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Interaction between midlife blood glucose and APOE genotype predicts later Alzheimer pathology

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Abstract

Elevated blood glucose and the apolipoprotein (APOE) ε4 allele have both been associated with increased dementia risk; however, the neuropathological mechanisms underlying these associations remain unclear. We examined the impact of APOE genotype and midlife blood glucose on post-mortem vascular and Alzheimer's disease (AD) neuropathology. Ninety-four participants from the Framingham Heart Study without diagnosed diabetes underwent health examination at midlife and brain autopsy at death. Histopathological measures of vascular and AD neuropathology were obtained and analyzed. Results demonstrated that, among APOE ε4 carriers, elevated blood glucose was associated with more severe AD pathology. There was no such relationship with vascular pathology. In a relatively healthy sample with low vascular risk burden, midlife elevated blood glucose was associated with greater AD pathology among APOE ε4 carriers. A better understanding of interactive effects of APOE genotype and vascular risk on neuropathology has implications for identification of individuals at risk for decline and long-term preventive treatment.

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Conflicts

There are no conflicts to report.

Keywords

Alzheimer's disease; glucose; diabetes; vascular risk; apolipoprotein E (APOE); neuropathology

INTRODUCTION

Although many factors regarding the underlying medical problems that may potentiate Alzheimer's disease (AD) are poorly understood, recent research has established links between AD and common cerebrovascular risk factors including diabetes [1]. Several studies have found that, even among nondiabetic individuals, elevated blood glucose is associated with increased risk for dementia [2, 3]. However, despite relatively consistent epidemiological findings linking diabetes and elevated blood glucose to dementia, there are few neuropathological studies examining the underlying neuropathological substrate of this association, and findings have generally been mixed. For example, although diabetes has been linked to cerebral infarcts [4–6], its association with AD neuropathology (amyloid plaques and neurofibrillary tangles [NFT]) is less clear [4, 5, 7–9]. Discrepant findings across studies have been attributed to methodological differences regarding risk factor characterization and assessment, as well as failure to account for potentially confounding variables such as presence of vascular pathologies [1, 9].

To our knowledge, there are no published reports investigating how the apolipoprotein E (APOE) ε4 allele— a well-established risk factor for AD—may modify the relationship between elevated blood glucose and neuropathology in nondiabetic individuals. Furthermore, most investigations of dementia and diabetes or blood glucose have focused on late-life versus midlife exposure despite growing awareness of the importance of a life-long perspective of dementia prevention and, in particular, the need for studies to follow participants beginning in midlife, prior to the typical later-life onset of AD. Thus, in a well-characterized cohort of Framingham Heart Study participants, we examined the combined impact of midlife elevated blood glucose and APOE genotype as related to AD neuropathology, vascular lesions, and clinical diagnosis.

MATERIALS AND METHODS

Participants

The Framingham Heart Study (FHS) began in 1948 to identify risk factors for cardiovascular disease. Participants in the current study were members of the Original cohort and Offspring cohort, which includes biological children of the Original cohort and Offspring spouses. Original and Offspring cohorts have undergone health examinations every two years since 1948 and every four to eight years since 1971, respectively. Participants in the current study are those individuals from these two cohorts that later came to autopsy through the Framingham Brain Donation Program.

Midlife blood glucose and other vascular risk factor data were extracted from the examination closest to midlife (50±5 years). Clinical diagnosis is the diagnosis closest in time to death and is determined at a consensus conference. The diagnostic protocol involves

review of medical information from five key sources: (1) FHS health examinations, (2) FHS neurological examinations, (3) neuropsychological evaluations, (4) outside medical records, and (5) a post-mortem family interview inquiring about changes in cognitive and functional status as well as the time course of these changes. Information from these sources is reviewed to determine presence of cognitive impairment and dementia, timing of onset and diagnosis, and subtype of dementia or cognitive impairment. Individuals identified as demented met Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria [10]. Those categorized as AD met National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorder Association criteria for possible, probable or definite AD [11]. The diagnosis of vascular dementia was determined based on Alzheimer's Disease Diagnostic and Treatment Centers and National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria [12]. Clinical diagnostic criteria for other forms of dementia such as Lewy Body disease and frontotemporal dementia were also carefully specified based on published criteria [13, 14]. Mild cognitive impairment (MCI) diagnosis was based on subjective and/or objective cognitive impairment in one or more cognitive domains with essentially normal functional status [15].

