 2004, with the purpose of clarifying the risk factors associated with disease in the elderly. 6119 individuals without dementia at the start of the study had data on the *IL6-*174G>C polymorphism. For the individuals who were diagnosed with dementia or AD during follow-up, no significant association found with *IL6*-174 genotype: dementia GC/CC hazard ratio of 1.02/1.08; AD GC/CC hazard ratio of 1.05/1.15 [37].

In India, Mansoori and colleagues found that the *IL6*-174G allele is associated with a protective effect, whereas the C allele associated with an increased risk. The study, which consisted of 74 AD individuals and 46 vascular dementia (VaD) individuals (mean age 66 years; average 30% female, 70% male), and 113 healthy controls (mean age 64 years; 36% female, 64% male), analyzed the correlation between dementia and the *IL6*-174 polymorphism alone as well as the interaction of the *IL6-*174 polymorphism with the *APOE* *ε4* allele. No direct causal relationship was observed between the *IL6*-174 *C* allele and AD or VaD, but the frequency of the C allele was higher (p-value 0.021) and the frequency of the GG genotype was lower (p-value 0.026) in VaD individuals compared to controls. In addition, the influence of *IL6*-174 *C* allele combined with the *APOE* *e4* allele was found to increase the risk of both AD (p-value 0.001, OR 13.75) and VaD (p-value 0.001, OR 14.74) [40].

A study in the Brazilian population comprising 200 AD individuals who were at least 65 years of age (mean age 75.31; 70 male and 170 female) and 165 elderly controls (mean age 71.67; 55 male and 110 female) also found an association between AD and the *IL6*. The investigators reported no significant effect of the *IL6* genotype individually on AD (p-value 0.48); however they observed that the *IL6-*597A/174G haplotype was significantly associated with a protective effect on AD (p-value 0.0023, OR 0.15) [41].

In contrast to the studies in Brazil and India, researchers in Italy and Japan reported that the G allele was associated with increased risk for AD and that the C allele is protective. Using data on 128 AD individuals (mean age 70.5 years) and 83 healthy controls (mean age 69.1 years) from the Japanese population , investigators reported that the frequency of the *G* allele as well as the GC genotype in the *IL-6* promoter region was significantly higher in AD individuals compared to controls (p-value 0.03 and 0.00005, respectively) [42]. This finding indicates that the IL6-174G haplotype may be associated with an increased risk for AD.

A study in the Italian population by Flex and colleagues investigated SNPs of eleven genes involved in the expression of proinflammatory cytokine, including *IL6-*174 G/C, *ILIB*-31 T/C, and *TNF⍺-*308 G/A. The study consisted of 533 individuals (mean age 76.6 years; 180 males and 353 females) with sporadic AD and 713 controls (mean age 76.7 years; 268 males and 445 females). The participants were also matched for comorbidities. The results showed that the *IL6-*174 GG genotype was associated with a nearly 4-fold increased risk for AD (OR 3.92, p-value 0.001), suggesting a finding similar to the Japanese study that the *G* allele may increase risk for AD. The study also showed a nearly 3-fold increased risk associated with the *IL1B-*31 TT genotype (OR 2.78, p-vale 0.022). Six other genes, *CCL2*, *CCL3*, *SELE*, *ICAM1*, *MMP3*, and *MMP9*, that encode proinflammatory cytokines were found to also be significantly associated with AD. However, unlike other studies discussed later, no significant association was found between the *TNF⍺-*308 SNP AD risk [43]. The results of this study suggest that numerous genes involved in proinflammatory cytokine production and expression are likely involved in AD pathology.

Epigenetic functions may also be important in regulating the CNS [44]. IL-1 is a proinflammatory cytokine thought to be involved in the progression of dementias through epigenetic changes. Because microglia play a critical role in surveillance of the CNS, as well as in normal function and survival of neurons, epigenetic modification of genes that regulate the expression of microglia may lead to cognitive decline. The mechanism by which this occurs is not well understood. However, researchers think that aging is associated with reduced efficiency of microglia and may be correlated with the epigenetic regulation, specifically hypomethylation, of *IL-1β* [13].

A few studies have also implicated TNF⍺ as a potential contributor to cognitive decline, especially in the pathogenesis of AD. In AD brains, the *TNF⍺* gene has been shown to be overactive, and several studies hypothesize that in AD, amyloid-β (Aβ) may activate microglia, which then induce proinflammatory cytokine secretion, including TNF⍺ [19]. However, hypotheses about mechanisms by which TNF⍺ further exacerbates AD pathology vary. For example, Medeiros and colleagues hypothesize that TNF⍺ may be involved in the activation of additional downstream inflammatory pathways that further increase amyloid-β plaques and tau neurofibrillary tangles, such as the upregulation of *COX-2* (official symbol *PTGS2*). This study administered Aβ1-40 to TNFR1 (official symbol TNFRSF1A) knockout mice after treating them with either PBS (the placebo) or AbTNF⍺ (an anti-TNF⍺ antibody) and NS398 (a *COX-2* inhibitor), then 1) allowed the mice to become familiar with a water maze, which acted as the memory reference, then 2) 24 hours later tested their ability to find the platform in the maze. They found that mice treated with AbTNF⍺ and NS398 had significant improvements in cognitive deficits caused by Aβ1-40 compared to those treated with the vehicle [45]. By demonstrating that inhibition of TNF⍺ improves cognitive function, these findings support the hypothesis that TNF⍺ is involved in the pathogenesis of AD.

