Type 2 Diabetes, APOE Gene, and the Risk for Dementia and Related Pathologies

The Honolulu-Asia Aging Study

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Type 2 diabetes may be a risk factor for dementia, but the associated pathological mechanisms remains unclear. We evaluated the association of diabetes alone or combined with the apolipoprotein E (APOE) gene with incident dementia and neuropathological outcomes in a population-based cohort of 2,574 Japanese-American men enrolled in the Honolulu-Asia Aging Study, including 216 subjects who underwent autopsy. Type 2 diabetes was ascertained by interview and direct glucose testing. Dementia was assessed in 1991 and 1994 by clinical examination and magnetic resonance imaging and was diagnosed according to international guidelines. Logistic regression was used to assess the RR of developing dementia, and log-linear regression was used to estimate the incident rate ratio (IRR) of neuropathological outcomes. Diabetes was associated with total dementia (RR 1.5 [95% CI 1.01–2.2]), Alzheimer's disease (AD; 1.8 [1.1–2.9]), and vascular dementia (VsD; 2.3 [1.1–5.0]). Individuals with both type 2 diabetes and the APOE ε4 allele had an RR of 5.5 (CI 2.2–13.7) for AD compared with those with neither risk factor. Participants with type 2 diabetes and the ε4 allele had a higher number of hippocampal neuritic plaques (IRR 3.0 [CI 1.2–7.3]) and neurofibrillary tangles in the cortex (IRR 3.5 [1.6–7.5]) and hippocampus (IRR 2.5 [1.5–3.7]), and they had a higher risk of cerebral amyloid angiopathy (RR 6.6, 1.5–29.6). Type 2 diabetes is a risk factor for AD and VsD. The association between diabetes and AD is particularly strong among carriers of the APOE ε4 allele. The neuropathological data are consistent with the clinical results. *Diabetes* 51:1256–1262, 2002

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ABI, ankle-brachial index; AD, Alzheimer's disease; AGE, advanced glycation end product; apoE, apolipoprotein E; CAA, cerebral amyloid angiopathy; CASI, Cognitive Abilities Screening Instrument; CHD, coronary heart disease; CVD, cerebrovascular disease; DLB, dementia with Lewy bodies; HAAS, Honolulu-Asia Aging Study; IRR, incident rate ratio; LB, Lewy body; NFT, neurofibrillary tangle; NP, neuritic plaque; VsD, vascular dementia.

Type 2 diabetes is one of the most common metabolic disorders, and its prevalence increases with age. Pathological complications of diabetes affect several organs, including the brain (1). Diabetes is associated with atherosclerosis of the cerebral arteries (2) and leads to important cerebral vascular changes that cause a decrease in cerebral blood flow (3). Furthermore, hyperglycemia is accompanied by an accelerated rate of advanced glycation end product (AGE) formation, which is associated with increased amyloid deposition, tau formation, and oxidative stress (4). Longitudinal population-based studies (5–7) have shown that diabetes is associated with dementia as well as with the subtypes vascular dementia (VsD) and Alzheimer's disease (AD). However, results are not consistent, and several questions need to be addressed. Two lines of evidence would help to clarify the association of diabetes and dementia.

First, there may be a modification of the risk in diabetic individuals with a particular genetic susceptibility. The major gene associated with AD is the apolipoprotein E (APOE) gene; in particular, the ε4 allelic variant is associated with an increased risk for cognitive impairment and AD (8), whereas its role in VsD is unclear (9). Apolipoprotein E (apoE) plays a critical role in lipid metabolism and brain physiology (10).

Second, investigations of the association of diabetes with neuropathological markers that are common to AD would help to support findings based on clinical diagnosis and to identify possible mechanisms underlying the association. Although there is substantial neuropathological evidence that diabetes is associated with cerebral infarct lesions and stroke (11,12), there are few data on the association of diabetes to neuropathological markers common in AD (13). The Honolulu-Asia Aging Study (HAAS) provides the unique opportunity to examine the association of diabetes to clinically diagnosed subtypes of dementia. In addition, an autopsy study on a subsample of the same cohort provides the basis to examine the association between diabetes, apoE, and neuropathological data.

RESEARCH DESIGN AND METHODS

The Honolulu Heart Program (HHP) is a longitudinal study on heart disease and stroke based on a cohort of 8,006 Japanese-American men born between 1900 and 1919 who were living in Oahu, Hawaii, when the study began in 1965. Respondents were identified using selective service registration records from