Of the 164 participants who came to autopsy between 1997 and 2013, we included those individuals who had midlife blood glucose and APOE genotype data. Individuals were excluded for missing data, APOE ε2/ε4 genotype, and diagnosis of diabetes at midlife. For Original cohort participants (who had not been asked to fast for blood glucose test), diabetes mellitus was defined as blood glucose ≥200 mg/dL or current treatment for diabetes. For Offspring cohort participants (who were asked to fast for blood glucose test), diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or use of an anti-diabetic therapy. Together these inclusion and exclusion criteria resulted in a final sample of 94 participants for the current study. See Figure 1. The protocol was approved by the Institutional Review Board of Boston University Medical Center. All participants provided written informed consent.

Blood Glucose and Vascular Risk Factors

Elevated blood glucose was defined as fasting glucose ≥100 mg/dL (offspring cohort) or random glucose ≥140 mg/dL (original cohort) [16]. No participants were taking anti-diabetic medication. To assess overall vascular risk burden, additional vascular risk factors were assessed at midlife. Obesity was defined as body mass index ≥30 kg/m^2; hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications; history of cardiovascular disease was based on history of coronary heart disease (myocardial infarction, angina pectoris, coronary insufficiency), cardiac failure, and intermittent claudication [17]; serum total cholesterol was measured; and cigarette smokers were identified based on current smoking at midlife.

Neuropathological Assessment

Brain autopsies were performed by a single neuropathologist (ACM), who was blind to all clinical information, using established Framingham Brain Donation Program protocols. Details of the neuropathological assessment have been previously published [15]. Briefly,

brains were received fresh and examined grossly. The frontal, temporal and occipital poles were removed from one hemisphere and snap frozen at −80 C. The remaining tissue was fixed in 4% periodate-lysine-paraformaldehyde at 4 degrees Celsius for a minimum of two weeks. Tissue blocks were taken from 30 regions including two levels of the midbrain; two levels of pons; medulla; two levels of the cerebellum; the olfactory bulb; subcortical regions of the brain including the caudate, putamen, nucleus accumbens, amygdala, entorhinal cortex, globus pallidus, substantia innominata, anterior hippocampus, hippocampus at the level of the lateral geniculate, and two levels of the thalamus; the pre- and post-central gyrus; the frontal lobe including inferior frontal, superior frontal, and dorsolateral middle frontal regions, and anterior cingulate; the temporal lobe including anterior temporal, superior temporal, superior temporal posterior regions; posterior cortex including the posterior cingulate, calcarine, inferior parietal, superior parietal and visual association regions.

Tissue blocks were embedded in paraffin, cut into 10-μm thick sections, and stained with luxol fast blue, hematoxylin and eosin, Bielschowsky silver method, and immunocytochemistry for phosphorylated tau protein (AT8) and Aβ protein.

Alzheimer's Disease Lesions

Neurofibrillary tangles (NFT) were counted using AT8 immunostained sections and rated on a semi-quantitative scale for three medial temporal structures (amygdala, entorhinal cortex, hippocampus) and five neocortical regions (inferior parietal, middle frontal, superior temporal, calcarine, visual association cortices). For analytical purposes, we focused on MTL and cortical NFT densities given that they are areas thought to be affected early and later in the course of AD, respectively. The four point semiquantitative scale ranges from 1+ (indicating 1–10 NFT/microscopic field for the MTL structures and a maximum density of 1 NFT per 200 × microscopic field for the cortical regions) to 4+ (corresponding to ≥31 NFT/field for MTL structures and ≥10 NFT/field for cortical regions). Density of diffuse and neuritic plaques was determined by Aβ immunostaining and Bielschowsky silver stain in these same regions [15]. Brains were staged for the degree of NFT pathology using a modification of Braak and Braak's scheme [15]. Neuropathological diagnosis of AD was performed based on recommendations of the National Institute on Aging (NIA)-Reagan criteria [19] and Braak and Braak Stage.