A study by Vural *et al.* further supports the hypothesis that the pro-inflammatory cytokines IL-1, IL-6, and TNF⍺ may increase the risk for AD. In a study of 101 individuals with sporadic AD (ages 65-99 with mean age 77.3; 65% female, 35% male) and 138 controls (ages 62-95 with mean age 73; 67% female, 33% male) in the Turkish population, polymorphisms in the promoter region of *TNF⍺-*308, *IL6-*174, and *IL10-*1082 were analyzed. The *IL6*-174 genotype was not associated with increased AD risk; however, the *TNF⍺-308* AA genotype (OR 2.2, p=0.27) and the *IL10-*1082 AG genotype (OR 2.15, p=0.01) were associated with increased risk for AD by two-fold, although the OR for TNF⍺-308 SNP was not significant. Furthermore, the combination of the *TNF⍺-*308 *A* allele and either the *IL10-*1082 *A* allele or the *IL6-*174 *C* allele was found to significantly increase risk for AD (OR 6.5 and 2.94, respectively)[46]. These findings support the hypothesis that these pro-inflammatory cytokines are involved in the cognitive decline associated with AD. Additionally, they suggest that there may be a compounding effect of having at least two of the risk alleles.

Liao *et al.* (2004) further investigated the mechanism by which TNF⍺ contributes to AD pathogenesis; their results suggest that elevated levels of TNF⍺ may lead to the disruption of β-secretase and *𝛾*-secretase.They used transfected cells optimized for a *𝛾* -secretase assay to understand the mechanism of signaling cascades involving proinflammatory cytokines that might regulate the cleavage of APP by *𝛾* -secretase. They found that stimulation using TNF⍺ significantly increased the activity of *𝛾*-secretase by approximately 100% and the production of Aβ by 160% (1.6-fold increase) [47, 48].They further found that this regulation is elicited through the JNK-dependent MAP-kinase signaling pathway, indicating either JNK or TNF⍺ as a potential target for AD therapy [47].

Another study by Yamamoto *et al.* (2007) further supports the conclusion that TNF⍺ stimulates APP cleavage. The study used mutant transgenic mice and found that TNF⍺ stimulates the expression of the β-site APP cleavage enzyme (BACE1) and thus increases production of amyloid-β. The study also reported that activation of TNF⍺ inhibits the ability of microglia to clear amyloid-β, further compounding the pathogenesis of AD [49].

In summary, three proinflammatory cytokine loci, *IL1B1*-31, *IL6*-174, and *TNF⍺-*308, are hypothesized to be involved in the pathogenesis of cognitive decline, especially with AD. Previous studies have reported conflicting results of the relationship between allelic variants at these loci and risk of cognitive decline and suggest this relationship may vary by ethnicity. However, further research using larger sample sizes is still needed to resolve the divergence in these previously reported study results.

## Statins

### Cardiovascular Disease

Statins are a class of drug used to lower cholesterol. Cholesterol level is controlled primarily by hepatocytes. Hepatocytes uptake LDL-cholesterol from the blood circulation through the use of various apolipoproteins, lipoprotein receptors, and lipid processing enzymes. The LDL-receptor gene regulates the number of receptors on the hepatocytes, and the expression of LDL receptor gene is regulated by amount of cholesterol in the blood. When the intracellular cholesterol level is low, expression of the LDL-receptor gene increases, leading to increased production of LDL-receptors. A larger number of receptors enable increased uptake of extracellular cholesterol from the blood, and thus lowered blood LDL-cholesterol level [50, 51]. In addition to LDL-receptors, the enzyme HMG-CoA reductase controls the intracellular rate of cholesterol synthesis. When active, there is an increased production of cholesterol, which decreases the expression of LDL-receptor gene and reducing cholesterol uptake into the liver (and thus increasing the blood LDL-cholesterol level). Statins work by inhibiting HMG-CoA reductase, which lowers intracellular cholesterol synthesis, leading to an increased number of LDL-receptors [51]. This increase allows for increased extracellular cholesterol uptake into the liver, thereby reducing the level of cholesterol in the blood.

### Current Pharmacogenomic Applications of Statins

As of 2013, nearly 30% of adults over the age of 40 and 40% of adults over 65 in the U.S. were taking a cholesterol lowering medication, approximately 93% of whom were taking a statin [52, 53]. Given that statin use is so prevalent, understanding other potential effects of the drug is crucial. Evidence of adverse effects among people taking statins prompted the FDA to introduce drug safety warnings for statins in 2012. These side effects included serious liver injury, cognitive impairment such as memory loss and confusion, increased blood-glucose levels, muscle injury, and contraindications [6].