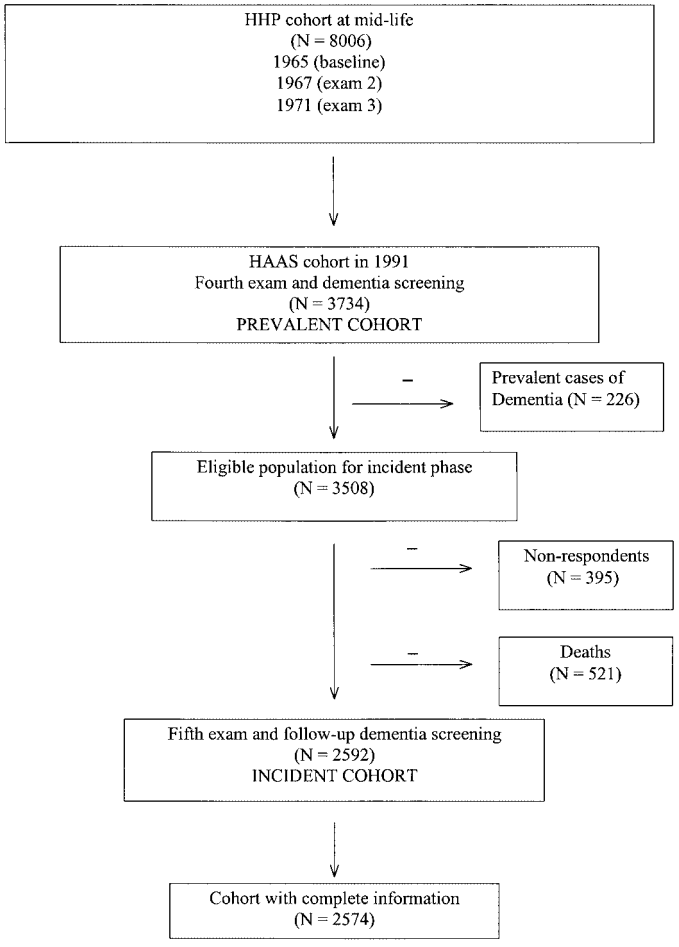

FIG. 1. Selection process of the cohort for the diabetes-dementia study: the HAAS.

World War II. The baseline exam was followed by two other exams in 1967–1970 and 1971–1974 (14).

Research on dementia began in 1991 at the fourth examination, when the HAAS was established (15) to investigate the risk factors associated with neurodegenerative disorders and aging (Fig. 1). A total of 3,734 surviving individuals (80% participation rate) were examined for cognitive function at the fourth exam (1991–1993, prevalent phase) and again at the fifth exam (1994–1996, incident phase). During the prevalent phase, a total of 226 cases of dementia were found. Before the incident phase, 521 (14%) subjects died and 395 (10%) subjects could not be reexamined. Therefore, 2,592 individuals were reexamined. As a part of the study, subjects were asked to participate in an autopsy program. To date, autopsy specimens have been collected on ~20% of the fourth exam cohort, and complete information for the analysis was available on 216 men.

The study was approved by Institutional Review Board of the Kuakini Medical Center and the Honolulu Department of Veterans Affairs, and informed consent for the HAAS and the autopsy study were collected from the participants. At the time of death, consent for the autopsy was also obtained from a family representative.

Diagnosis of dementia. In the prevalent and incident phases, all subjects were administrated the 100-point Cognitive Abilities Screening Instrument (CASI) (16), a well recognized instrument to assess cognitive function validated among Japanese and Western sample populations (17). In the prevalent phase, CASI score and age were used to identify a subgroup for dementia evaluation (15). At the follow-up exam, subjects with a CASI score less than an education-adjusted cutoff (77 for those with low education and 79 for those with high education) or an absolute drop ≥9 CASI points (n = 749) underwent a specific dementia examination (18). At each exam, evaluation of clinical dementia included a proxy interview, detailed neuropsychological assessment, neurological examination, and neuroimaging. Final diagnosis of clinical dementia was determined by a consensus committee that included the study neurologist and at least two other physicians expert in geriatric medicine and dementia.

Dementia was diagnosed according to DSM-III R criteria (19). Probable and possible AD were diagnosed following the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (20), and diagnosis of VsD was based on the California Alzheimer's Disease Diagnostic and Treatment Centers guidelines (21). Diagnosis of dementia due to multiple etiologies included AD with cerebrovascular disease (CVD) for which probable or possible AD was either the primary or secondary cause of dementia and that was accompanied by probable or possible VsD. For these diagnoses, clinical criteria and neruoimaging data were used, as suggested by the DSM-IV criteria (22). Diagnosis of VsD without apparent AD included individuals with probable VsD and additional non-AD disease (depression, alcohol abuse, B_{12} deficiency, or subdural hematoma). Dementia caused by other medical conditions included: Parkinson's disease (n = 10), dementia with Lewy bodies (DLB; n = 5), Pick's disease, head trauma, B_{12} deficiency, hypothyroidism, progressive supranuclear palsy, and other unknown causes (total number of subjects = 13). Clinical diagnosis of DLB was based on the guidelines from the consortium on DLB (23,24).

Incidence of dementia was evaluated after an average (means ± SD) period of 2.91 ± 0.30 years. For the analysis, dementia was grouped as total dementia, which included all causes of dementia; AD, with and without apparent CVD (n = 76), which included AD without apparent CVD (n = 51); and VsD without apparent AD (n = 33). AD with apparent CVD included 19 subjects (76%) with AD as the primary cause of dementia and 6 (24%) with VsD as the primary cause.

Using established neuropathological criteria (25), 25% of clinical AD cases met the criteria for definite AD and 65% for definite or probable AD. Among all the VsD cases, 68% had neuropathological evidence of atherosclerosis and did not have substantial AD-related pathologies. Lewy bodies (LBs) in the cortical area were found in ~10% of the autopsy group; however, there was only one autopsied case diagnosed with VsD with cortical LB, and there were no diagnosed AD cases with cortical LB (24).

Autopsy substudy. Baseline characteristics of the autopsy group were comparable to those of the whole cohort, except the autopsied sample was older and by design included a higher percentage of individuals clinically demented before death (data not shown) (24). A detailed description of neuropathological methods and clinical pathological correlations has been published (26). Briefly, evaluation of brain infarcts was performed on the parenchymal and meningeal tissue from neocortical areas (frontal, temporal, parietal, and occipital lobes). Brain infarcts were classified by the extent of tissue damage and labeled as "large infarcts" for lesions larger than 1 cm, or as "lacunes" for lesions 1 cm or smaller. Infarcts and lacunes were identified during macroscopic examination. Cerebral amyloid angiopathy (CAA) in the parenchyma of the neocortical tissue was evaluated by βA4 amyloid immunostaining (27) and graded from "mild" to "severe" depending on the number of positive vessels per area (28). To improve the statistical power, CAA was categorized as present or absent. The autopsy rates were similar for the major clinically diagnosed subtypes of dementia in this study (24).