Vascular Lesions

Participant brains were assessed for forms of vascular pathology including: 1) chronic macroscopic infarcts (>10 mm), 2) macroscopic lacunes (small [<10 mm] infarcts) 3) microinfarcts (encephalomalacic lesions ≤2 mm in diameter not identifiable on gross inspection of the brain), 4) degree of cerebral arteriosclerosis (hyaline thickening of arteriolar wall evaluated in four regions of deep white matter and basal ganglia), and 5) degree of atherosclerosis in the circle of Willis. A semi-quantitative measure of vascular pathology severity was calculated based on the presence versus absence and severity of the above forms of pathology (Atherosclerotic Injury Score). The presence and severity of cerebral amyloid angiopathy (CAA) was assessed semi-quantitatively in middle frontal, inferior parietal, superior temporal, and calcarine cortices.

Statistical Analyses

Multivariate linear and logistic regression analyses were performed to examine associations among blood glucose, APOE genotype, and neuropathology. APOE genotype was coded as ε4 carrier (i.e., individuals with at least one copy of the APOE ε4 gene) versus non-carrier (i.e., participants with no copies of the APOE ε4 gene). Main effects and interactions were examined in separate models. Models with interaction terms were performed in order to examine the modifying effect of APOE genotype on the relationship between midlife blood glucose level and neuropathological burden. All models were adjusted for age at blood glucose assessment, sex, time from midlife vascular risk factor assessment to death, and obesity, smoking status, systolic blood pressure, and total cholesterol at midlife. Logistic regression analyses were conducted to examine the associations among blood glucose, APOE genotype, and clinical diagnosis (i.e., normal cognition, MCI, or dementia).

Three sets of secondary analyses were performed. First, analyses were performed adjusting for late life diabetes to determine whether findings were due to late life diabetes onset in people with elevated blood glucose at midlife. Second, analyses were performed adjusting for late life elevated blood glucose to determine whether results were due to late life elevated blood glucose regardless of the presence or absence of diabetes. Third, analyses additionally adjusting for cohort given that our definition of elevated blood glucose was not uniform across the original and offspring cohorts (i.e., data from the original cohort consisted of casual glucose samples whereas the offspring cohort consisted of fasting glucose samples). Significance levels of 0.05 were used for all tests. Analyses were performed using Statistical Analyses System software version 9.3 (SAS Institute, Cary, NC).

RESULTS

Demographic variables and participant characteristics

Midlife blood glucose and vascular risk factors were assessed at a mean age of 50 years. The mean age at death was 84 years. During the period of time between blood glucose assessment and death, 20 participants sustained a stroke and 41 developed dementia. Of those who sustained a stroke, five had elevated blood glucose at midlife (two APOE ε4 carriers and three noncarriers) and 15 did not have elevated blood glucose at midlife (four APOE ε4 carriers and 11 noncarriers). At the midlife assessment approximately 20% of the sample was classified as having elevated blood glucose. Nearly half of the participants had no vascular risk factors (elevated blood glucose, hypertension, cardiovascular disease, current smoking) and only 20% had two or more vascular risk factors. Approximately 25% of participants were APOE ε4 carriers (Table 1). APOE ε4 carriers and non-carriers did not significantly differ in terms of the proportion of individuals who had elevated blood glucose at midlife (30.4% of APOE ε4 carriers and 16.9% of non-carriers; Fisher's exact $p = 0.08$) and at late life (38.1% of APOE ε4 carriers and 35.7% of non-carriers; $\chi^2 = 0.04$, $p = 0.85$). It should be noted that late life blood glucose data was missing for 18% of the sample.

Effects of midlife elevated blood glucose and APOE genotype on neuropathology

Across all participants, elevated midlife blood glucose was significantly associated with greater density of medial temporal NFT (β±SE: 1.97±0.88, $p<.05$). There were no other

significant main effects of elevated blood glucose (*p*-values>.05; Table 2). Across all participants, the presence of the APOE ε4 allele was significantly associated with AD pathology, including greater density of medial temporal NFT (β±SE: 2.13±0.77, p≤ .01) and cortical NFT (β±SE: 5.73±1.90, p≤.01), presence of cortical diffuse plaques (OR[95% CI]: 6.86[1.26–37.50], p<.05), medial temporal neuritic plaques (OR[95% CI]: 5.77[1.52–21.89], p≤.01), cortical neuritic plaques (OR[95% CI]: 10.23[1.75–59.94], p≤.01), and higher Braak staging (β±SE: 1.24±0.44, p≤.01). The presence of the APOE ε4 allele was also significantly associated with the presence of CAA (OR[95% CI]: 16.76[1.85–152.13], p<.05). APOE genotype was not associated with the presence of vascular pathology (*p*-values >.05). All models were adjusted for age, sex, time between midlife vascular risk assessment and death, and presence of additional vascular risk factors at midlife.