One specific adverse effect was the development of myopathy with the use of the drug simvastatin. The *SLCO1B1* gene encodes the anion transporter OATP1B1, which regulates the hepatic uptake of statins. A single-nucleotide polymorphism (SNP) in the *SLCO1B1* gene, c.521T>C, has been shown to be associated with statin-induced myopathy. This particular variant results in reduced ability of the transporter to localize at the plasma membrane, which reduces the hepatic uptake of statins. As a result, statins are not efficiently cleared from the blood, thus increasing the plasma concentration of statins[54]. Statin-induced myopathy is dose-dependent, and primarily limited to simvastatin use. CPIC has developed guidelines for simvastatin based on genotype. As standard practice, physicians prescribe a starting dose of 80mg for simvastatin. The 2014 CPIC guidelines recommend that individuals with the *SLCO1B1* TC or CC genotype should either be prescribed a lower dose or should consider a different statin [54].

### Statins and Dementia

Statins may have pleiotropic effects, that is, the ability to have more than one beneficial effect. For example, in addition to lowering cholesterol, statins may also protect against dementia, especially after long-term statin use in patients who were not cognitively impaired at the start of treatment [7].

The hypotheses regarding the molecular mechanism by which statins may have a protective effect against dementias vary. One hypothesis is that statins reduce amyloid-β secretion by reducing the expression of amyloid precursor protein (APP) and phosphorylated APP (P-APP) [55]. Another hypothesis is that statins inhibit activation of microglia in the CNS [56].By inhibiting microglia activation, proinflammatory cytokine production associated with aging would be reduced and the resulting microglial dysfunction would be minimized. If microglia were able to continue functioning properly, a protective effect against cognitive decline would be established because CNS surveillance, neuronal function and neuronal survival would be unaffected.

### Statins and Proinflammatory Cytokines

In addition to the cholesterol-lowering capability of statins, statins are also thought to produce an anti-inflammatory response that may reduce the risk of AD. This is hypothesized to occur through the lowering of proinflammatory cytokines levels (released from microglia) in the brain, which may then reduce amyloid deposition and neurofibrillary tangles [57].

In a study by Zhang *et al.* (2013), AD rat models were treated with atorvastatin, and their cognitive ability tested using the Morris Water Maze. Immunohistochemistry staining of IL1β, IL6, and TNF⍺ was also performed to determine expression of these proteins in brain tissue. The study found that the escape latency of atorvastatin treated AD rats was significantly shorter than non-treated AD rats, indicating that statins may be able to decrease impaired learning and memory cognitive impairment associated with AD. Furthermore, the study not only showed that the AD rats had significantly higher levels of IL1β, IL6, and TNF⍺ compared to the control group, but the atorvastatin treated AD rats had reduced levels of these cytokines compared to the AD group [58].

Another study by Boimel *et al.* (2009) used transgenic mouse models of neurofibrillary tangles to examine the relationship between microglia and AD pathological hallmarks and effect of statins on AD pathology. They used immunohistochemistry to detect phosphorylated tau and a T-maze to assess cognitive function (spatial short-term memory). They found that short-term simvastatin treated mice had significantly reduced burden of neurofibrillary tangles (25-31% reduction, p<0.02), reduced microglial burden (28.7% reduction, p<0.0001) and better T-maze performance (p=0.049) compared to non-treated mice, and that the difference was even greater in mice that were treated at a younger age (before the onset of neurofibrillary tangles) and for a longer duration (30% reduction in tangles, p=0.005). Mice treated with atorvastatin also showed significant reductions in tangles (53-59%, p<0.0001) and in microglial burden (20.3% reduction, p<0.0001). They also showed that there was a strong positive correlation between microglial burden and neurofibrillary tangles (R=0.8) [59]. These results indicate that statins may inhibit microglial function, leading to reduced tauopathy in AD.

A study by Li *et al.* (2007) that performed brain autopsies on 110 AD participants (36 of whom were statin users) aged 65-79 years from the Adult Changes in Thought Study further supports statin-induced reduction in neurofibrillary tangles. They found that statin users had a significantly lower burden of both plaques and neurofibrillary tangles and an overall lower risk for AD pathology (as determined by Braak stage and CERAD rating; OR=0.20, 95% CI 0.05-0.86) [60]. All of the above studies support the hypothesis that the anti-inflammatory properties of statins may reduce the pathology of AD.

# Specific Aims

Based on the studies above, the possible beneficial (or adverse) effects of statin use on cognitive function is unclear. Furthermore, the relationship between statin use and variants at the *APOE* locus and proinflammatory cytokine loci is unclear. My overall hypothesis was that statin use was associated with improved cognitive function and that this effect differed among individuals with different genotypes at the *APOE* locus and the three proinflammatory cytokine loci. To test this hypothesis, I used genotype and phenotype data on 4938 participants from the Long Life Family Study (LLFS), a two-generation family study consisting of 4953 participants. Because of the large difference in mean age between the two generations (90 versus 61 years old) and the well-known strong relationship between age and cognition, the analyses were done on each generation separately.