Assessment of diabetes. Diabetes was assessed on the basis of self-report of a doctor's diagnosis of diabetes, use of oral hypoglycemic medications or insulin intake, or fasting and postchallenge glucose levels measured at the fourth exam. At the time of the study, only subjects who did not report diabetes (with no gastrectomy, no active peptic ulcer, and no stomach cancer) received a 75-g glucose drink (n = 1,729). In these subjects, diabetes was defined according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (29) and included individuals with fasting blood glucose ≥126 mg/dl or with 2-h postload glucose ≥200 mg/dl. A total of 551 subjects without previously reported diabetes were not tested for 2-h postload glucose level and were classified based on the fasting glucose level. However, among older adults, there are a significant number of individuals with normal fasting glucose and isolated postload hyperglycemia with vascular risk factors as high as those with diabetes (30). To evaluate the effect of the lack of information on the 2-h postload glucose level, we performed a sensitivity analysis. We tested the effect of diabetes on dementia under the hypothesis that nondiabetic individuals with normal fasting glucose level and missing data for the 2-h postload glucose were all diabetic; therefore, 1,451 (56.4%) subjects were classified as diabetic. We also evaluated isolated 2-h hyperglycemia separately to test whether elevated postload glucose was a sign of incipient AD (31). There were 202 subjects (7.8%) with normal fasting glucose and isolated elevated 2-h glucose.

Measure of covariates. APOE genotyping was performed by PCR amplification followed by restriction enzyme digestion (32) at the Bryan Alzheimer's Disease Research Center at Duke University. Participants were categorized as APOE ε4–positive if they carried at least one copy of the ε4 allele and ε4-negative otherwise. As a result of possibly opposing effects of ε2 and ε4

TABLE 1
Characteristics of the cohort that reached exam 5

	No diabetes	Diabetes	*P**
n	1,674	900	
Age (years)†	76.9 ± 4.0	77.0 ± 4.1	>0.2
Education (years)	10.7 ± 3.1	10.7 ± 3.2	>0.2
Systolic blood pressure (mmHg)‡	129.7 ± 15.8	134.0 ± 16.7	<0.001
Diastolic blood pressure (mmHg)‡	81.8 ± 9.4	83.5 ± 9.9	<0.001
BMI (kg/m^2)‡	23.6 ± 2.7	24.4 ± 2.8	<0.001
Antihypertension treatment	38.9	48.9	<0.001
Total cholesterol (mg/dl)‡	216.2 ± 30.9	220.2 ± 32.9	0.003
APOE ε4 (%)	18.3	18.0	>0.2
Fasting glucose (mg/dl)‡	102.2 ± 8.3	132.3 ± 39.5	<0.001
2-h glucose (mg/dl)‡§	138.5 ± 32.7	247.0 ± 55.0	<0.001
Fasting insulin (μIU/ml)‡	13.5 ± 8.4	20.2 ± 27.2	<0.001
2-h insulin (μIU/ml)‡§	115.8 ± 92.3	120.3 ± 89.2	<0.001
Diabetes medications			
Oral hypoglycemic (%)	—	24.7	
Insulin (%)	—	5.8	
Alcohol (g/day)‡	11.3 ± 19.5	11.0 ± 20.6	>0.2
Smoking status (%)‡			
Former	34.9	33.9	>0.2
Current	27.7	27.5	>0.2
ABI‡	1.05 ± 0.1	1.04 ± 0.2	0.112
Prevalent CHD (%)‡	12.4	20.4	<0.001
Prevalent stroke (%)‡	3.6	4.8	0.192
Incident dementia (%)‖	4.5	5.9	0.138
Incident VsD (%)‖	1.0	1.9	0.080
Incident AD (%)‖	2.2	3.5	0.127
Incident AD without CVD (%)‖	1.8	2.2	>0.2

Data are means ± SD unless otherwise specified. *Age-adjusted *P* values from linear and logistic regression models; †variable measured at exam 4; ‡variable measured at midlife; §among diabetic subjects the variable was available only for newly detected cases at exam 4; ‖variable measured at exam 5.

alleles on dementia (33), individuals with genotype ε2ε4 (n = 36) were excluded from the analyses.

We measured several covariates as possible confounders. At the fourth exam, we controlled the analysis for age, education, and midlife systolic and diastolic blood pressure, cholesterol, BMI, smoking status, and alcohol intake. We used midlife values because they are less influenced by preclinical dementia status. Blood pressure was the mean of the three exams; at each exam the blood pressure represented the average of three measures made 5 min apart. Total cholesterol levels were determined with an Autoanalyzer 1 N24B cholesterol method (34). BMI (kg/m^2) was calculated from participants' height and weight at each exam and averaged. Midlife self-reported smoking was categorized by smoking status (never, former, or current) and by pack/years of cigarette exposure (packs of cigarettes a year × years of smoking). Alcohol intake was recorded as grams of alcohol per day (g/day) and recoded as drinks per day (none, <1 drink/day [13.2 g], 1–2 drinks/day, and ≥3 drinks/day). History of diabetes medication and treatment with insulin were self-reported at each exam up to the fourth exam and included in the analysis as an indicator of the severity of the underlying metabolic impairment associated with diabetes. Use of antihypertensive medications was self-reported by the subjects from exam 1–3 and included the presentation of medication vials at exam 4; the variable was dichotomized (no/yes). Ankle-brachial index (ABI) was measured at the fourth exam, and the values were dichotomized, with a cutoff of 0.9; values below this point were interpreted as indicators of generalized atherosclerosis (35). Stroke and coronary heart disease (CHD) history were assessed at baseline in 1965 and throughout the entire follow-up, up to 1996. Stroke was ascertained as to certainty and type by a neurologist, who reviewed hospital and study records.