There were significant interactions whereby APOE genotype modified the relationship between midlife elevated blood glucose and neuropathology (Table 2). Specifically, among APOE ε4 carriers, elevated blood glucose was associated with significantly greater density of medial temporal NFT (β±SE: 7.41±2.26, p=0.006) and higher Braak staging (β±SE: 3.54±1.12, p =0.007). See Table 3 and Figure 2.

Effects of midlife elevated blood glucose and APOE genotype on neuropathological and clinical diagnosis

Although there were no main effects of midlife elevated blood glucose on neuropathological or clinical diagnosis (*p*-values >.05), there were significant main effects of APOE genotype such that APOE ε4 carriers were significantly more likely to be diagnosed with AD based upon Braak staging and NIA-Reagan neuropathologic criteria. In addition, APOE ε4 carriers were significantly more likely to be clinically diagnosed with dementia (all forms) and AD specifically (Table 4). APOE genotype did not significantly modify the relationship between midlife elevated blood glucose and neuropathological or clinical diagnosis (*p*-values >.05).

Secondary analyses adjusting for late life diabetes, late life elevated blood glucose, and cohort effect

When analyses were performed a second time adjusting for late life diabetes, all findings remained statistically and qualitatively similar. When analyses were performed a third time additionally adjusting for late life elevated blood glucose, the majority of the findings remained similar. The association between cortical diffuse plaques and APOE genotype, association between elevated blood glucose and medial temporal NFT density, interaction between elevated blood glucose and APOE genotype on Braak and Braak stage, and associations between APOE genotype and clinical diagnosis no longer met statistical significance. When analyses were performed a fourth time additionally adjusting for cohort/type of blood glucose test (i.e., random versus fasting), findings were generally not changed. The one exception was related to the association between midlife elevated blood glucose and Braak Stage among APOE ε4 carriers (see Table 3). After controlling for cohort effect/type of glucose test, there was no longer a statistically significant association and instead a trend toward an association between elevated blood glucose and higher Braak stage among APOE ε4 carriers was revealed (β±SE: 2.90±1.62, p=.098).

DISCUSSION

In this prospective, community-based sample, we found that midlife elevated blood glucose predicted the later development of AD tangle pathology in individuals with the APOE ε4 genotype. Presence of the APOE ε4 allele significantly modified the association between elevated blood glucose and AD tangle pathology independently of other known risk factors for AD, including age, history of smoking, and midlife obesity, hypertension, and high cholesterol. Importantly, findings did not change when adjusting for presence of late life diabetes, suggesting that results were not due to late life diabetes in people with elevated glucose during midlife. These results suggest that higher levels of blood glucose—even decades before diagnosis and death—and even among nondiabetics—may exert deleterious effects on the aging brain among those at genetic risk for AD.

Most previous studies that have examined associations between glucose metabolism and neuropathological outcome have focused on diabetes itself, and far fewer have specifically examined the impact of APOE genotype on this association. Our findings are in line with autopsy studies wherein individuals with both diabetes and the APOE ε4 allele demonstrated an elevated risk of AD-associated neuropathology [5, 7]. Our findings also parallel those from various cohorts that demonstrate a significant interaction of APOE ε4 genotype and diabetes that increases the risk of cognitive decline [20–23], and they extend these findings to a relatively healthy sample of middle-aged adults with elevated blood glucose versus diagnosed diabetes.

Suggested mechanisms by which diabetes may affect the brain and negatively impact cognition include changes to brain vasculature, alterations in cerebral insulin signaling, insulin resistance, glucose toxicity, oxidative stress, accumulation of advanced glycation end products (AGEs), increased inflammation, and/or ischemia [24, 25]. The precise mechanism by which elevated blood glucose and APOE genotype interact to increase AD neuropathology is unclear. It has been suggested that AGEs and APOE may play a role in neurodegeneration given co-localization of AGEs in NFTs, senile plaques, and CAA in AD and other neurodegenerative diseases [26]. Indeed, APOE ε4 is associated with a 3-fold greater AGE-binding activity compared to the APOE ε3 isoform indicating that age by APOE ε4 interactions may contribute to the formation of dense amyloid deposits as well as NFTs [27]. Interestingly, although APOE ε4 carriers with elevated blood glucose at midlife demonstrated greater density of medial temporal NFT and higher Braak staging, there were no significant interactions on density of neuritic or diffuse plaques. These findings dovetail with evidence showing that hyperinsulinemia and impaired cortical insulin resistance may be linked to tau hyperphosphorylation [28, 29]. Collectively, these findings indicate that altered glucose metabolism may also be linked to processes promoting tau accumulation.