## Specific Aim 1

To assess whether statin use is associated with cognition, I:

Analyzed the effect of several covariates, include age, sex, and education on cognitive measures by each generation separately.

Added statin use/non-use to the above model to assess whether the measures of cognition differ between statin users and non-users.

## Specific Aim 2

To assess whether possible effects of statin use on cognitive measures differs among genotypes at *APOE* and three proinflammatory cytokine loci, I:

Calculated the frequencies of genotypes at each of the four loci and tested for Hardy-Weinberg equilibrium.

Used general linear univariate models to assess the relationship between specific genotypes at these loci and cognitive outcomes (while simultaneously incorporating effects of age, sex, and education).

Added a variable for statin users and non-users, as well as an interaction term for statin use and each of the four genotypes.

# Methods

## Description of the Data Set

Data from the Long Life Family Study (LLFS) was used to test these hypotheses. LLFS is a collaboration among five research institutions to understand the genetic and environmental factors associated with longevity and healthy aging. The study consists of phenotypic measurements including physical activity and cognition, blood measurements, body measurements, information on medications taken, and genotype.

The initial recruitment phase of the Long Life Family Study took place in the United States and Denmark between 2006 and 2009. Four universities, University of Pittsburgh, Columbia University, Boston University, and University of Southern Denmark were involved in the recruitment and research for the study. The study consisted of 539 families that spanned two generations, including the older proband/sibling generation, their children, and the children’s spouses (Figure 1), for a total of 4953 people [61]. Participants were recruited based on elderly probands, mostly 90 years or older. These probands reported information on the longevity of their family, specifically of their parents and siblings. From these reports, families that showed exceptional longevity were selected to participate in the study. Exceptional longevity was determined based on the family longevity selection score (FLoSS). FLoSS is a combination of two elements, “1) an estimated family longevity score built from birth, gender, and nation specific cohort survival probabilities and 2) a bonus for older living siblings” [62].



Figure 1 Minimum Family structure of participants in the LLFS

## Data Analysis

To assess the relationship of statins and genotypes with cognitive outcomes, the following cognitive traits measured in the LLFS study were analyzed: animal recall, vegetable recall, digit span forward, digit span backward, immediate memory test, delayed memory test, and the Mini-Mental State Exam (MMSE) score [63]. A higher score on each test indicates better cognition. Analysis of MMSE was only done in the probands/sibling generation because it is not a useful measure of cognition among younger individuals. Three covariates known to affect cognitive measures – age, sex, and education level – were incorporated into the analysis models. Next, the possible associations between the cognitive traits, statin use, and genotypes at *APOE* and the three proinflammatory cytokine genes, *TNF⍺, IL6,* and *IL1B*, were investigated.

IBM SPSS Statistics software was used to analyze the data. To asses my hypothesis that statin use is associated with cognition, I first analyzed the possible effects of several covariates (age, sex, and education level) on the seven measures of cognitive function within each LLFS generation separately using one-way ANOVA. I next added a variable for statin use/non-use into the model to assess whether the measures of cognition differ between statin users and non-users.

To test my hypothesis that possible effects of statin use on cognitive measures differs among genotypes at *APOE* or at each of the three proinflammatory cytokine loci separately, I first calculated the frequencies of genotypes at *APOE* and each of the three loci and tested for Hardy-Weinberg equilibrium. Second, I used the general linear model approach to assess the relationship between the cognitive measures statin use. Next, I included age, sex, and education level as covariates in this model. Finally, for each cognitive measure I assessed the effects of age, sex, education, statin use, genotype, and statin use by genotype for each of the four loci separately.

# Results

## Characteristics of the Population

A total of 4,938 participants were enrolled in the study, split between two generations. Characteristics of the study population are provided in Table 1. The second generation consisted of 1,691 probands and their siblings, of whom 44.5% were male and 55.5% were female, with an average age of 89.59 years. The third generation comprised of the offspring of the second generation and their spouses (the spouses served as controls for the original study); the average age was 60.62 years and were 45.0% male and 55.0% female. Overall, the study population was largely of European descent (approximately 99% of participants were Caucasian). Statin use varied across generations; with 16.1% statin use in generation two and 10.7% statin use in generation three. As expected, mean cognitive scores were higher across all measures in the offspring generation.

The study population was tested for Hardy-Weinberg equilibrium (HWE) in *APOE* and the three proinflammatory cytokine loci, stratified by proband/sibling and offspring/spouse generations. A Chi-squared Goodness of Fit test was used to determine HWE (Table 1). Neither generation was in HWE for any of the four loci (p<0.001). Because of primary interest in the ε3 and ε4 alleles, ε2 groups were excluded from all subsequent analyses.

