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APOE ε4, but not diabetes, is a risk factor for Alzheimer's disease

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Abstract

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Alzheimer's Disease is an increasingly prevalent neurodegenerative disorder that systematically destroys cognitive skills and is the sixth leading cause of death in the United States. Apolipoprotein E ε4 allele (APOE ε4) and diabetes have previously been shown to increase the risk for Alzheimer's Disease (AD), mild cognitive impairment (MCI), and various other types of dementia. There is some evidence that having both APOE ε4 and diabetes increases the risk of AD, and that APOE ε4 modifies the relationship between diabetes and AD. We hypothesize that diabetes may modify the risk of AD in individuals with one or two APOE ε4 alleles. Thus, our primary aim was to examine the modifying effect of diabetes on the relationship between APOE ε4 and risk of AD. The National Alzheimer's Coordinating Center (NACC) data set used in this study is a publicly available sample designed to provide clinical evaluations, neuropathology data, and MRI data. Cognitive status was determined by clinical evaluation in Alzheimer's Disease Centers (ADCs). Presence of APOE ε4 alleles was determined by genotype sequencing. Diabetes was based on patient self-report. There were 33,456 subjects in the sample, with 24,336 individuals having APOE genotype data. Risk of cognitive impairment diagnosis was estimated using a multinomial logistic regression model adjusted for various demographic and clinical factors. Compared with those who had no APOE ε4 alleles, those with two copies of APOE ε4 had significantly higher odds of AD diagnosis (odds ratio, 8.75; 95% CI, 7.38-10.38). Diabetic individuals, on the other hand, did not have significantly higher odds of AD diagnosis (odds ratio, 1.09; 95% CI, 0.93-1.27) when compared to non-diabetic individuals. Compared with non-diabetic individuals with two APOE ε4 alleles, those with diabetes and two copies of APOE ε4 did not have significantly higher odds of AD diagnosis (odds ratio, 0.99; 95% CI, 0.55-1.75). Our results suggest that APOE ε4, but not diabetes is a risk factor for AD. Furthermore, there is no interaction between APOE ε4 and diabetes in relation to AD since diabetes does not modify the risk of AD in individuals with one or two APOE ε4 alleles.

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Table of Contents

I. Introduction …….. 8

II. Methods …… 11

III. Results ……... 15

IV. Discussion ……. 21

V. Tables and Figures …..…… 26

Table 1: Demographic information and neuropsychological test scores

Table 2: Performance on the Mini Mental State Exam

Table 3: Risk of cognitive impairment associated with APOE ε4 and diabetes

Figure 1: Odds ratios for adjusted model. Individuals effects of APOE ε4 and diabetes

Figure 2A: Predicted Probabilities for APOE ε4

Figure 2B: Predicted Probabilities for diabetes

Figure 3: Odds ratios for adjusted model calculated with zero APOE ε4 alleles and no diabetes as the baseline

Figure 4A: Odds ratios for adjusted model. Modifying Effect of APOE ε4 on diabetes

Figure 4B: Odds ratios for adjusted model. Modifying effect of diabetes on APOE ε4

VI. References ……... 35

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder that typically results in memory problems, decline in non-memory aspects of cognition, and impaired reasoning or judgement. AD is the most common type of dementia, accounting for 60 to 80 percent of dementia cases, while vascular dementia, Lewy Body dementia (LBD), and frontotemporal dementia (FTD) account for most of the remaining cases (Alzheimer's Association 2016). Due to the wide-ranging overlap of behavioral symptoms in AD and other dementias, clinicians use several methods to diagnose AD. Neuropsychological testing and personal questions about changes in ability to carry out daily activities can indicate cognitive impairment. In order to rule out possible causes other than AD, however, standard blood tests and brain scans are carried out (National Institutes of Aging 2017). However, given that AD can be definitively diagnosed only after death by examining pathological changes, there is substantial room for improvement in the early and accurate detection of AD. The many risk factors linked to AD may be a starting point for this improvement.

Even with a high prevalence, AD continues to be misdiagnosed due to its complex underlying pathophysiology and ambiguous risk factors (Solomon and Murphy 2005). One of the best characterized genetic risk factors is the *APOE* ε4 allele. There are three different types of alleles for the human APOE gene, ε2, ε3, and ε4, with ε4 representing the greatest risk for AD (Liu et al. 2013; Strittmatter et al. 1993). In fact, 80% of familial and 64% of sporadic AD cases have at least one APOE ε4 allele compared to 31% of control subjects, making APOE ε4 a strong risk factor for late-onset AD (Corder et al. 1993). APOE ε4 carriers are twice as likely to have declined on a global cognitive score as non-carriers and APOE ε4 is associated with greater, faster, and earlier cognitive decline (Bretsky et al. 2003; Blair et al. 2005; Martins et al. 2005).

This temporally mediated effect suggests that the processes by which the *APOE* genotype mediates dementia risk are operative well in advance of overt dementia, meaning that *APOE*-ε4 allele carriage can predict cognitive decline at an early stage (Dik et al. 2001). Meanwhile, the effect of the APOE ε4 allele on normal aging is less clear, but seems to be negatively associated with episodic memory, executive functioning, and overall global cognitive ability in cognitively healthy patients (Small et al. 2000; Wilson et al. 2011; Small et al. 2004). These behavioral symptoms are also accompanied by structural changes such as increased levels of vascular and plaque Aβ deposits, faster hippocampal loss, and cerebral hypometabolism (Drzezga et al. 2009; Moffat et al. 2000; Schuff et al. 2008; Cohen et al. 2001; Liu et al. 2014; Schmechel et al. 1993).

Growing literature also suggests that cardiovascular disease (CVD) and CVD risk factors such as diabetes are associated with AD. There are two main types of diabetes: Type 1 and Type 2. While both ultimately result in increased blood glucose levels, the mechanisms vastly differ. In Type 1 diabetes (T1DM), the pancreas is incapable of making insulin which results in increased blood glucose levels since insulin is responsible for transporting glucose from the blood into the cells. On the other hand, in Type 2 diabetes mellitus (T2DM), insulin receptors in the body do not respond properly to insulin, thus leading to both increased insulin and glucose in the blood. This increase in insulin levels in the absence of functional insulin receptors often results in the desensitization of insulin receptors in the brain. In fact, insulin receptor desensitization may be one of the pathological changes that explains the relationship between T2DM and AD (Ristow 2004). Since insulin acts as a growth factor in the brain and is neuroprotective against neuronal oxidative stress, insulin receptor desensitization may facilitate the development of AD (Holscher et al. 2011). Many of the changes in brain insulin and insulin-like growth factor (IGF) signaling represent early and progressive abnormalities and could lead

to potential early diagnosis of AD (Monte et al. 2008). Recently, several population-based studies have attempted to characterize the physiological relationship between diabetes and AD. Even with considerable disagreement between studies, however, the general trend points to diabetes being a risk factor for AD, MCI, and vascular dementia (Biessels et al. 2006; Cheng et al. 2012). Similarly, diabetes is also associated with lower levels of global cognition, episodic memory, semantic memory, and visuospatial ability (Arvanitakis et al. 2004). More specifically, cognitive dysfunction in Type 1 diabetes is characterized by diminished mental speed and flexibility, while Type 2 diabetes negatively impacts learning and memory (Brands et al. 2005). These behavioral changes are explained by reductions in hippocampal volume in individuals with diabetes as compared to control subjects as well as hippocampal and amygdalar atrophy in patients with T2DM (Heijer et al. 2003; Gold et al. 2007).

While APOE ε4 and diabetes are fairly well characterized risk factors of AD, the interaction between them is less well understood. Previous studies have suggested that the combination of APOE ε4 and diabetes increases the risk of AD more than each factor alone (Irie et al. 2008; Peila et al. 2002). While the mechanism for this interaction is not yet clear, there is reason to believe that insulin is the common factor. Brain insulin levels are increased in both the presence of an APOE ε4 allele and T2DM (Henderson et al. 2011). Due to this uncontrolled increase in insulin, insulin-degrading enzyme (IDE), which is responsible for breaking down both insulin and amyloid-β peptide, is flooded with insulin (Qiu et al. 2006). This, in turn, promotes neuritic plaque formation and dramatically increases amyloid deposition, thus providing a mechanism for the increase in risk of AD in the presence of both T2DM and the APOE ε4 allele (Messier et al. 2003). Nevertheless, relatively few studies have examined the modifying effect of diabetes on the association between APOE ε4 and cognitive impairment.