Statistical analysis. The incident cohort included participants who reached the 1994 exam without previously diagnosed dementia. Of these, 2,574 had complete information on dementia and major risk factors. Cohort characteristics were compared by diabetes status using age-adjusted general linear models for continuous variables and age-adjusted logistic regression models for dichotomous outcomes. Logistic regression analysis was used to calculate the estimated RR and 95% CI of diabetes on incidence of dementia and dementia subtypes. Analyses were adjusted for potential confounders. Two different models were examined: in the first, age, education, APOE ε4, diabetes medications, alcohol, and smoking status were included; in the second we added midlife systolic blood pressure, cholesterol, BMI, ABI, stroke, and CHD.

Based on the hypothesis that APOE ε4 modifies the risk of diabetes for dementia, we stratified the analysis based on ε4 and diabetes status (36). Nondiabetic individuals with no APOE ε4 allele were used as the reference group and were compared with those with diabetes and APOE ε4 allele alone or combined. Participants were assigned to four groups: (no diabetes and no APOE ε4 allele, diabetes, ε4 allele, and diabetes with ε4 allele). The significance of the interaction term between diabetes and APOE ε4 was tested by comparing the likelihood ratio of the full regression model, including diabetes and APOE ε4 cross-product, versus the reduced model without the cross-product term.

Neuropathological data were available for 216 subjects, with complete information on risk factors. Multiple logistic regression was performed to compare the presence of CAA among groups with different APOE ε4 and diabetes status. To evaluate the association of APOE ε4 and diabetes to brain infarct, neuritic plaques (NPs), and neurofibrillary tangles (NFTs) counts we used a log-linear regression model (Poisson regression). This approach assumes that the counts of each outcome variable follow an overdispersed Poisson distribution. Overdispersion of the data were assessed by comparing the mean and the variance of the different outcomes (37). All Poisson models were adjusted for the same variables included in the previous analysis plus the time interval between the fourth exam and death. Statistical analysis was performed by using Stata software, version 6.0 (Stata, College Station, TX).

RESULTS

Among the 2,574 participants with complete information, 900 (35%) were classified as diabetic. Compared with nondiabetic subjects, those with diabetes had significantly higher levels of blood pressure, BMI, and cholesterol; had more CHD; and more frequently took antihypertensive medications (Table 1).

After adjustment, compared with nondiabetic subjects, those with diabetes had a significantly increased risk for

TABLE 2
Risk for incident dementia associated with diabetes: the HAAS

	Unadjusted	Adjusted*	Adjusted†
Total dementia	1.4 (1.0–1.9)	1.5 (1.0–2.2)	1.5 (1.01–2.2)
VsD	1.9 (0.9–3.8)	2.2 (1.1–4.7)	2.3 (1.1–5.0)
AD‡	1.5 (0.9–2.4)	1.7 (1.01–2.8)	1.8 (1.1–2.9)
AD without CVD	1.3 (0.7–2.2)	1.5 (0.8–2.7)	1.6 (0.9–3.0)

Data are RR (95% CI). Participants without diabetes served as the reference group to calculate RR and 95% CI. *Analyses were adjusted for age, education, APOE E ε4 status, diabetes medications, alcohol, and smoking status; †analyses were adjusted as in the column to the left, plus midlife systolic blood pressure, cholesterol, BMI, ABI, stroke, and CHD; ‡AD definition includes subjects with concomitant CVD contribution to dementia.

total dementia, AD, and VsD (Table 2); the risk associated with AD without CVD was higher but not statistically significant. When all subjects with missed 2-h glucose data were considered as diabetic, the association between diabetes and dementia maintained the same direction and magnitude as the main analysis. Diabetic subjects had an RR of 1.6 (95% CI 1.0–2.3) for dementia, 2.0 (1.0–3.5) for VsD, 1.5 (1.0–2.4) for AD, and 1.5 (0.8–2.7) for AD without CVD. Compared with the nondiabetic group, the RR for those with isolated 2-h hyperglycemia was 1.1 (0.5–2.1) for total dementia, 0.9 (0.2–4.1) for VsD, 1.2 (0.5–2.9) for AD, and 0.8 (0.2–2.8) for AD without CVD (Table 3). Furthermore, by eliminating these subjects from the diabetes group, the RR for those with diabetes was 1.6 (1.1–2.4) for total dementia, 2.9 (1.3–6.5) for VsD, 1.9 (1.1–3.2) for AD, and 1.8 (0.9–3.5) for AD without CVD.

We then stratified diabetes on APOE ε4 status. In the first adjusted model, the combined effect of diabetes and the ε4 allele was significantly stronger than in the reference group for total dementia and for the three dementia subtypes (Table 4). After adjusting for confounders, the diabetes and ε4 combined RR for VsD was reduced and became nonsignificant (RR 2.8 [95% CI 0.8–9.8]). The risk for all AD and AD without CVD increased (4.4 [1.9–10.0] and 5.5 [2.2–13.7], respectively).