Table 1 Characteristics of the study population

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristic** | | **Probands & Siblings**  **[Gen 2]**  **(N = 1691)** | | **Offspring & Spouses**  **[Gen 3]**  **(N = 3157)** |
| Age in years,  Mean ± std.dev. | | 89.59 ± 6.81 | 60.62 ±8.39 | |
| Sex, % F | | 55.5 | 55.0 | |
| Statin Use, % | | 16.1 | 10.7 | |
| Education level, % | ≤ High School | 47.8 | 14.3 | |
| > High School | 52.2 | 85.7 | |
| **Cognitive Data** | | **mean ± std.dev.** | | |
| Digit Forward | | 7.44 ± 2.24 | 8.54 ± 2.19 | |
| Digit Backward | | 5.32 ± 2.15 | 6.75 ± 2.33 | |
| Animal Recall | | 14.04 ± 5.33 | 22.12 ± 5.79 | |
| Vegetable Recall | | 10.17 ± 4.22 | 15.09 ± 4.44 | |
| Immediate Memory | | 8.01 ± 4.50 | 13.20 ± 3.97 | |
| Delayed Memory | | 5.92 ± 4.55 | 11.77 ± 4.31 | |
| MMSE | | 23.35 ± 4.28 | N/A | |
| **Genetic Data** | | **count (%)** | | |
| *APOE* genotype | | **N=1562** | **N=3102** | |
| ε2/ε2 | | 11 (0.7) | 22 (0.7) | |
| ε2/ε3 | | 254 (16.3) | 441 (14.2) | |
| ε2/ε4 | | 29 (1.9) | 58 (1.9) | |
| ε3/ε3 | | 1073 (68.7) | 1966 (63.4) | |
| ε3/ε4 | | 190 (12.2) | 572 (18.4) | |
| ε4/ε4 | | 5 (0.3) | 43 (1.4) | |
| HWE Chi-square value  (p-value) | | Χ2=3250.80  (p<0.001) | Χ2=5394.17  (p<0.001) | |
| IL6-174 genotype  (SNP rs1800795) | | **N=1533** | **N=3044** | |
| CC | | 261 (17.0) | 539 (17.7) | |
| CG | | 703 (45.9) | 1447 (45.9) | |
| GG | | 569 (37.1) | 1058 (37.1) | |
| HWE Chi-square value  (p-value) | | Χ2=201.0  (p<0.001) | Χ2=307.3  (p<0.001) | |
| IL1B-31 genotype  (SNP rs1143634) | | N=1533 | N=3044 | |
| AA | | 75 (4.9) | 159 (4.9) | |
| AG | | 561 (36.6) | 1106 (36.3) | |
| GG | | 897 (58.5) | 1779 (58.4) | |
| HWE Chi-square value  (p-value) | | Χ2=668.5  (p<0.001) | Χ2=999.3  (p<0.001) | |
| TNFα-308 genotype  (SNP rs1800629) | | **N=1473** | **N=2920** | |
| AA | | 34 (2.3) | 65 (2.0) | |
| AG | | 337 (22.9) | 788 (27.0) | |
| GG | | 1102 (74.8) | 2067 (63.3) | |
| HWE Chi-square value  (p-value) | | Χ2=1233.9  (p<0.001) | Χ2=1632.9  (p<0.001) | |

## Statins and Cognition

I first assessed, within each generation, whether statin use varied by sex, age, or education (Table 2). Mean age of statin users was higher in the generation 3 compared to non-users (64.2 years vs. 60.2 years), whereas in generation 2 mean age of statin users was lower than non-users (87.2 years vs. 90.1 years); this difference was statistically significant in both generations (p<0.001). Statin use was higher among males in both generations: 58.8% in generation 2 and 52.1% in generation 3; this difference was statistically significant for both generations (p≤0.005). Statin use differed across education level within each generation; the proportion of statin users who received education beyond high school was: 55.4% of probands/siblings and 79.6% of offspring/spouses; this difference was statistically significant in the offspring/spouses (p=0.001) but not in probands/siblings (p=0.257). I also used t-tests to assess whether means of the cognitive measures varied between statin users and non-users, within generation (Table 2). All cognitive measures were higher in probands taking statins compared to those not taking statins; these differences were statistically significant for digit forward (p-value<0.001), digit backward (p-value=0.005), and MMSE (p-value <0.001). In contrast, all cognitive measures were lower for the offspring and spouses taking statins compared to those not taking statins and were statistically significant for digit backward (p-value=0.015), animal recall (p-value=0.008), and vegetable recall (p-value=0.011) (Figure 2).