More importantly, previous studies that have reported an interaction between diabetes and APOE ε4 in relation to AD have examined limited populations (Peila et al. 2002). The present study seeks to further elucidate this interaction between APOE ε4 and diabetes by analyzing a more representative patient population so that the findings can be more applicable to the general populace. It is reasonable to expect, based on past evidence, that individuals with one or two APOE ε4 alleles would be at an even higher risk of cognitive impairment if they were also diabetic.

Methods

Sample

Data was obtained from the National Alzheimer's Coordinating Center (NACC) which is part of the National Institutes of Aging (NIA) and has maintained a cumulative database based on clinical evaluations, neuropathology data, and MRI imaging ("Information and Resources" 2010). The data is consisted of a clinic-based population who have undergone a standardized evaluation. The database includes cognitively normal subjects, subjects with AD, and individuals with other types of cognitive impairment. The data set used in this study included variables from the Uniform Data Set (UDS) and Neuropathology Data Set (NP). The UDS is made up of data from a prospective, standardized, and longitudinal clinical evaluation of the subjects in the NIA's Alzheimer's Disease Center (ADC) Program. The NP contains autopsy data for a subset of UDS subjects. Data collection and storage procedures have been described in detail previously (Beekly et al. 2007).

Procedures

Initially, the NACC data set was used to evaluate the risk of cognitive impairment diagnosis for individuals with copies of the APOE ε4 allele or diabetes independently. This initial analysis ensured that the individual effects of APOE ε4 and diabetes on cognitive impairment could be isolated and well-characterized. Subsequently, the interaction between APOE ε4 and diabetes as they relate to risk of cognitive impairment diagnosis was examined. These relationships were quantified by odds ratios (ORs) obtained from multinomial logistic regression analysis. Statistical analyses were conducted using R. Tables and figures were made using SPSS and Microsoft Excel.

Of the 108,501 observations present in the longitudinal data set, only the first visit was selected for each patient. This resulted in a final sample consisting of 33,456 participants. This filter was employed to ensure a cross-sectional study design, at least until the major relationships between APOE ε4, diabetes, and cognitive impairment in this data set were understood. In the future, however, longitudinal data analysis using techniques such as generalized estimating equations (GEE's) could provide more information as to how these interactions evolve over time. The number of APOE ε4 alleles was presented as either 0, 1, or 2 from the genetic portion of the UDS. Diabetes was based on patient self-report and was spilt into three categories: Absent, Remote/Inactive, Recent/Active. The individuals who identified as Remote/Inactive were excluded, making diabetes a binary variable: no diabetes or diabetes. Diagnosis of cognitive impairment was also split into three categories: subjects with no cognitive impairment, subjects with cognitive impairment and no Alzheimer's disease, and subjects with cognitive impairment and Alzheimer's disease. The cognitive impairment without AD group included individuals with MCI or dementia outside of AD, while the cognitive impairment with AD group consisted

exclusively of individuals with AD. The etiologic diagnosis of AD was based on the National Institutes of Aging-Alzheimer's Association (NIA-AA) criteria, and is characterized by gradual onset of symptoms over months to years as well as a clear history of worsening cognition (McKhann et al. 2011). Given that this data set and the most widely-used criteria for AD diagnosis (NIA-AA) were both provided by the NIA, the cognitive impairment diagnosis in this sample is presumed to be extremely accurate. Other covariates included in the adjusted model (Table 3), such as congestive heart failure, hypertension, hypercholesterolemia, thyroid disease, vitamin B12 deficiency, atrial fibrillation, heart attack/cardiac arrest, years smoked cigarettes, and alcohol abuse, were self-report (Morris et al. 2006). Neuropsychological tests were completed as part of the standardized evaluation and played a part in the diagnosis of cognitive impairment (Weintraub et al. 2009).

Statistical Analysis

Risk of cognitive impairment was estimated using multinomial logistic (multi logit) regression models. Multinomial logistic regression is used to model how a categorical outcome variable depends on a set of predictors. Specifically, the multinomial logistic regression model is an extension of binary logistic regression and is used when the dependent variable has a polytomous (having more than two outcomes) response (Aldrich and Nelson 1984). Since the outcome variable in this study, diagnosis of cognitive impairment, had three levels, multi logit regression was chosen. Multinomial logistic regression requires that one category of the dependent variable is chosen as the reference level, which was no cognitive impairment in our study. Since the choice of this reference level drives the interpretation of the multi logit results, it afforded us the statistical dexterity that is essential when investigating subtle, complex questions.

Multi logit regression models the log odds of the outcomes (no cognitive impairment, cognitive impairment without AD, and cognitive impairment with AD) as a linear combination of the independent variables. Therefore, the logit for each non-reference category *j* = 1 (cognitive impairment without AD) or 2 (cognitive impairment with AD) against the reference category *j* = 0 (no cognitive impairment) can be modeled as:

$$\ln\left(\frac{\pi^{j}}{\pi^{0}}\right) = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k$$

by exponentiating both sides we obtain the odds for each non-reference category as compared to the reference category:

$$\left(\frac{\pi^{j}}{\pi^{0}}\right) = e^{\beta_0+\beta_1 x_1+\cdots+\beta_k x_k}$$

where $\beta_1, \ldots, \beta_k$ are population parameters for the independent variables. Thus, the odds for cognitive impairment without AD against no cognitive impairment with APOE ε4 and diabetes as the independent variables can be modeled as:

$$\left(\frac{\pi^{1}}{\pi^{0}}\right) = e^{\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_4 x_1 x_2+\beta_5 x_1 x_3}$$

where x_1 is presence of diabetes (1 is diabetes and 0 is no diabetes), x_2 is the presence of one APOE ε4 allele (1 is one APOE ε4 allele and 0 is zero APOE ε4 alleles), x_3 is the presence of two APOE ε4 alleles (1 is two APOE ε4 alleles and 0 is zero APOE ε4 alleles), β_4 is the parameter for the interaction between diabetes and one APOE ε4 allele, and β_5 is the parameter for the interaction between diabetes and two APOE ε4 alleles. The odds ratio for individuals with

one APOE ε4 allele versus zero APOE ε4 alleles in terms of being diagnosed with cognitive impairment without AD as compared to no cognitive impairment is:

$$OR = \frac{(\pi^1|x_1=0,x_2=1,x_3=0)\Big/(\pi^0|x_1=0,x_2=1,x_3=0)}{(\pi^1|x_1=0,x_2=0,x_3=0)\Big/(\pi^0|x_1=0,x_2=0,x_3=0)} = \frac{e^{\beta_0+\beta_2}}{e^{\beta_0}} = e^{\beta_2}$$

The same method was used to calculate all ORs.

To summarize, the independent variables were the number of APOE ε4 alleles and diabetes. The dependent variable was diagnosis of cognitive impairment. Two models are presented: the basic model controls for gender (male/female), race (White/Black/American Indian or Alaska Native/Native Hawaiian or Other Pacific Islander/Asian), education (years), and age (years), and the adjusted model controls for gender (male/female), race (White/Black/American Indian or Alaska Native/Native Hawaiian or Other Pacific Islander/Asian), education (years), age (years), congestive heart failure (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), thyroid disease (yes/no), vitamin B12 deficiency (yes/no), atrial fibrillation (yes/no), heart attack/cardiac arrest (yes/no), years smoked cigarettes (years), and alcohol abuse (yes/no). Covariates were selected based on their theoretical relevance to the independent variables and dependent variable.