We investigated the independent and combined effect of diabetes and the ε4 allele on different pathological outcomes. Diabetes alone was significantly associated with the number of brain infarcts, whereas APOE ε4 alone was associated with the number of NFTs in the cortex and in the hippocampus and with the risk for CAA (Table 5). After full adjustment, the group with both diabetes and the ε4 allele had a higher number of hippocampal NPs and hippocampal and cortical NFTs. The risk for CAA was significantly higher for those with both risk factors.

DISCUSSION

The present study provides important evidence that type 2 diabetes is a risk factor for the development of both VsD and AD. Our data show that association between diabetes and AD is particularly strong among carriers of the APOE ε4 allele. The association between the two risk factors and neuropathological findings support our results on clinical outcomes and provide clues for the pathological mechanism of dementia.

These findings are different from those previously published on the same cohort (38), when no association between midlife diabetes and prevalence of dementia and dementia subtypes identified in 1991 was found. In the previous study, fewer participants had self-reported diabetes (n = 259, 7%). The prevalence of diabetes is known to increase with age (39). In this cohort, there was a more than twofold increase of self-reported cases of diabetes between the third and fourth exams, which were 20 years apart. The small number of diabetic subjects included in the previous analyses could have limited the power to find an association between diabetes and subtypes of dementia.

Our results are based on a large number of participants and were population-based. Covariates were well characterized over the time and often based on multiple measurements. Diabetes diagnosis included the direct assessment of diabetes status during the examination, reducing the possibility of misclassification.

Our study included a large number of autopsies. In the present cohort, clinical diagnosis and autopsy data showed a good agreement (24). However, a recent study found that vascular and AD-type pathologies are related to the degree of cognitive decline without a clear cut-point for the diagnosis of dementia (40). Therefore, we evaluated multiple pathological outcomes to assess the effect of diabetes on the brain independently from the diagnosis of dementia. Autopsy data were available for only 20% of the participants. A selection bias could have been introduced if this group included more demented diabetic subjects compared with those without autopsy. However, the comparison between the two groups with and without autopsy indicated that the frequency of diabetic subjects with dementia was similar.

TABLE 3
Adjusted RR for dementia of type 2 diabetes defined by different criteria

	Elevated 2-h only*	Incident diabetes†	ADA definition‡	Prevalent diabetes§
Total dementia	1.1 (0.5–2.1)	1.4 (0.8–2.4)	1.6 (1.1–2.4)	1.6 (0.97–2.5)
VsD	0.9 (0.2–4.1)	2.3 (0.8–6.2)	2.9 (1.3–6.5)	2.3 (0.9–5.8)
AD	1.2 (0.5–2.9)	1.4 (0.7–2.9)	1.9 (1.1–3.2)	2.0 (1.1–3.6)
AD without CVD	0.8 (0.2–2.8)	0.8 (0.3–2.3)	1.8 (0.9–3.5)	2.2 (1.1–4.2)

Data are RR (95% CI). Subjects without diabetes were adopted as the reference group. Analyses were adjusted for age, education, ε4 status, diabetes medication, midlife systolic blood pressure, cholesterol, BMI, ABI, and stroke. AD definition includes subjects with concomitant CVD contributing to dementia. *Subjects with normal fasting glucose but with 2-h glucose ≥200 mg/dl; †subjects with no reported diabetes but with fasting glucose ≥126 mg/dl or 2-h glucose ≥200 mg/dl; ‡American Diabetes Association (ADA) suggestion for epidemiological studies on diabetes includes subjects with reported diabetes or fasting glucose concentration ≥126 mg/dl; §subjects with self-reported diabetes.

TABLE 4
Risk of incident dementia associated with diabetes and APOE ε4: the HAAS

	Unadjusted	Adjusted*	Adjusted†
Total dementia			
Diabetes only	1.3 (0.9–2.0)	1.2 (0.8–1.9)	1.4 (0.9–2.2)
APOE ε4 only	1.4 (0.8–2.4)	1.6 (0.9–2.7)	1.4 (0.8–2.5)
Diabetes/ε4	1.9 (1.1–3.6)	2.3 (1.2–4.4)	2.6 (1.3–5.1)
ε4 × diabetes interaction term $P > 0.2$‡			
VsD			
Diabetes only	1.6 (0.7–3.5)	2.0 (0.8–4.7)	1.9 (0.8–4.6)
APOE ε4 only	0.7 (0.2–2.9)	0.8 (0.2–3.6)	0.7 (0.1–3.1)
Diabetes/ε4	2.6 (0.8–7.9)	3.7 (1.1–12.5)	2.8 (0.8–9.8)
ε4 × diabetes interaction term $P > 0.2$‡			
AD§			
Diabetes only	1.5 (0.9–2.6)	1.4 (0.8–2.4)	1.6 (0.9–2.8)
APOE ε4 only	1.9 (0.9–3.7)	2.1 (1.1–4.3)	2.0 (0.9–4.0)
Diabetes/ε4	2.7 (1.3–5.8)	3.4 (1.5–7.5)	4.4 (1.9–10.0)
ε4 × diabetes interaction term $P > 0.2$‡			
AD without CVD			
Diabetes only	1.0 (0.5–2.1)	1.0 (0.5–2.0)	1.1 (0.5–2.4)
APOE ε4 only	1.7 (0.8–3.9)	2.0 (0.9–4.6)	1.7 (0.7–4.2)
Diabetes/ε4	3.3 (1.5–7.6)	4.2 (1.8–9.9)	5.5 (2.2–13.7)
ε4 × diabetes interaction term $P = 0.114$‡			

Data are RR (95% CI). Participants with no diabetes and no ε4 served as the reference group to calculate RR and 95% CI. *Adjusted for age, education, smoking status, and alcohol use; †adjusted for age, education, smoking status, alcohol, midlife cholesterol, systolic blood pressure, BMI, ABI, stroke, and CHD; ‡ε4 × diabetes interaction term represents the cross-product between ε4 and diabetes, the analysis was adjusted as in the model in the † footnote; §AD definition includes subjects with concomitant CVD contribution to dementia.