Table 2 Statin use/non-use by age, sex, education, and measures of cognition

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Probands & Siblings**  **[Gen 2]** | | | **Offspring & Spouses**  **[Gen 3]** | | |
| **Statin User:** | | **No**  N=1419 | **Yes**  N=272 | **p-value** | **No**  N=2898 | **Yes**  N=349 | **p-value** |
| **Sex,** % F | | 58.3 | 41.2 | **<0.001** | 55.8 | 47.9 | **0.005** |
| **Age,** mean ± std.dev. | | 90.1 ± 6.8 | 87.2 ± 6.3 | **<0.001** | 60.2 ± 8.4 | 64.2 ± 7.3 | **<0.001** |
| **Education Level,** % | ≤ High School | 48.4 | 44.6 | 0.257 | 13.5 | 20.4 | **0.001** |
| > High School | 51.6 | 55.4 | 86.5 | 79.6 |
| **Cognitive Test**,  mean | Digit Forward | 7.35 | 7.93 | **<0.001** | 8.55 | 8.43 | 0.328 |
| Digit Backward | 5.25 | 5.66 | **0.005** | 6.78 | 6.46 | **0.015** |
| Animal Recall | 13.95 | 14.51 | 0.115 | 22.21 | 21.35 | **0.008** |
| Vegetable Recall | 10.10 | 10.54 | 0.084 | 15.16 | 14.51 | **0.011** |
| Logical Immediate Memory | 7.99 | 8.10 | 0.741 | 13.24 | 12.90 | 0.134 |
| Logical Delayed Memory | 5.90 | 6.05 | 0.616 | 11.82 | 11.38 | 0.072 |
| MMSE | 25.18 | 26.25 | **<0.001** | N/A | N/A | N/A |

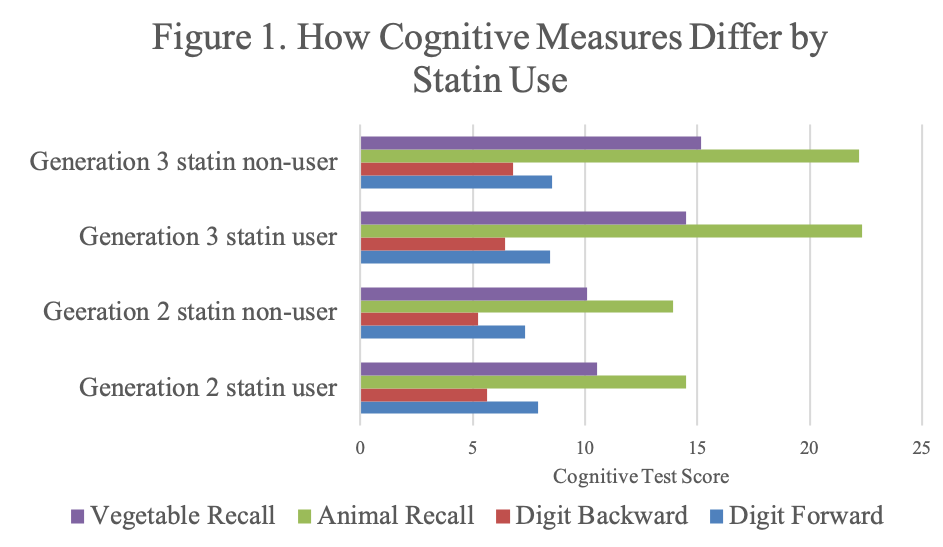


Figure 2 Cognitive Measures by statin use

I then analyzed the effect of three covariates, age, sex, and education, on cognitive measures within each generation. Age and education level were significantly associated with all cognitive measures in both generations (p≤0.009 and p<0.001, respectively), and sex was significant for four out of six cognitive measures in the proband/sibling generation (p≤0.02) and five out of six of the measurers in the offspring/spouse generation (p<0.05). Because age, sex, and education were significantly associated with cognitive outcomes and statin use, I analyzed the effect of statins on cognition including the covariates age, gender, and education (Table 3). After inclusion of the covariates in the models, statin use was only associated with the cognitive measure digit forward in the proband/sibling generation (p=0.038).

Table 3 Effect of statin use on cognitive outcome (including confounding variables in the analysis)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Probands & Siblings**  **[Gen 2]** | | | **Offspring & Spouses**  **[Gen 3]** | | |
| **Statin User:** | | **No**  N=1419 | **Yes**  N=272 | **p-value** | **No**  N=2898 | **Yes**  N=349 | **p-value** |
| **Cognitive Test**,  mean | Digit Forward | 7.40 | 7.70 | **0.038** | 8.53 | 8.54 | 0.985 |
| Digit Backward | 5.30 | 5.47 | 0.210 | 6.76 | 6.60 | 0.228 |
| Animal Recall | 14.11 | 13.67 | 0.173 | 22.14 | 21.98 | 0.612 |
| Vegetable Recall | 10.16 | 10.27 | 0.656 | 15.09 | 15.08 | 0.977 |
| Logical Immediate Memory | 8.09 | 7.62 | 0.098 | 13.16 | 13.28 | 0.706 |
| Logical Delayed Memory | 6.00 | 5.55 | 0.118 | 11.76 | 11.85 | 0.708 |
| MMSE | 25.31 | 25.59 | 0.311 | N/A | N/A | N/A |

A Chi Square Test for Independence was used to determine the association between statin use and *APOE* genotype for individuals with *ε3* or *ε4* alleles (Table 4). Some of the cells had expected values less than five, so a Fisher’s Exact test was done instead of the standard Chi-square test. Statin use was significantly associated with *APOE* genotype for the offspring generation: 3.9% of statin users were homozygous ε4/ε4, whereas only 1.1% of nonusers were homozygous ε4/ε4(p=0.001).