Results/Current Data

An important first step in the analysis was to characterize the individuals in our dataset. Demographic information and performance on cognitive tests is summarized in Table 1 and is stratified according to the two independent variables: number of APOE ε4 alleles for non-diabetic and diabetic individuals. Of the 24,336 participants with genetic information, 14,449

(59%) had zero copies of the APOE ε4 allele, 8,261 (34%) had one APOE ε4 allele, and 1,626 (7%) had two copies of the APOE ε4 allele. Therefore, the percentage of patients with at least one APOE ε4 allele was 41%, which was higher than the previously-reported range of 24-30% (Haan et al. 1999). The number of individuals classified into the APOE genotypes among 24,108 participants was as follows: 2/2 (106), 2/3 (2,115), 3/3 (12,093), 2/4 (638), 3/4 (7,544), 4/4 (1,612). As the number of APOE ε4 alleles increased, performance across all neuropsychological measures decreased in both non-diabetic and diabetic individuals. Among the 33,091 individuals who had available data on diabetic status, 4,204 (13%) self-reported as actively having diabetes, which was within the 12-14% estimated prevalence of diabetes among US adults (Menke et al. 2015). Compared to non-diabetic individuals, participants with diabetes performed worse on all the neuropsychological tests. There was no significant effect of race on the presence of APOE ε4 alleles, but there was a higher prevalence of diabetes among minorities, particularly African-Americans. This finding is consistent with the general trend seen in previous studies (Carter 1996). Table 1 showed the demographic diversity of the sample while hinting at a larger negative effect of APOE ε4 than diabetes on cognitive performance.

In order to plainly characterize the cognitive performance of the sample, Table 2 presents performance on the Mini Mental State Exam (MMSE) according to gender, race, diabetic status, cognitive status, and number of APOE ε4 alleles. The MMSE was chosen due its ubiquity in clinical diagnosis (Shulman et al. 2006). There are no appreciable differences in performance for gender, race, and diabetic status. However, cognitive status and the number of APOE ε4 alleles have more pronounced effects on MMSE performance. Scores significantly declined as the severity of cognitive impairment diagnosis increased from no cognitive impairment to cognitive impairment with AD. Similarly, there was a downward trend in MMSE performance as the

number of copies of the APOE ε4 allele increases. The results in Table 2 provide further evidence for the general trend of APOE ε4 having a larger detrimental effect than diabetes on cognitive performance.

With the general trends from Tables 1 and 2 in mind, the odds of cognitive impairment diagnosis were estimated for APOE ε4 and diabetes. Table 3 shows the results of multinomial logistic regression analysis regarding the association of diabetes and APOE ε4 with cognitive impairment diagnosis. Two models were run to confirm that the effects of diabetes and APOE ε4 were consistent even as covariates were added. The basic model controlled for demographic factors, while the adjusted model included both demographic and clinical factors. The effects of APOE ε4 and diabetes on cognitive status were consistent between the two models, suggesting that the overall trends were robust. The adjusted model was a better fit (lower AIC) and thus will be the focus here. Individuals with 1 APOE ε4 allele had 1.26 (95% CI 1.15-1.38) times higher odds of having cognitive impairment without AD versus no cognitive impairment, compared with the odds for people with 0 APOE ε4 alleles. This effect was slightly more pronounced in individuals with 2 APOE ε4 alleles, as they had 1.95 (95% CI 1.58-2.41) times higher odds of having cognitive impairment without AD versus no cognitive impairment, compared with the odds for individuals with 0 APOE ε4 alleles. The effect of APOE ε4 on the odds of having cognitive impairment with AD compared to no cognitive impairment were similar but more pronounced. Subjects with 1 APOE ε4 allele had an OR of 2.78 (95% CI 2.57-3.01) and individuals with 2 APOE ε4 alleles had an OR of 8.75 (95% CI 7.38-10.38) for cognitive impairment with AD as compared to no cognitive impairment. Therefore, there is an increase in risk for both cognitive impairment without AD and AD as the number of APOE ε4 alleles increases.

On the other hand, participants with diabetes had an OR of 1.10 (95% CI 0.94-1.28) for cognitive impairment without AD and an OR of 1.09 (95% CI 0.93-1.27) for cognitive impairment with AD when compared to non-diabetic individuals. Thus, diabetes trended toward increasing the odds of cognitive impairment diagnosis, but ultimately did not increase risk.

Consequently, it was important to more thoroughly examine the effects of APOE ε4 and diabetes on cognitive impairment diagnosis using a different measure: predicted probabilities. Predicted probabilities were calculated based on the adjusted model while holding all predictors except APOE ε4 and diabetes at their respective mean over the whole sample. This method isolates the variable of interest (APOE ε4 or diabetes) and gives a snapshot of the sample at some fixed values for the other covariates in the model. To visualize the trends seen in Figure 1 more clearly, the predicted probabilities of cognitive status for both APOE ε4 (Figure 2A) and diabetes (Figure 2B) are shown. Figure 2A shows that individuals with 0 APOE ε4 alleles are the most likely to be diagnosed with no cognitive impairment (Pr = 0.49), but the majority of subjects with 0 APOE ε4 alleles are cognitively impaired to some extent (Pr = 0.51). As the number of APOE ε4 alleles increase, the probability of being diagnosed with either no cognitive impairment or cognitive impairment without AD decreases, while the probability of being diagnosed with cognitive impairment with AD increases. This effect results in individuals with 2 APOE ε4 alleles having a 70% chance of being diagnosed as having cognitive impairment with AD. Figure 2A confirms the significant effect of APOE ε4 seen in Figure 1 while showing that the presence of APOE ε4 alleles disproportionately increases the likelihood of a cognitive impairment with AD diagnosis. Similarly, Figure 2B confirms the findings in Figure 1 by showing that diabetes has no effect on cognitive status. That is to say, the probability of being diagnosed as any of the

three cognitive statuses is identical for diabetic and non-diabetic subjects, and thus diabetes does not increase the probability of being diagnosed with cognitive impairment.

Subsequently, with a sufficient grasp on how APOE ε4 and diabetes individually affect the odds of cognitive impairment diagnosis, the effect of both factors together was investigated. Figure 3 shows the ORs for all possible combinations of APOE ε4 and diabetes with no diabetes/0 APOE ε4 alleles as the baseline. This allowed for a visual representation of odds ratios of the five groups (no diabetes/1 APOE ε4 allele, no diabetes/2 APOE ε4 alleles, diabetes/0 APOE ε4 alleles, diabetes/1 APOE ε4 allele, diabetes/2 APOE ε4 alleles) for both cognitive impairment without AD and cognitive impairment with AD. The OR's for diabetes/0 APOE ε4 alleles, no diabetes/1 APOE ε4 allele, and no diabetes/2 APOE ε4 alleles are identical to the ones presented in Table 3 for diabetes, 1 APOE ε4 allele, and 2 APOE ε4 alleles respectively. The key groups then are diabetes/1 APOE ε4 allele and diabetes/2 APOE ε4 alleles. Compared with participants who had neither diabetes nor APOE ε4, those with both diabetes and 1 APOE ε4 allele had significantly higher odds of being diagnosed with cognitive impairment with AD (OR, 2.41; 95% CI, 1.97-2.94), but not cognitive impairment without AD (OR, 1.19; 95% CI, 0.94-1.50). Similarly, compared with participants who had neither diabetes nor APOE ε4, those with both diabetes and 2 APOE ε4 alleles had significantly higher odds of being diagnosed with cognitive impairment with AD (OR, 8.68; 95% CI, 4.95-15.06), but not cognitive impairment without AD (OR, 1.50; 95% CI, 0.73-3.11). Therefore, these data suggest that having both diabetes and APOE ε4 increases the risk of AD.

At this stage, it was appropriate to test for an interaction between APOE ε4 and diabetes as it relates to their prospective effects on cognitive impairment diagnosis. Figure 3 offers an initial glimpse at this question. The OR of having cognitive impairment with AD for subjects

with both diabetes and 1 APOE ε4 allele (OR, 2.41; 95% CI, 1.97-2.94) was not higher than the OR for subjects with no diabetes and 1 APOE ε4 allele (OR, 2.78; 95% CI, 2.57-3.01). This effect is also seen for 2 APOE ε4 alleles and cognitive impairment without AD, suggesting that there is no interaction between diabetes and APOE ε4 as they relate to any type of cognitive impairment. In other words, diabetes may not modify the relationship between APOE ε4 and cognitive impairment diagnosis.