Previous prospective studies have shown an association between diabetes and VsD and between diabetes and AD (5–7,10). The Canadian Study on Health and Aging had the largest number of VsD cases. The study showed an RR associated to diabetes that was comparable to the one found in our study. Two major population-based studies in different ethnic groups have shown an association between diabetes and incidence of AD, providing similar results (6,7).

This is the first study to examine the interaction between diabetes and the APOE gene in relation to incident dementia and subtypes. Diabetes is associated with multiple metabolic and hemodynamic defects that cause micro- and macrovascular damage (3), leading to a reduced cerebral blood flow and an impaired vascular reactivity (3). These pathological changes may cause hypoxic and ischemic conditions.

VsD is characterized by small and large brain infarcts usually associated with vascular changes (41), and although ischemic lesions are not a unique trait of VsD (42), they might be a good indicator of CVD pathogenesis. Diabetes is a well-known risk factor for lacunar infarction (11) and stroke (12); therefore, its association to VsD and brain infarcts was not surprising. Interestingly, the combined effect of diabetes and ε4 allele was associated with a slightly higher risk for VsD. Diabetes and the apoE ε4 isoform may interact by worsening the atherosclerotic risk profile. This could explain why the combined effect of the two risk factors was not significant after adjusting for cholesterol, systolic blood pressure, stroke, CHD, BMI, and ABI in our study.

The present study also evaluated the role of diabetes as a risk factor for AD. AD is characterized by altered glucose metabolism (4). Some studies have reported higher 2-h glucose levels among patients with impending AD (31) compared with control subjects. Therefore, the observation that hyperglycemia is associated with incident AD could reflect, rather than affect, a preclinical AD status. To examine this question further, we estimated the risk for AD in people with isolated elevated 2-h glucose levels. We found that their risk for AD did not differ from nondiabetic participants. In addition, by removing those subjects from

TABLE 5
Regression analysis of diabetes and APOE ε4 status on infarcts, NP, NFT, and CAA

	Diabetes only	APOE ε4 only	Diabetes/APOE ε4
Lacune*	1.0 (0.6–1.8)	0.9 (0.4–1.9)	1.5 (0.8–3.5)
Large infarct*	1.8 (1.1–3.0)	1.6 (0.7–3.6)	1.2 (0.5–3.2)
NP (cortex)*	0.8 (0.5–1.4)	1.1 (0.6–2.3)	1.5 (0.7–3.1)
NP (hippocampus)*	1.2 (0.5–2.3)	2.0 (0.6–6.6)	3.0 (1.2–7.6)
NFT (cortex)	1.0 (0.6–2.4)	2.9 (1.1–7.8)	3.5 (1.6–7.5)
NFT (hippocampus)*	1.0 (0.6–1.4)	1.7 (1.1–2.8)	2.4 (1.5–3.7)
CAA†	0.6 (0.3–1.2)	3.6 (1.0–10.6)	6.6 (1.5–29.6)

Data are IRR (95% CI) or RR (95% CI). Participants with no diabetes and no ε4 served as the reference group to calculate RR and 95% CI. Analyses were adjusted for age at exam 4, interval between exam 4 and death, education, smoking status, alcohol, midlife cholesterol and systolic blood pressure, BMI, and ABI. *Poisson regression, IRR, †logistic regression, RR.

the diabetes group, the association between diabetes and AD become stronger, suggesting that our approach for the analysis of diabetes and AD relationship was conservative.

The association of diabetes with AD, which includes AD cases with or without CVD, was stronger than the association of diabetes with AD and no CVD. This result would suggest that the association between diabetes and AD could also be mediated by the vascular changes caused by the diabetes, similar to VsD. The fact that there were no changes in the association after adjustment for vascular risk factors suggest that other diabetes-dependent mechanisms may be involved. Hyperglycemia in diabetes is usually associated with accelerated AGE formation. Different groups have demonstrated that AGE-related modifications decrease protein solubility and increase protease resistance (43) of several proteins present in the pathological lesions associated with AD (44). AGE product accumulation has been demonstrated in NPs and NFTs in different brain areas of patients with AD (45). Indeed, it appears that aggregation of amyloid-β is accelerated by AGE-mediated cross-linking of extracellular proteins. Furthermore, AGEs may contribute to the microtubule-associated tau protein stabilization and tangle formation (46).

APOE ε4 significantly modified the risk for AD in diabetic subjects. The joint effect of diabetes and APOE ε4 was synergistic, causing a more than fivefold increase in the risk for AD for subjects with diabetes and APOE ε4 compared with those without the two risk factors. Colocalization of AGE and apoE has been reported in senile plaques, NFTs, and CAA of patients with AD and other types of dementia (47). The APOE ε4 allele has a reduced ability to repair neuronal damage and a decreased antioxidant activity compared with the ε2 and ε3 forms (48). ε4 contributes to stabilize β-amyloid deposits, and autopsy data have confirmed that ε4 is related to a higher degree of insoluble amyloid accumulation compared with the other two common polymorphisms (49). Furthermore, the apoE ε4 isoform shows a threefold greater AGE-binding activity than the ε3 isoform, suggesting that AGE-ε4 interaction may contribute to the pathogenesis of the dense amyloid deposits and extracellular NFT (50). We found that among ε4 carriers, there was a significant association between diabetes and the number of NPs in the hippocampus, the number of NFTs in the cortex and the hippocampus, and the risk of having CAA. In most of the subjects, the number of NPs and NFTs associated with the combined presence of the ε4 allele and diabetes seems to be the result of the independent contribution of the two risk factors (additive effect). In the case of the NFTs in the hippocampus, a moderate but significant interaction between the two risk factors seems to be present. Most of all, the risk of having CAA is higher for ε4 carriers with diabetes than those with only diabetes or ε4, indicating a synergistic interaction between the two risk factors. This suggests that the pathological link between APOE ε4, diabetes, and AD may be partially due to an increased risk of CAA formation. Based on these results, we could speculate that a higher prevalence of CAA associated with ε4 and diabetes may affect the clinical expression of AD pathology, in agreement with the findings on this same cohort in which subjects with AD and CAA had a worst cognitive function than those with AD without CAA (27).