Table 4 Frequency of statin use by APOE status

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***APOE* status** | **Probands & Siblings**  N=1268 | | **Offspring & Spouses**  N=2581 | |
| Statin use: | non-user | user | non-user | user |
| **ε3/ε3** | 895 (68.3%) | 178 (70.9%) | 1761 (63.6%) | 205 (61.9%) |
| **ε3/ε4** | 149 (11.4%) | 41 (16.3%) | 493 (17.8%) | 79  (23.9%) |
| **ε4/ε4** | 4  (1.6%) | 1  (0.4%) | 30  (1.1%) | 13  (3.9%) |
| p-value | 0.175 | | **<0.001** | |

## Pharmacogenetic Effect of Statins on Cognition

To determine if the relationship between statin use and cognition varied by genotype in *APOE* and three proinflammatory cytokine loci, *IL6*-174 (SNP rs1800795), *IL1B*-31 (SNP rs1143634), and *TNFɑ*-308 (SNP rs1800629), a general linear model was used. For each of the six cognitive traits, the following variables were included in the model: age, sex, education, statin use, each locus (separately), and locus by statin interaction. When *APOE* and each proinflammatory cytokine locus was analyzed for association with cognition alone (i.e. without the statin interaction), the majority of loci were not significant predictors of cognition (except *IL6*-174 and *TNFɑ*-308 were significant predictors of the digit backward trait (in the proband/sibling generation and the offspring/spouse generation, p=0.050 and p=0.034 respectively). Similarly, the only significant loci by statin interaction effect on cognitive measures were *IL6-*174 and *IL1B1*-31 statin on animal recall in the proband/sibling generation, p=0.021 and p=0.047, respectively. With the exception of the delayed memory trait in the proband/sibling generation, (p=0.042; Figure 3), I observed no significant interaction effect of *APOE* and statins on the cognitive measures (Table 5).

Table 5 Analysis Results (p-values) of statin by genotype interactions for each cognitive trait by generation (after adjusting for age, sex, and education)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Probands & Siblings** | | | **Offspring & Spouses** | | |
|  | Statin | *APOE* | Statin x *APOE* | Statin | *APOE* | Statin x *APOE* |
| **Digit Forward** | 0.333 | 0.559 | 0.643 | 0.777 | 0.006 | 0.159 |
| **Digit Backward** | 0.477 | 0.046 | 0.533 | 0.584 | 0.053 | 0.202 |
| **Animal Recall** | 0.189 | 0.334 | 0.507 | 0.572 | 0.995 | 0.701 |
| **Vegetable Recall** | 0.427 | 0.094 | 0.494 | 0.380 | 0.632 | 0.763 |
| **Immediate Memory** | 0.730 | 0.721 | 0.243 | 0.746 | 0.238 | 0.302 |
| **Delayed Memory** | 0.321 | 0.749 | **0.042** | 0.866 | 0.146 | 0.176 |
| **MMSE** | 0.727 | 0.261 | 0.852 | N/A | N/A | N/A |



Figure 3 Effect of statin x *APOE* interaction on Delayed Memory in the Proband/Sibling Generation (adjusted for age, sex, and education)

# Discussion

## Conclusions of the Study

Frequency of statin use was significantly higher among men compared to women in both the proband/sibling generation and the offspring/cohort generation. In contrast with the probands/siblings, statin use was significantly lower in offspring/spouses who received post-high school education. The reasons for this observation are unknown, but I postulate that the younger generation may have had better access to higher education, and thus may have more knowledge of medical treatments and better access to health facilities.

Before the inclusion of other covariates, my results indicated higher cognitive scores among older individuals (the probands) taking statins and lower scores among younger individuals taking statins (the offspring). This disparate result is consistent with previous reports, that the relationship between statins and cognition is unclear and may differ by age. Hypotheses regarding these opposing results may be developed, e.g. long-term statin use is beneficial on cognitive outcome, whereas short-term statin use has little or no effect, or different types of memory (e.g. verbal versus mathematical) are affected by statins at younger versus older ages.

However, after the inclusion of age, sex, and education as covariates, statin use was only associated with digit forward in the proband/sibling generation and vegetable recall in the offspring/spouse generation. Several prior studies that were conducted with non-demented individuals included some, but not all of these covariates. For example, one study by Rockwood *et al.* (2002) stratified by age (similar to Generation 2 and 3 in this study) but did not also adjust for age within the cohort, thus their conclusion was incomplete [30]. In contrast, previous studies showing a significant protective effect of statin use on AD-related cognition did adjust for all of these covariates (age, sex, education, and/or *APOE*), suggesting that reported evidence of a significant protective effect of statins on AD-related cognition may be more reliable.