While Figure 3 provides a workable visual representation, further analysis was needed to formally examine the interaction between diabetes and APOE ε4. Figure 4A meets the criteria by presenting ORs for two comparisons, 1 APOE ε4/diabetes versus 0 APOE ε4/diabetes and 2 APOE ε4/diabetes versus 0 APOE ε4/diabetes. Among subjects with diabetes, individuals with 1 APOE ε4 allele (OR, 2.21; 95% CI, 1.74-2.80) and 2 APOE ε4 alleles (OR, 7.99; 95% CI, 4.48-14.04) have significantly higher odds of having cognitive impairment with AD versus no cognitive impairment, compared with the subjects who had 0 APOE ε4 alleles. This analysis provided evidence that APOE ε4 cannot modify the relationship between diabetes and AD in our sample because there is no relationship between diabetes and AD. Figure 4B displays the flip side of the interaction by presenting ORs for diabetes/1 APOE ε4 versus no diabetes/1 APOE ε4 and diabetes/2 APOE ε4 versus no diabetes/2 APOE ε4. Individuals with diabetes do not have significantly higher odds of having cognitive impairment with AD versus no cognitive impairment for either 1 APOE ε4 allele (OR, 0.87; 95% CI, 0.71-1.06) or 2 APOE ε4 alleles (OR, 0.99; 95% CI, 0.55-1.75), compared with the subjects who had 0 APOE ε4 alleles. Thus, Figure 4B shows that when APOE ε4 alleles and diabetes are present together, the odds of being diagnosed with cognitive impairment without AD and cognitive impairment with AD do not increase beyond the odds for the APOE ε4 allele alone.

Discussion

The present study provides evidence that APOE ε4 is a strong risk factor for the development of AD. On the other hand, diabetes was not a risk factor for AD in our study. As such, in our sample, there is no interaction between APOE ε4 and diabetes in relation to AD. That is to say, diabetes does not modify the relationship between APOE ε4 and AD. Our results are in conjunction with previous findings in identifying the APOE ε4 allele as a risk factor for AD and other types of cognitive impairment (Liu et al. 2013; Strittmatter et al. 1993; Corder et al. 1993; Tervo et al. 2004). Our findings, however, are different from previous studies which suggest that diabetes is a risk factor for MCI and AD (Cheng et al. 2012; Biessels et al. 2006). Therefore, our results suggest that APOE ε4, but not diabetes is a risk factor for AD, and that APOE ε4 should be used as the standard for early detection techniques. Our findings also provide clues into the pathological mechanisms of AD and inform future study designs that may investigate APOE ε4, diabetes, and cognition.

The present findings are based on a large number of subjects who completed a thorough clinical evaluation. The covariates used in the adjusted model were extensive and were well-characterized. APOE genotype sequencing and diagnosis of cognitive impairment were carried out by Alzheimer's Disease Centers (ADCs), maximizing the likelihood of an accurate diagnosis especially when distinguishing AD from other types of cognitive impairment, such as MCI, LBD, and FTD.

Previous studies that have identified diabetes as a risk factor for MCI and AD have been fairly contradictory. In fact, ten of the sixteen studies that have investigated diabetes and risk of AD concluded that diabetes could not increase the risk of AD (Cheng et al. 2012). Similarly, one of the two studies that investigated diabetes and risk of MCI concluded that diabetes does not

increase the risk of MCI. A meta-analysis of these studies, however, revealed that diabetes is a risk factor for both AD and MCI (Cheng et al. 2012). The reasons why our results do not identify diabetes as a risk factor for cognitive impairment might be that our study relied on patient self-report for diabetes diagnosis and did not distinguish between T2DM and T1DM. The inability to separate T2DM and T1DM, however, might not be a major issue. One reason is that just 4% of the 29.1 million Americans with diabetes have T1DM. This low prevalence of T1DM is mirrored in our sample where just nine of the 112 subjects (8%) with information on diabetes type had T1DM. More specifically, while more population-based studies that investigate the relationship between T1DM and AD diagnosis are necessary, T1DM has been shown to impair aspects of cognition (Brands et al. 2005). In addition, both insulin resistance, seen in T2DM, and insulin deficiency, seen in T1DM, play important roles in AD pathology (Li et al. 2015; Jolivalt et al. 2010). Therefore, any relatively minor effect that T1DM might have on AD diagnosis seems to be similar to the effect of T2DM on risk of AD.

Given the strengths of this sample, however, our finding that diabetes is not risk factor for AD should also be used to critique previous studies. For example, since diabetes is a stronger risk factor for vascular dementia than for AD and there are similarities between the clinical representations of vascular dementia and AD, it could be the case that AD is misdiagnosed as vascular dementia in other studies (Cheng et al. 2012; Gorelick et al. 1996). In addition, other factors related to diabetes, such as obesity, hyperinsulinemia, and metabolic syndrome, but not diabetes itself could be affecting risk of AD (Li et al. 2015). Therefore, while it is certainly possible that our self-reported diabetes diagnosis resulted in an incorrect assessment of the relationship between diabetes and AD, it is also possible that previous studies have misdiagnosed vascular dementia as AD or ignored factors such as metabolic syndrome, resulting in an

overestimation of the effect of diabetes on AD. Thus, in order to definitively characterize the effect of diabetes on AD, a future study needs to pull from the strengths of our study, accurate AD diagnosis and a large, diverse sample, while fixing the weaknesses, use serum insulin or HbA1c levels to diagnose diabetes.

The finding, in our sample, that APOE ε4, but not diabetes, is not a risk factor for AD implies that the mechanisms through which APOE ε4 and diabetes affect AD pathology are separate. Most of the adverse pathological effects seen in AD, such as oxidative stress, decreased neuronal growth, and decreased synaptic plasticity, have Aβ as the common factor. However, both diabetes and APOE ε4 cause insulin resistance and a subsequent increase in Aβ production and Aβ oligomer formation in the brain (Aulston et al. 2013; Henderson et al. 2011). Thus, there must be pathway other than insulin resistance through which APOE ε4 acts on Aβ. The answer seems to be that the major clearance pathways of Aβ, outside of IDE, are impaired for the apoE4 isoform. More specifically, receptor-mediated clearance of Aβ by cells in the brain parenchyma, along the interstitial fluid drainage pathway, and through the blood brain barrier (BBB) is impaired in apoeE4 isoforms when compared to apoE3 (Bu 2009). In addition to these Aβ-mediated pathways which are separate from insulin resistance, the apoE4 isoform also leads to increased lysosomal leakage and subsequent apoptosis as well as increased neuron-specific proteolysis resulting in an abundance of neurotoxic apoE4 fragments in the cytosol, where they are associated with cytoskeletal disruption and mitochondrial dysfunction (Mahley et al. 2006). Therefore, there are pathways outside of insulin resistance that are affected by APOE ε4. A possible explanation for our results is that having the APOE ε4 allele disproportionately activates these pathways that insulin is not involved in, and insulin by itself plays a small role in AD pathology. This explanation would imply that the pathological pathways that APOE ε4 activates

are more potent than the insulin resistance pathway that both diabetes and APOE ε4 activate. In order to test this possible explanation, more studies that characterize and quantify the pathological and behavioral effects of these various pathways are needed.