In conclusion, our results showed an association between diabetes and both VsD and AD. The study analyzed for the first time the role of diabetes and APOE ε4 allele combined presence in the pathogenesis of dementia, especially for AD. The pathological data in the autopsy subgroup confirm the results on clinical outcomes.

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REFERENCES

1. McCall AL: The impact of diabetes on the CNS (Review). *Diabetes* 41:557–570, 1992
2. Kameyama M, Fushimi H, Udaka F: Diabetes mellitus and cerebral vascular disease. *Diabetes Res Clin Pract* 24 (Suppl.):S205–S208, 1994
3. Mankovsky BN, Metzger BE, Molitch ME, Biller J: Cerebrovascular disorders in patients with diabetes mellitus. *J Diabetes Complications* 10:228–242, 1996
4. Munch G, Schinzel R, Loske C, Wong A, Durany N, Li JJ, Vlassara H, Smith MA, Perry G, Riederer P: Alzheimer's disease-synergistic effects of glucose deficit, oxidative stress and advanced glycation endproducts. *J Neural Transm* 105:439–461, 1998
5. Kuusisto J, Koivisto K, Mykkanen L, Helkala E-L, Vanhanen M, Hanninen T, Kervinen K, Kesaniemi YA, Riekkinen PJ, Laakso M: Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein e4 phenotype: cross sectional population based study. *BMJ* 315:1045–1049, 1997
6. Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ: Risk of dementia among persons with diabetes mellitus: a population-based cohort study. *Am J Epidemiol* 145:301–308, 1997
7. Ott A, Stolk RP, van Hardkamp F, Pols HAP, Hofman A, Breteler MMB: Diabetes mellitus and the risk of dementia. *Neurology* 53:1937–1942, 1999
8. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD: Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90:9649–9653, 1993
9. Hebert R, Lindsay J, Verreault R, Rockwood K, Hill G, Dubois MF: Vascular dementia: incidence and risk factors in the Canadian study of health and aging. *Stroke* 31:1487–1493, 2000
10. Dallongeville J, Lussier-Cacan S, Davignon J: Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J Lipid Res* 33:447–454, 1992
11. Lodder J, Boiten J: Incidence, natural history, and risk factors in lacunar infarction. *Adv Neurol* 62:213–227, 1993
12. Stegmayr B, Asplund K: Diabetes as a risk factor for stroke: a population perspective. *Diabetologia* 38:1061–1068, 1995
13. Heitner J, Dickson D: Diabetics do not have increased Alzheimer-type pathology compared with age-matched control subjects. *Neurology* 49: 1306–1311, 1997
14. Kagan A, Harris BR, Winkelstein W Jr, Johnson KG, Kato H, Syme SL, Rhoads GG, Gay ML, Nichaman MZ, Hamilton HB, Tillotson J: Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: demographic, physical, dietary and biochemical characteristics. *J Chronic Dis* 27:345–364, 1974
15. White L, Petrovitch H, Ross GW, Masaki KH, Abbott RD, Teng EL, Rodriguez BL, Blanchette PL, Havlik RJ, Wergowske G, Chiu D, Foley DJ, Murdaugh C, Curb JD: Prevalence of dementia in older Japanese-American men in Hawaii: the Honolulu-Asia Aging Study. *JAMA* 276:955–960, 1996
16. Teng EL, Hasegawa K, Homma A, Imai Y, Larson E, Graves A, Sugimoto K, Yamaguchi T, Sasaki H, Chiu D, et al: The Cognitive Abilities Screening Instrument (CASI): a practical test for cross-cultural epidemiological study studies of dementia. *Int Psychogeriatr* 6:45–58, 1994
17. Graves AB, Larson EB, Kukull WA, White LR, Teng EL: Screening for dementia in the community in cross-national studies: comparison between the Cognitive Avilities Screeinig Instrument and the Mini-Mental State Examination. In *Alzheimer's Disease*: *Advances in Clinical and Basic*