One proposed model of neurodegeneration suggests that amyloid-β (Aβ) protein deposition drives AD pathogenesis and secondarily induces the formation of abnormal tau protein, followed by tau-mediated neuronal injury, metabolic and structure brain changes, and culminating with cognitive and functional impairments [30]. However, other evidence suggests that Aβ is not required to develop neurodegeneration within AD-affected regions[31]. Furthermore, recent studies suggest that the various biomarkers of

neurodegeneration (FDG-PET, CSF tau, structural MRI) are not interchangeable [32]. That is, different 'neuronal injury' biomarkers showed important disagreements in classifying subjects and in predicting progression [32]. Our findings suggest that the mandatory initial appearance of amyloidosis prior to tau alterations may be altered in the presence of APOE ε4 and elevated blood glucose. That is, genetic and neural injury risks may trump amyloidosis in the AD pathophysiological cascade.

Although diabetes has been shown to be associated with increased risk for cerebral infarcts [5, 8, 33] and vascular dementia [34], we did not observe such an association with elevated blood glucose in our sample. However, a recent study that modeled diabetic status in midlife and cognitive functioning and MR measures of brain volume, infarcts, and white matter hyperintensities in late life found that global brain volume (rather than markers of vascular pathology) was in the causal pathway for the association between diabetes and cognitive decline [35]. Furthermore, we previously found that, in a sample of patients with autopsy-confirmed AD who had mild CVD, diabetes predicted the presence of CVD only when considered in combination with additional vascular risk factors [36]. Such findings highlight the importance of investigating aggregate risk in the vascular contribution to dementia. However, it may be the case that some individuals in our sample, with an average age of 50, may not have yet expressed abnormalities in glucose metabolism.

After additionally adjusting statistical models for late life elevated blood glucose, the majority of findings remained similar including the significant interaction between midlife elevated blood glucose and APOE genotype on medial temporal neurofibrillary tangles; however, some findings were attenuated and no longer statistically significant. Both midlife and late life diabetes have been associated with increased risk of Alzheimer's disease [37] and findings from a meta-analysis suggested that the magnitude of association is similar in both age groups [38]. Although both midlife and late life elevated blood glucose may represent important risk factors, we focused on midlife elevated blood glucose given that many previous studies of dementia risk have not included midlife data as well as the hope that midlife may present opportunities for earlier intervention aimed at preventing cognitive decline. It is possible that including midlife and late life elevated blood glucose as an independent variable and covariate, respectively, in our models may have led to overadjustment in our secondary analyses.

Strengths of the current study include its well-characterized, community-based sample; prospective study design; intensive, ongoing follow-up for several decades; and pathologic diagnoses made blind to clinical or demographic information [39]. However, despite these strengths, there are some weaknesses that are important to note. First, we did not find any significant interactive effects of midlife elevated blood glucose and APOE genotype on diagnosis, and findings may have differed if we had examined diagnosis at an earlier age. In addition, the number of participants in some subgroups was relatively small and the sample was generally healthy with relatively few vascular risk factors, which may have limited our findings. Moreover, we measured blood glucose levels, and results may have differed if we examined a different glucose measure (e.g., glycated hemoglobin level). In addition, due to different collection procedures (i.e., random/casual versus fasting) and therefore different thresholds for elevated blood glucose and diabetes across participants, we did not investigate

blood glucose as a continuous variable. Given power considerations, we were unable to examine gene-dose effects or the specific influence of the APOE ε2 allele. Finally, as is typical among neuropathology studies, we assessed segments of brain tissue in only one hemisphere. We may have therefore underestimated existing pathology—particularly evidence of CVD that may be patchy and irregularly distributed throughout the brain—and, as such, a more exhaustive autopsy approach may be more useful to quantify this type of neuropathology.