This is the first study to investigate the effects of APOE ε4 and diabetes on AD and other types of dementia using a single large and diverse sample. The sample sizes in similar studies have ranged from 826-2,574 subjects compared to the 33,456 subjects in this study. Perhaps more important than the size of the data set is its diversity, as multiple races and genders are represented. In comparison, the Honolulu-Asia Aging Study which concluded that T2DM modifies the relationship between APOE ε4 and dementia only enrolled Japanese-American men (Peila et al. 2002). The large sample size and diversity of our dataset means that the findings can be generalized to a larger subset of the population. Our dataset provided APOE genotype data for 24,336 subjects and stratified the presence of an APOE ε4 allele into three categories: zero copies, one copy, and two copies. The three levels are in contrast with most other similar studies which show the presence of an APOE ε4 allele as a binary variable (yes or no) by combining one copy and two copies into the yes category. This not only dampens the effect of having 2 APOE ε4 alleles, but it also fails to show the relationship between risk of cognitive impairment and number of APOE ε4 alleles. The nuance missing in previous studies is provided in our results where subjects with 2 APOE ε4 alleles (OR = 8.75) have more than three-fold higher odds of a cognitive impairment with AD diagnosis than subjects with 1 APOE ε4 allele (OR = 2.78). These types of distinctions become crucial in the clinic when diagnosing a continuous spectrum of cognitive disorders. Therefore, even with some limitations, this is the most comprehensive study of its kind because of the representative sample, accuracy and stratification of cognitive impairment diagnosis, and separation of one and two APOE ε4 alleles.

The immediate future for this study will be to assess the effects of diabetes and APOE ε4 on cognition in the same NACC dataset. Since diabetes and APOE ε4 have previously been shown to decrease cognitive performance, it would be informative to observe whether the findings seen thus far are replicated when cognitive performance replaces cognitive impairment diagnosis as the dependent variable. In addition, MRI data is available for a subset of the population, meaning that the effect of diabetes and APOE ε4 on hippocampal volume, brain volume, and neuritic plaque levels can be investigated. A holistic approach is necessary since the vast number of variables in flux when investigating the three levels of APOE ε4, two types of diabetes, and continuous spectrum of cognition can often produce complex results. This analysis would make the clinical findings even more convincing by providing a physiological basis for the cognitive and behavioral observations. Now that the major trends between APOE ε4, diabetes, and cognitive impairment diagnosis have been examined, this study will become even more applicable to individual patient diagnosis by taking advantage of the longitudinal aspect of this data set.

Table 1: Demographic Information and neuropsychological measures (n=33,456)

Variable	No Diabetes (*n* =28887)			Diabetes (*n* =4204)		
	0 APOE ε4 Alleles	1 APOE ε4 Allele	2 APOE ε4 Alleles	0 APOE ε4 Alleles	1 APOE ε4 Allele	2 APOE ε4 Alleles
Age (years), mean ± SD (*n*)	72.3 ± 11.3 (12540)	71.8 ± 10.2 (7308)	69.5 ± 8.7 (1457)	73.3 ± 8.9 (1774)	72.8 ± 8.3 (874)	70.5 ± 7.2 (155)
Gender, *n* (%)						
Male	5335 (42.5)	3148 (43.1)	680 (46.7)	854 (48.1)	421 (48.2)	75 (48.4)
Female	7205 (57.5)	4160 (56.9)	777 (53.3)	920 (51.9)	453 (51.8)	80 (51.6)
Race, *n* (%)						
White	10896 (88.0)	6264 (86.9)	1241 (86.3)	1188 (67.0)	556 (65.6)	103 (71.0)
Black or African-American	1122 (9.1)	818 (11.3)	168 (11.7)	395 (23.6)	263 (31.0)	37 (25.5)
American Indian or Alaska Native	51 (0.4)	19 (0.3)	6 (0.4)	28 (1.7)	12 (1.4)	1 (0.7)
Native Hawaiian or Other Pacific Islander	8 (0.1)	4 (0.1)	1 (0.1)	3 (0.2)	1 (0.1)	-
Asian	304 (2.5)	104 (1.4)	22 (1.5)	60 (3.6)	16 (1.9)	4 (2.8)
Education (years), mean + SD (*n*)	15.2 ± 3.4 (12463)	15.1 ± 3.3 (7265)	15.3 ± 3.2 (1450)	13.9 ± 4.1 (1758)	14.0 ± 3.7 (870)	14.1 ± 3.2 (152)
Cognitive Status, *n* (%)						
No Cognitive Impairment	5908 (47.1)	2320 (31.7)	235 (16.1)	709 (40.0)	254 (29.1)	24 (15.5)
Cognitive Impairment w/o Alzheimer's Disease	3163 (25.2)	1561 (21.4)	249 (17.1)	500 (28.2)	212 (24.3)	27 (17.4)
Cognitive Impairment w/ Alzheimer's Disease	3469 (27.7)	3427 (46.9)	973 (66.8)	565 (31.8)	408 (46.7)	104 (67.1)
APOE genotype, *n* (%)						
2/2	93 (0.7)	-	-	13 (0.7)	-	-
2/3	1821 (14.5)	-	-	294 (16.6)	-	-
3/3	10626 (84.7)	-	-	1467 (82.7)	-	-
2/4	-	550 (7.5)	-	-	88 (10.1)	-
3/4	-	6758 (92.5)	-	-	786 (89.9)	-
4/4	-	-	1457 (100)	-	-	155 (100)
Mini Mental State Exam, mean + SD (*n*)	26.2 ± 5.3 (11919)	24.5 ± 6.4 (6943)	22.9 ± 6.7 (1390)	25.7 ± 5.2 (1701)	24.4 ± 6.3 (833)	23.2 ± 5.4 (150)
Total Number of Story Units Recalled-Immediate, mean + SD (*n*)	10.5 ± 5.4 (11340)	8.5 ± 5.6 (6498)	6.6 ± 5.1 (1290)	9.8 ± 5.0 (1621)	8.4 ± 5.2 (780)	6.2 ± 4.9 (144)
Total Number of Story Units Recalled-Delayed, mean + SD (*n*)	9.0 ± 5.7 (11318)	6.7 ± 5.9 (6478)	4.3 ± 5.3 (1284)	8.1 ± 5.3 (1618)	6.7 ± 5.4 (781)	4.1 ± 4.7 (144)
Digit Span Forward Trials Correct, mean + SD (*n*)	7.9 ± 2.4 (11479)	7.7 ± 2.3 (6603)	7.6 ± 2.4 (1305)	7.3 ± 2.4 (1642)	7.3 ± 2.2 (794)	6.9 ± 2.4 (145)
Digit Span Forward Length, mean + SD (*n*)	6.4 ± 1.3 (11473)	6.3 ± 1.3 (6602)	6.2 ± 1.3 (1304)	6.1 ± 1.3 (1642)	6.1 ± 1.2 (794)	5.8 ± 1.4 (145)
Digit Span Backward Trials Correct, mean + SD (*n*)	6.0 ± 2.4 (11452)	5.6 ± 2.4 (6579)	5.2 ± 2.5 (1297)	5.3 ± 2.3 (1636)	5.2 ± 2.2 (793)	4.9 ± 2.1 (145)
Digit Span Backward Length, mean + SD (*n*)	4.4 ± 1.4 (11450)	4.2 ± 1.4 (6579)	4.0 ± 1.5 (1297)	4.1 ± 1.4 (1636)	4.0 ± 1.3 (793)	3.9 ± 1.3 (145)
Total Number of Animals named in 60 seconds, mean + SD (*n*)	16.7 ± 7.0 (11636)	15.3 ± 7.0 (6674)	14.4 ± 6.8 (1317)	15.3 ± 6.3 (1658)	14.8 ± 6.0 (801)	13.1 ± 6.0 (144)
Total Number of Vegetables named in 60 seconds, mean + SD (*n*)	11.9 ± 5.4 (11418)	10.6 ± 5.4 (6566)	9.4 ± 5.1 (1298)	11.0 ± 4.8 (1634)	10.4 ± 4.8 (793)	8.5 ± 4.5 (145)
WAIS-R Digit Symbol Task, mean + SD (*n*)	40.5 ± 16.2 (10702)	37.5 ± 16.9 (6054)	34.5 ± 16.8 (1170)	34.4 ± 14.6 (1539)	34.1 ± 14.7 (738)	30.3 ± 15.5 (139)
Boston Naming Task, mean + SD (*n*)	24.6 ± 6.1 (11362)	23.5 ± 6.7 (6519)	23.3 ± 6.7 (1291)	23.5 ± 6.2 (1617)	22.8 ± 6.9 (788)	22.2 ± 6.7 (146)

Table 2: Performance on the Mini Mental State Exam (MMSE; mean ± SD)

	Level 0	Level 1	Level 2	Level 3	Level 4
Gender[a]	24.8 ± 6.0 (13283)	25.3 ± 6.1 (17606)	-	-	-
Race[b]	25.3 ± 6.0 (24975)	24.6 ± 5.9 (4352)	23.5 ± 5.9 (251)	22.7 ± 7.9 (25)	25.6 ± 5.2 (584)
Diabetic Status[c]	25.2 ± 6.1 (26663)	24.8 ± 5.9 (3913)	-	-	-
Cognitive Status[d]	28.9 ± 1.5 (11692)	25.2 ± 5.6 (7516)	21.3 ± 6.8 (11681)	-	-
Number of APOE ε4 alleles[e]	26.2 ± 5.3 (13746)	24.5 ± 6.4 (7846)	22.9 ± 6.5 (1552)	-	-

a. male (0), female (1). b. white (0), black/African American (1), American Indian/Native Alaskan (2), Native Hawaiian/Pacific Islander (3), Asian (4). c. No Diabetes (0), Diabetes (1). d. No Cognitive Impairment (0), Cognitive Impairment without AD (1), Cognitive Impairment with AD (2). e. no copies (0), one copy (1), two copies (2).