Research. Corain B, Iqbal K, Nicolini M, Winbald B, Wisniewski H, Zatta P, Eds. New York, John Wiley & Sons, 1993, p. 113–119
18. Havlik RJ, Izmirlian G, Petrovitch H, Ross GW, Masaki K, Curb JD, Saunders AM, Foley DJ, Brock D, Launer LJ, White L: APOE-epsilon4 predicts incident AD in Japanese-American men: the Honolulu-Asia Aging study. *Neurology* 54:1526–1529, 2000
19. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders*. 3rd ed., revised. Washington, DC, America Psychiatric Association, 1987
20. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services task force on Alzheimer's disease. *Neurology* 34:939–944, 1984
21. Chiu HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R: Criteria for the diagnosis of ischemic vascular dementia proposed by the state of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology* 42:473–480, 1992
22. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington, DC: America Psychiatric Association, 1994
23. McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, Salmon DP, Lowe J, Mirra SS, Byrne EJ, Lennox G, Quinn NP, Edwardson JA, Ince PG, Bergeron C, Burns A, Miller BL, Lovestone S, Collerton D, Jansen EN, Ballard C, de Vos RA, Wilcock GK, Jellinger KA, Perry RH: Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* 47:1113–1124, 1996
24. Petrovitch H, White LR, Ross GW, Steinhorn SC, Li CY, Masaki KH, Davis DG, Nelson J, Hardman J, Curb JD, Blanchette PL, Launer LJ, Yano K, Markesbery WR: Accuracy of Clinical Criteria for AD in the Honolulu-Asia Aging Study, a population based study. *Neurology* 57:226–234, 2001
25. Gearing M, Mirra SS, Hedreen JC, Sumi SM, Hansen LA, Heyman A: The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part X. Neuropathology confirmation of the clinical diagnosis of Alzheimer's disease. *Neurology* 45:461–466, 1995
26. Petrovitch H, White LR, Izmirilian G, Ross GW, Havlik RJ, Markesbery W, Nelson J, Davis DG, Hardman J, Foley DJ, Launer LJ: Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. *Neurobiol Aging* 21:57–62, 2000
27. Pfeifer LA, White LR, Nelson JS, Ross GW, Petrovitch H, Launer LJ: Association between cerebral amyloid angiopathy and dementia: the Honolulu-Asia Aging study (Abstract). *Neurology* (Suppl. 3):A323, 2000
28. Vinters HV, Gilbert JJ: Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of vascular amyloid changes. *Stroke* 14:924–928, 1983
29. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
30. Wahl PW, Savage PJ, Psaty BM, Orchard TJ, Robbins JA, Tracy RP: Diabetes in older adults: comparison of 1997 American diabetes association classification of diabetes mellitus with 1985 WHO classification. *Lancet* 352:1012–1015, 1998
31. Meneilly GS, Hill A: Alterations in glucose metabolism in patients with Alzheimer's disease. *J Am Geriatr Soc* 41:710–714, 1993
32. Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 31:545–548, 1990
33. Locke PA, Conneally PM, Tanzi RE, Gusella JF, Haines JL: Apolipoprotein E4 allele and Alzheimer disease: examination of allelic association and effect on age at onset in both early- and late-onset cases. *Genet Epidemiol* 12:83–92, 1995
34. Syme SL, Marmot MG, Kagan A, Kato H, Rhoads G: Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: introduction. *Am J Epidemiol* 102:477–480, 1975
35. Newman AB, Sutton-Tyrrell K, Vogt MT, Kuller LH: Morbidity and mortality in hypertensive adults with a low ankle/arm blood pressure index. *JAMA* 270:487–489, 1993
36. Ottman R: Gene-environment interaction: definitions and study designs. *Prev Med* 25:764–770, 1996
37. Frome EL, Checkoway H: Epidemiologic programs for computers and calculators: use of Poisson regression models in estimating incidence rates and ratios. *Am J Epidemiol* 121:309–323, 1985
38. Curb JD, Rodriguez BL, Abbott RD, Petrovitch H, Ross GW, Masaki KH, Foley D, Blanchette PL, Harris T, Chen R, White LR: Longitudinal association of vascular and Alzheimer's dementias, diabetes, and glucose tolerance. *Neurology* 52:971–975, 1999
39. Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Byrd-Holt DD: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Examination Survey, 1988–1994. *Diabetes Care* 21:518–524, 1998
40. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS): Pathological correlates to late-onset dementia in a multicenter, community-based population in England and Wales. *Lancet* 357:169–175, 2001
41. Erkinjuntti T: Cerebrovascular dementia: pathophysiology, diagnosis and treatment. *CNS Drugs* 12:35–46, 1999
42. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR: Brain infarction and the clinical expression of Alzheimer disease: the Nun Study. *JAMA* 277:813–817, 1997
43. Selkoe DJ, Ihara Y, Salazar FJ: Alzheimer's disease: insolubility of partially purified paired helical filaments in sodium dodecyl sulfate and urea. *Science* 215:1243–1245, 1982
44. Smith MA, Taneda S, Richey PL, Miyata S, Yan SD, Stern D, Stern D, Sayre LM, Monnier VM, Perry G: Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc Natl Acad Sci U S A* 91:5710–5714, 1994
45. Munch G, Mayer S, Michaelis J, Hipkiss AR, Riederer P, Muller R, Neumann A, Schinzel R, Cunningham AM: Influence of advanced glycation end-products and AGE-inhibitors on nucleation-dependent polymerization of beta-amyloid peptide. *Biochim Biophys Acta* 1360:17–29, 1997
46. Dickson DW, Sinicropi S, Yen SH, Ko LW, Mattiace LA, Bucala R, Vlassara H: Glycation and microglial reaction in lesions of Alzheimer's disease. *Neurobiol Aging* 17:733–733, 1996
47. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, Garruto R, Amano N, Makita Z: Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. *Am J Pathol* 153:1149–1155, 1998
48. Ma J, Yee A, Brewer HB Jr, Das S, Potter H: Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 372:92–94, 1994
49. Beffert U, Danik M, Krzywkowski P, Ramassamy C, Berrada F, Poirier J: The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease. *Brain Res Brain Res Rev* 27:119–142, 1998
50. Li YM, Dickson DW: Enhanced binding of advanced glycation endproducts (AGE) by the ApoE4 isoform links the mechanism of plaque deposition in Alzheimer's disease. *Neurosc Lett* 226:155–158, 1997