Despite these limitations, our finding of an interaction between elevated midlife glucose levels and APOE ε4 genotype is especially important when taking into consideration the public health impacts of diabetes and AD coupled with epidemiological data showing that rates of both disease states are expected to dramatically increase. In the search for reliable biomarkers of preclinical dementia, examination of the combination or interaction of risk factors in evaluating dementia risk may have potential use. Among older patients with diabetes, the effects of tighter glycemic control on cognition have been inconclusive [40–42]. However, it is possible that earlier intervention in individuals who do not yet have diabetes may be more effective, particularly in light of recent findings demonstrating that diabetes medications may reduce AD neuropathology [43]. The present findings, along with other studies, may inform future trials of glucose lowering in prediabetes during midlife, particularly among those who are already at risk for dementia by virtue of possession of the APOE ε4 allele.

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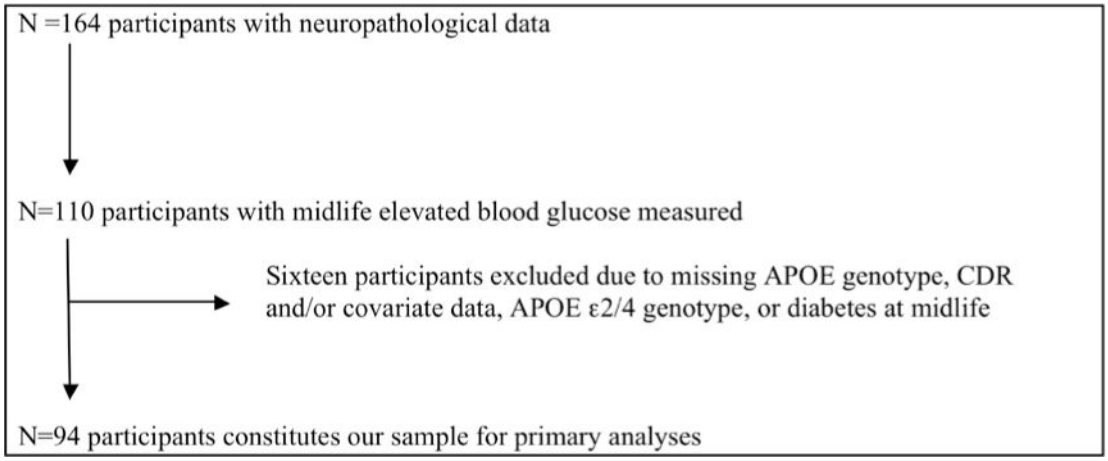