Table 3: Risk of cognitive impairment associated with diabetes and APOE ε4; OR (95% CI)

	Basic Model[a]	Adjusted Model[b]
Cognitive Impairment w/o AD		
1 APOE ε4 Allele	1.27 (1.17-1.37)	1.26 (1.15-1.38)
2 APOE ε4 Alleles	2.04 (1.69-2.45)	1.95 (1.58-2.41)
Diabetes	1.11 (0.98-1.27)	1.10 (0.94-1.28)
1 APOE ε4/Diabetes	1.24 (0.99-1.55)	1.08 (0.83-1.41)
2 APOE ε4/Diabetes	1.75 (0.96-3.21)	1.37 (0.66-2.86)
Diabetes/1 APOE ε4	1.09 (0.89-1.33)	0.94 (0.74-1.19)
Diabetes/2 APOE ε4	0.94 (0.79-1.12)	0.77 (0.36-1.64)
Cognitive Impairment w/ AD		
1 APOE ε4 Allele	2.71 (2.52-2.91)	2.78 (2.57-3.01)
2 APOE ε4 Alleles	8.69 (7.45-10.13)	8.75 (7.38-10.38)
Diabetes	1.13 (0.99-1.28)	1.09 (0.93-1.27)
1 APOE ε4/Diabetes	2.26 (1.85-2.76)	2.21 (1.74-2.80)
2 APOE ε4/Diabetes	7.43 (4.53-12.18)	7.99 (4.48-14.04)
Diabetes/1 APOE ε4	0.96 (0.52-1.78)	0.87 (0.71-1.06)
Diabetes/2 APOE ε4	0.96 (0.58-1.59)	0.99 (0.55-1.75)
AIC	47226.68	35782.39

Odds ratios are presented as 1 APOE ε4 vs. 0 APOE ε4, 2 APOE ε4 vs. 0 APOE ε4, diabetes vs. no diabetes, 1 APOE ε4/diabetes vs. 0 APOE ε4/diabetes, 2 APOE ε4/diabetes vs. 0 APOE ε4/diabetes, diabetes/1 APOE ε4 vs. no diabetes/1 APOE ε4, and diabetes/2 APOE ε4 vs. no diabetes/2 APOE ε4. a. Adjusted for gender, race, education, and age; b. Adjusted for gender, race, education, age, congestive heart failure, hypertension, hypercholesterolemia, thyroid disease, vitamin B12 deficiency, atrial fibrillation, heart attack/cardiac arrest, years smoked cigarettes, and alcohol abuse.

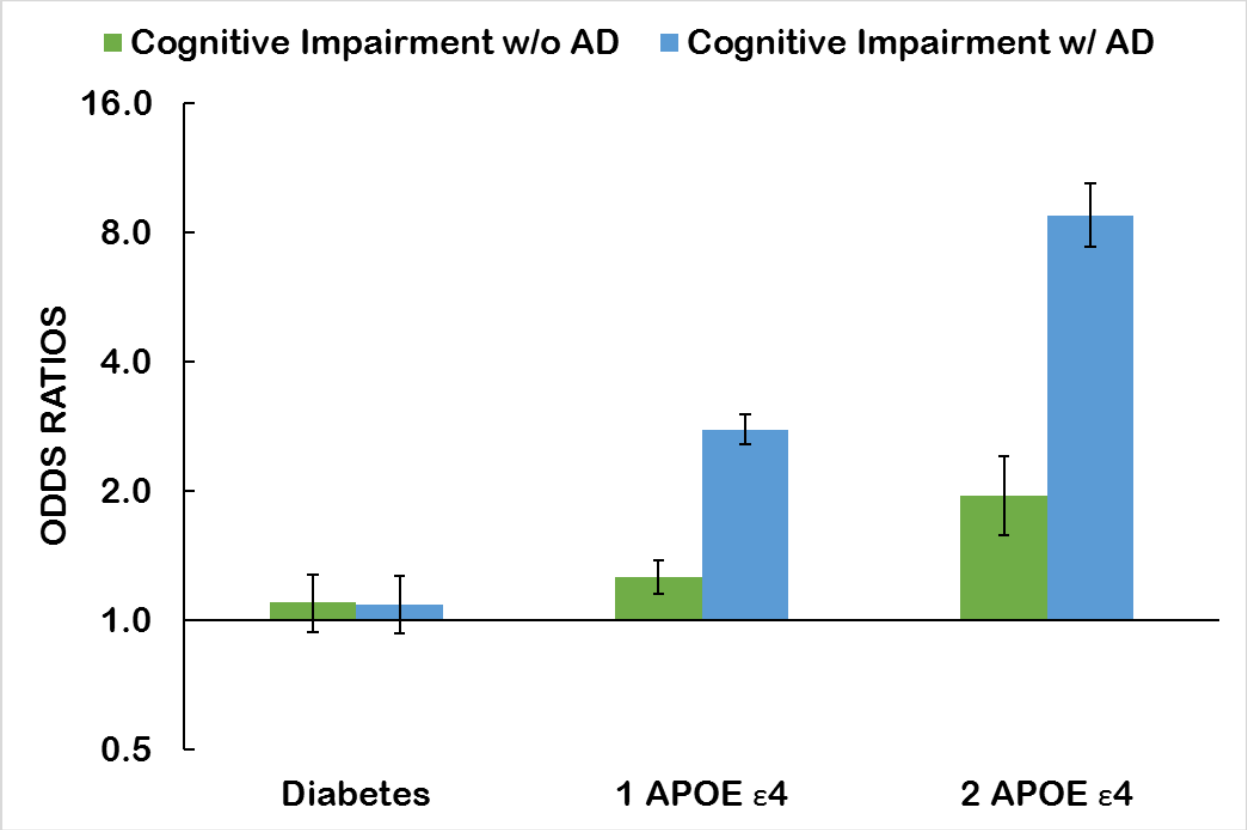

Figure 1: Odds Ratios for adjusted model. Error bars represent 95% CI's. Odds Ratios are presented as Diabetes vs. No diabetes, 1 APOE ε4 allele vs. 0 APOE ε4 alleles, and 2 APOE ε4 alleles vs. 0 APOE ε4 alleles. Odds ratios are plotted on a log scale.