Thus, the results of my analyses may give insight into the conflicting results of prior research, in which statins were shown to cause mild, reversible cognitive impairment in some studies but in other studies were shown to have a protective effect on cognition. AAs the current study is a preliminary, cross-sectional analysis, a causal relationship cannot be determined. However, my results may provide rationale for future studies comparing the effects of short and long-term statin use on cognition and/or further careful investigation into the role of age in this relationship. Furthermore, my analyses were based on a cohort of primarily healthy individuals without dementia, which may have affected the outcome of the study. For example, participants in this study had a much lower rate of statin use than the general population (11-16% compared to U.S. average of 30%), and the frequency of the *APOE* *ε2* and *ε3* alleles are much higher than in the general population (*ε3* allele95-97% in study participants vs. 75% in the general population, *ε2* allele 17-19% in study participants vs. 8% in the general population).

Because many of the robust studies in the literature that found a protective effect of statins on cognition were specifically looking at individuals with AD, and because the molecular process by which statins are thought to reduce dementia is by lowering the level of proinflammatory cytokines involved in the progression of AD, further research into the relationship between statin use and cognition in individuals in the early stages of AD (using a longitudinal study design) may be warranted.

Overall, my results do not demonstrate a robust genetic or pharmacogenetic effect of statins on cognition at *APOE* or any of the three proinflammatory cytokine loci analyzed. Given the conflicting reports in the literature, and the unique study population, the lack of association found here between statins, genotype, and cognition is not surprising. I did, however, find an association found between the *IL6*-174 SNP in the proband/sibling generation and *TNFɑ*-308 SNP in the offspring/spouse generation with the digit backward test (p≤0.05). I also found a significant interaction of statin use with *IL6*-174 and *IL1B*-31 with animal recall in the proband/sibling generation (p<0.05). These significant results may be spurious due to the number of analyses that were done and, as such, additional analyses are needed to see if this finding could be replicated. No significant interactions between statin use and *APOE* were found with any of the cognitive traits.

Given that the study population used here was recruited based on exceptional longevity, these results may not reflect the relationship of *APOE* or proinflammatory cytokine genes with the cognitive function associated with normal aging. To determine if there is a relationship between these genes, statin use, and cognitive decline in AD, future analyses should consider comparing individuals with early signs of AD progression to those without AD.

## Limitations and Future Directions

There were several limitations to this study. First, the study population was not in Hardy-Weinberg equilibrium. This may indicate significant violations of the HWE assumptions in the general population; however, because the study population was selected based on a specific trait of interest, that is, healthy aging, HWE may not hold [64]. Survivor bias may have skewed the data for study participants in the proband/sibling generation, who then passed on these genes to their children in offspring generation. Given that the average age of this generation was 90 years old, some of the participants may have died before some of the data was collected. This is especially true of *APOE* ε4/ε4homozygotes, who are predisposed to cardiovascular disease and dementia at an earlier age.

Second, the data used from the LLFS was cross-sectional, thus a temporal causal relationship cannot be determined. For specific associations that were significant in this study, further research utilizing more advanced study methods should be conducted. In an effort to reduce survivor bias and for a more robust study, future studies should consider recruiting participants between 40 and 50 years old and following them longitudinally.

Third, covariates other than age, sex, and education were not considered in the analyses; thus, the study may not be robust. In addition, the study population recruited families, thus all individuals were not independent and family structure was not incorporated into the analytical model in this study. This may have affected the significance level of the results, resulting in inflated p-values. However, since I detected few statistically significant results after incorporating several covariates, the violation of the assumption of independent observations was likely not critical. Nevertheless, family structure and confounders that might have influenced the results such as race, income, duration of statin use, effect non-statin lipid-lowering agents, effect of other medications, comorbidities such as cancer or stroke, smoking and alcohol use, psychiatric disorders such as depression, and physical health/mobility need to be considered in future analyses.

Fourth, a list of all the medication names was not provided in the codebook. As a result, the medication names were scanned manually. Moreover, many names were in Danish or spelled incorrectly. While I made considerable attempts to ensure all subjects taking a statin were designated as a statin-user, some individuals may have been missed.

## Public Health Significance

Statins have become the most highly prescribed class of medication to lower cholesterol and reduce the risk of heart disease, yet the side effects of statin use are still unclear. The pharmacogenetic effects of statins are even less understood, with a paucity of research on the topic; what research has been conducted used small sample sizes and yielded conflicting results. With the prevalence and incidence of cardiovascular disease and dementias in the U.S. and globally continually increasing, research on the potential adverse and beneficial effects of statin use is needed. The results of prior studies on immune and genetic involvement in Alzheimer’s disease indicate that statins may help in delaying the severe cognitive decline. Given that dementias, especially Alzheimer’s disease, place a large emotional and financial burden on families and a significant economic burden on the country, further pharmacogenetic research that may help reduce the cost associated with and improve the quality of life of those living with Alzheimer’s disease is warranted.

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