Figure 1.
Flow diagram of participant selection process

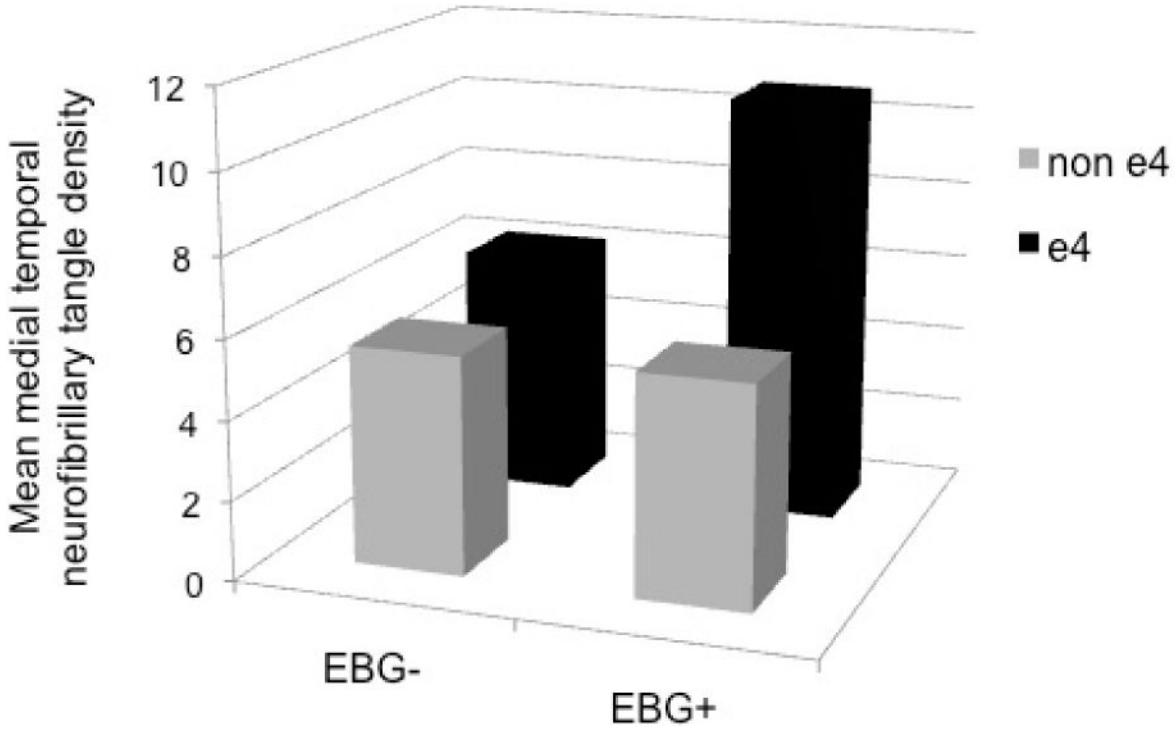

Figure 2. Mean medial temporal neurofibrillary tangle density by APOE genotype and elevated blood glucose

Mean medial temporal neurofibrillary tangle (NFT) density adjusted for age at blood glucose assessment, sex, time from midlife blood glucose assessment to death, and obesity, smoking status, systolic blood pressure, and total cholesterol at midlife. APOE genotype significantly modified the relationship between elevated blood glucose and medial temporal NFT density ($p = 0.017$). EBG = elevated blood glucose.

Table 1

Participant Demographics and Risk Factor Characteristics

Variable	
N	94
Demographics	
Age at vascular risk factor assessment (years; mean ± SD)	50±2
Time between vascular risk factor assessment and death (years; mean ± SD)	34±11
Cohort, n (%) Original (first generation) Offspring (second generation)	48 (51.1) 46 (48.9)
Women, n (%)	47 (50.0)
Education, n (%) No HS Degree HS Degree Some College College Degree	8 (8.8) 24 (26.4) 25 (27.5) 34 (37.3)
APOE genotype, n (%) 22 23 33 34 44 ε4 carrier, n (%)	1(1.0) 12 (12.8) 58 (61.7) 23 (24.5) 0 23 (24.5)
Vascular Risk Factors	
Systolic Blood Pressure (mmHg; mean ± SD) Total sample APOE ε4 carriers APOE ε4 non-carriers	123±17 119±15 125±17
Elevated blood glucose, n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	19 (20.2) 7 (30.4) 12 (16.9)
Hypertension, n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	24 (25.5) 7 (30.4) 17 (23.9)
History of CVD, n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	1 (1.1) 0 1 (1.4)
Atrial fibrillation, n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	1 (1.1) 1 (4.4) 0
Obesity (body mass index ≥30), n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	12 (12.8) 2 (8.7) 10 (14.1)
Smoking, n (%) Total sample APOE ε4 carriers APOE ε4 non-carriers	27 (28.7) 7 (30.4) 20 (28.2)
Aggregate Vascular Risk (based on sum of above risk factors [i.e., obesity, HTN, CVD, elevated blood glucose, Smoking], %) No vascular risk factors 1 vascular risk factor ≥2 vascular risk factors	44 (46.8) 31 (33.0) 19 (20.2)
Use of anti-hypertensive medication, n (%)	6 (6.5)

Variable	
Total sample APOE ε4 carriers APOE ε4 non-carriers	1 (4.4) 5 (7.3)
Cognitive Functioning	
Clinical Diagnosis, n (%) Normal MCI Dementia AD	37 (39.4) 16 (17.05) 41 (43.6) 29 (30.9)
Neuropathology	
Braak Stage, n (%) I–III, VII (Not AD) IV–VI (AD)	65 (69.2) 29 (30.9)
NIA Reagan, n (%) 1–2 (AD) 3–4 (Not AD)	36 (38.3) 58 (61.7)
Cortical neuritic plaques, n (%) Absent Present	29 (30.9) 65 (69.1)
Medial temporal neuritic plaques, n (%) Absent Present	38 (40.4) 56 (59.6)

Abbreviations: SD = standard deviation; HS = high school; APOE = apolipoprotein E; CVD = cardiovascular disease; HTN = hypertension; AD = Alzheimer's disease; NIA = National Institute on Aging