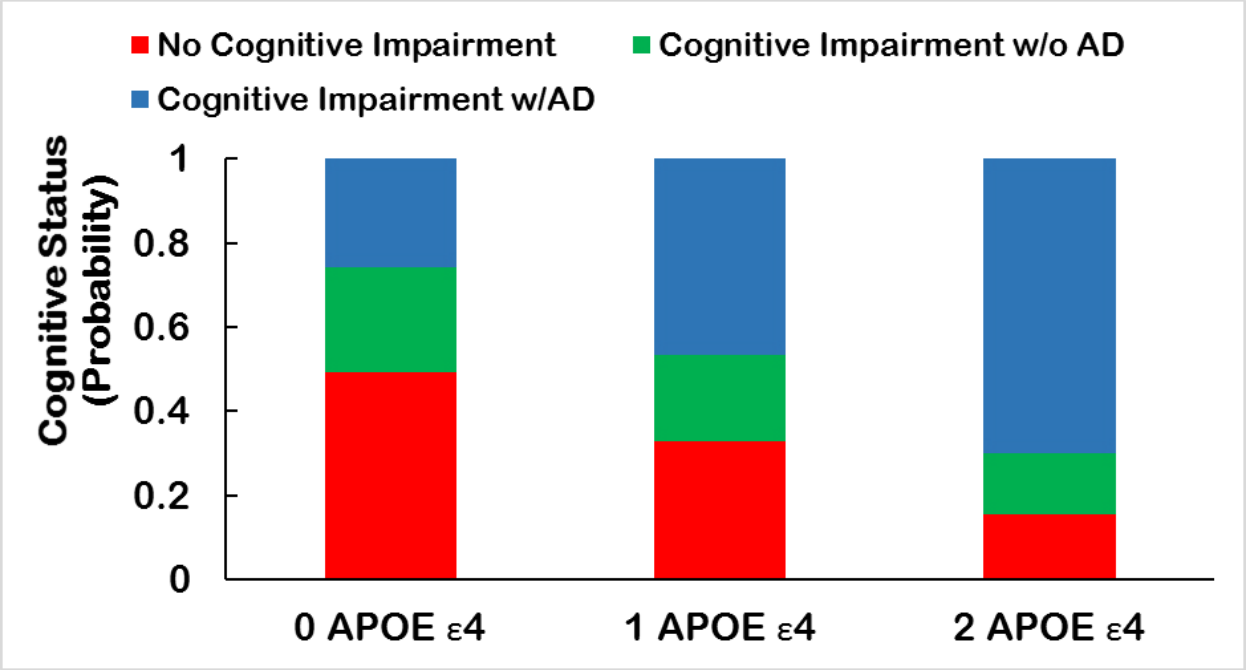

Figure 2A: Predicted Probabilities for APOE ε4. Probabilities are calculated using the adjusted model.

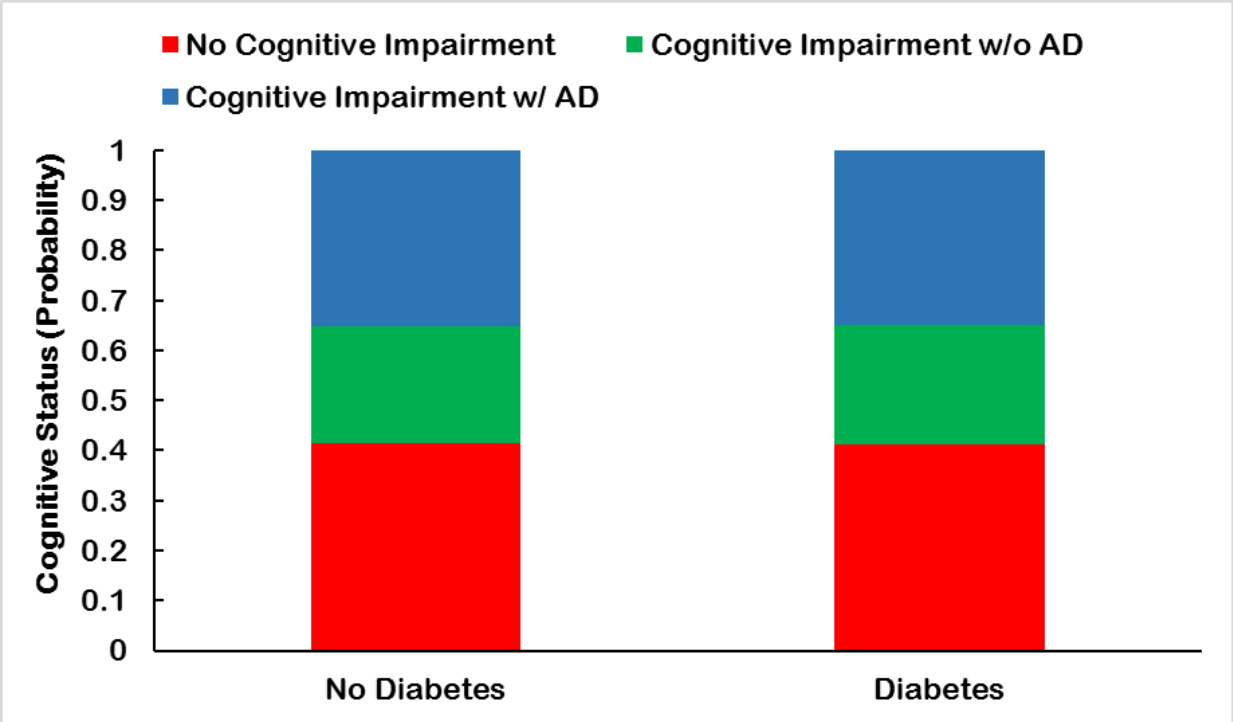

Figure 2B: Predicted Probabilities for diabetes. Probabilities are calculated using the adjusted model.

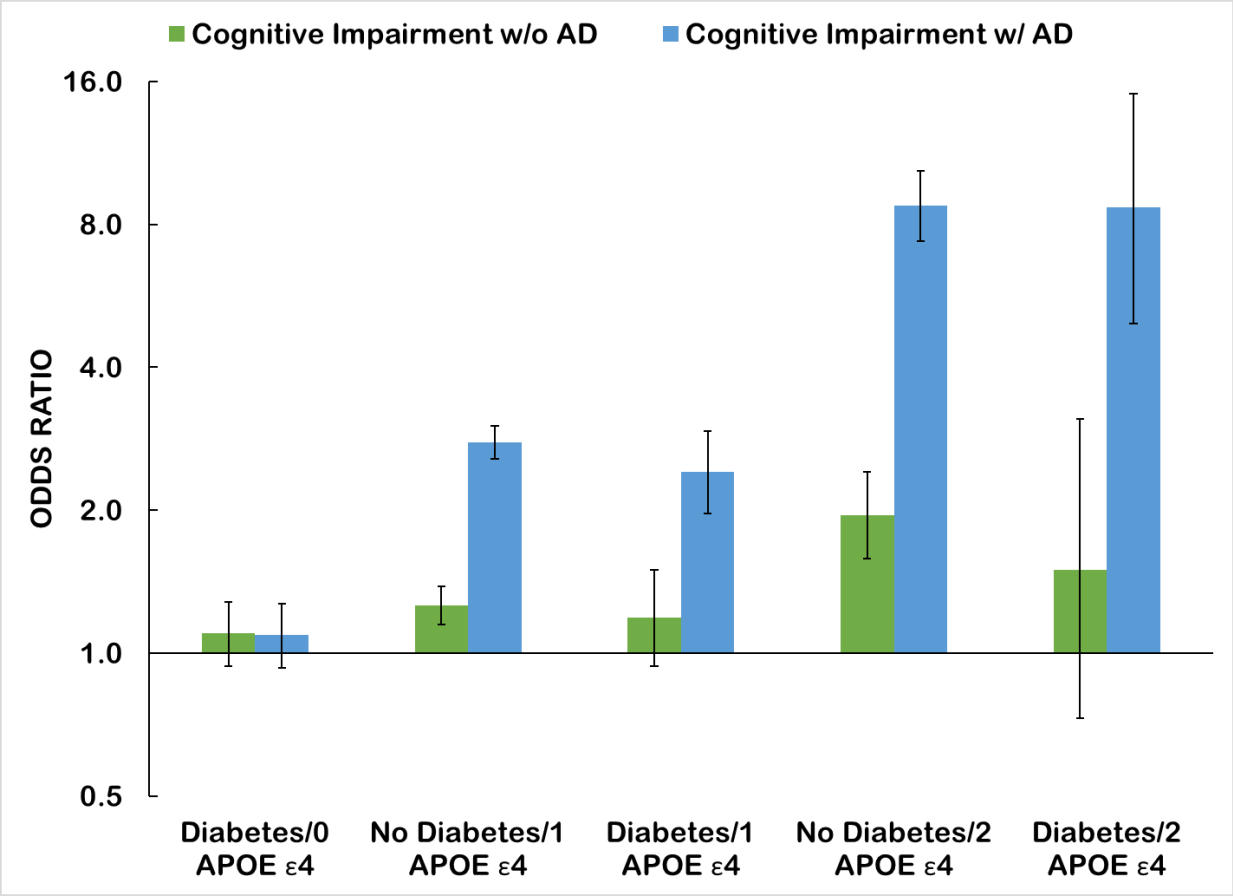

Figure 3: Odds Ratios for adjusted model. Error bars represent 95% CI's. Odds Ratios are presented with no diabetes/0 APOE ε4 alleles as the baseline. Odds ratios are plotted on a log scale.

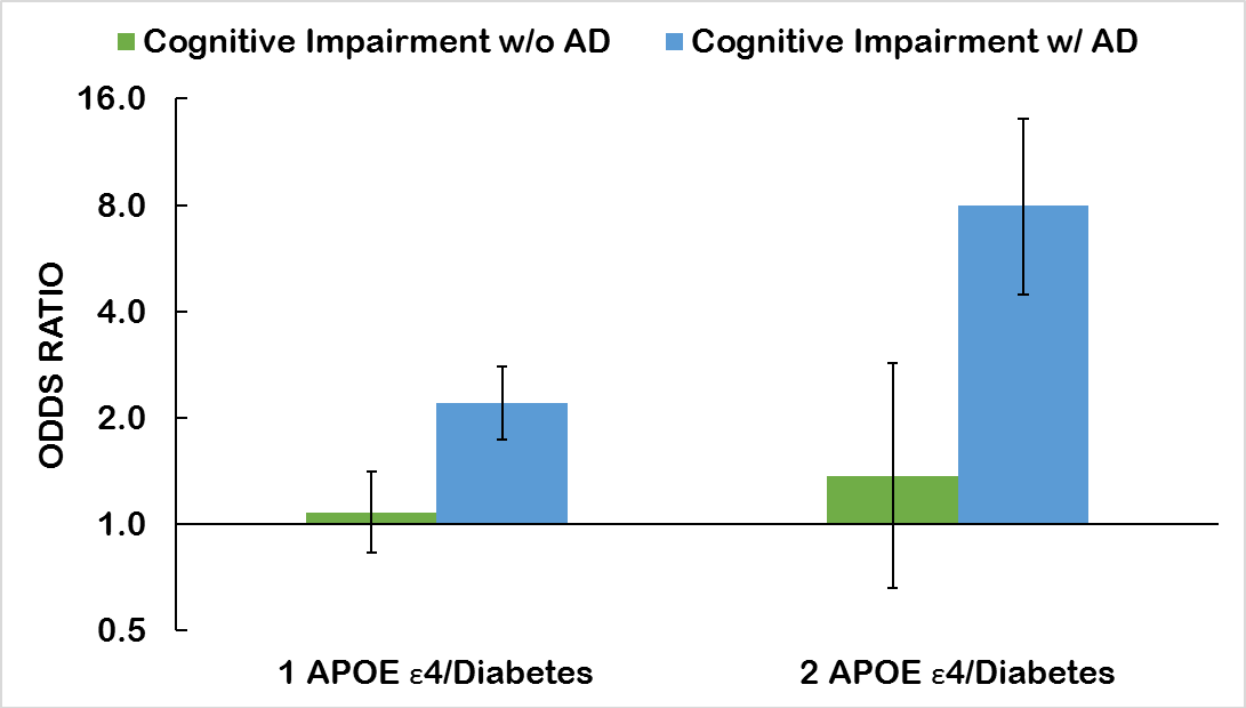

Figure 4A: Odds Ratios for adjusted model. Error bars represent 95% CI's. Odds Ratios are presented as 1 APOE ε4 allele/diabetes vs. 0 APOE ε4 allele/diabetes and 2 APOE ε4 alleles/diabetes vs. 0 APOE ε4 alleles/diabetes. Odds ratios are plotted on a log scale.

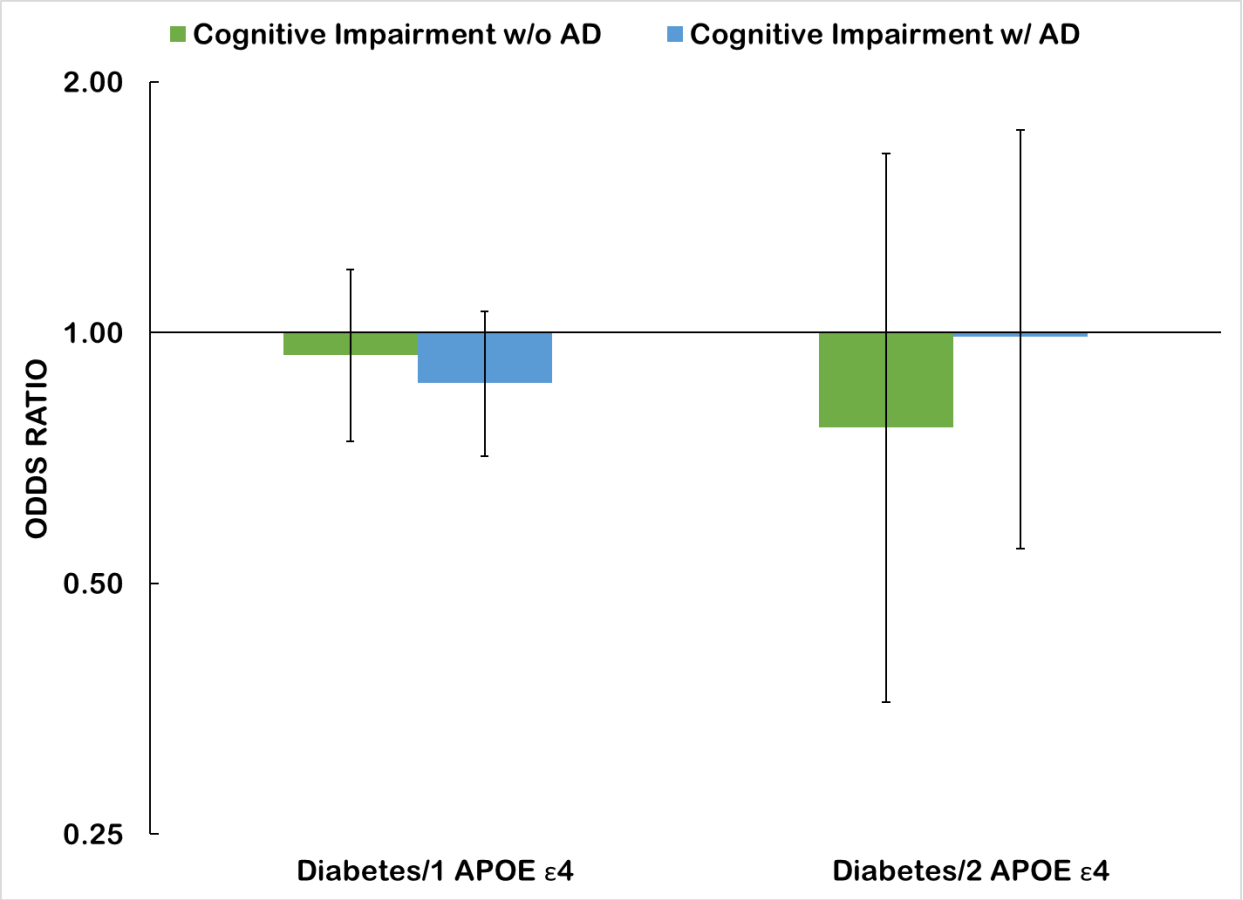

Figure 4B: Odds Ratios for adjusted model. Error bars represent 95% CI's. Odds Ratios are presented as diabetes/1 APOE ε4 vs. no diabetes/1 APOE ε4 allele and diabetes/2 APOE ε4 vs. no diabetes/2 APOE ε4. Odds ratios are plotted on a log scale.

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