Table 2

Main effect of APOE genotype, main effect of elevated blood glucose (EBG), and interaction between APOE genotype and EBG on neuropathology

	APOE ε4 β ± SE or OR [95% CI]	Elevated Blood Glucose (EBG) β ± SE or OR [95% CI]	Interaction between APOE genotype and EBG p value
AD pathology			
Medial temporal AT8 NFT density	**2.13 ± 0.77** **	**1.97 ± 0.88** *	**0.017**
Cortical AT8 NFT density	**5.73 ± 1.90** **	1.13 ± 2.23	0.179
Medial temporal diffuse plaques (>0)	3.56 [0.95–13.38]	0.93 [0.27–3.23]	0.965
Cortical diffuse plaques (>0)	**6.86 [1.26–37.50]** *	1.25 [0.34–4.60]	0.973
Medial temporal neuritic plaques (>0)	**5.77 [1.52–21.89]** **	1.04 [0.33–3.29]	0.964
Cortical neuritic plaques (>0)	**10.23 [1.75–59.94]** **	1.00 [0.28–3.56]	0.972
Braak and Braak Stage	**1.24 ± 0.44** **	−0.03 ± 0.52	**0.064**
Vascular pathology			
Atherosclerotic Injury Score	−0.17 ± 0.76	−0.08 ± 0.86	0.119
Atherosclerosis of the circle of Willis (>0)	1.14 [0.37–3.54]	2.87 [0.72–11.42]	0.741
Arteriosclerosis (>0)	1.23 [0.29–5.19]	2.48 [0.45–13.50]	0.973
Cerebral amyloid angiopathy			
CAA (>0)	**16.76 [1.85–152.13]** *	0.60 [0.18–2.00]	0.971

* $p<0.05$,

** $p\leq0.01$

β represents difference in adjusted means; SE = standard error; AD = Alzheimer's disease; NFT = neurofibrillary tangles; OR = odds ratio; CI = confidence interval; APOE = apolipoprotein E; CAA = cerebral amyloid angiopathy

Main effects and interactions were examined in separate models. All models adjusted for age; sex; time between midlife vascular risk factor assessment and death; and obesity, systolic blood pressure, total cholesterol, and smoking status at midlife.

Table 3

Association between midlife elevated blood glucose and neuropathology stratified by APOE ε4 status (for those interactions where $p < 0.10$)

	Elevated Blood Glucose β ± SE (p)
AD pathology	
Medial temporal AT8 NFT	0.30±1.04 (0.771)
APOE ε4−	**7.41±2.26 (0.006)**
APOE ε4+	
Braak and Braak Stage	−0.91±0.61 (0.140)
APOE ε4−	**3.54±1.12 (0.007)**
APOE ε4+	

β represents difference in adjusted means; SE = standard error; AD = Alzheimer's disease; NFT = neurofibrillary tangles; APOE = apolipoprotein E

All models are adjusted for age; sex; time between midlife vascular risk assessment and death; and obesity, systolic blood pressure, total cholesterol, and smoking status at midlife

Table 4

Main effect of APOE genotype, main effect of elevated blood glucose (EBG), and interaction between APOE genotype and EBG on neuropathological and clinical diagnosis

Neuropathological Diagnosis	**APOE *ε*4 OR [95% CI]**	**Elevated Blood Glucose (EBG) OR [95% CI]**	**Interaction between APOE genotype and EBG p value**
Alzheimer's disease (Braak and Braak Stage)	**4.46 [1.50–13.29]** **	1.62 [0.48–5.47]	0.200
Alzheimer's disease (NIA/Reagan)	**5.77 [1.88–17.70]** **	1.32 [0.41–4.30]	0.560
Clinical Diagnosis			
Cognitively normal	Ref	Ref	
Mild cognitive impairment	2.17 [0.40–11.94]	2.59 [0.41–16.33]	0.999
Dementia (all forms)	**5.63 [1.50–21.20]** **	2.73 [0.71–10.55]	0.963
Alzheimer's disease	**8.66 [1.68–44.77]** **	2.18 [0.45–10.62]	0.952

* $p<0.05$,

** $p\leq0.01$

Abbreviations: OR = odds ratio; CI = confidence interval; APOE = apolipoprotein E; NIA = National Institute on Aging

All models are adjusted for age; sex; time between midlife vascular risk assessment and death; and obesity, systolic blood pressure, total cholesterol, and smoking status at midlife