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Permutations of cerebrovascular pathologies in older adults with and without diabetes

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ABSTRACT

Permutations of cerebrovascular pathologies (CVP) in persons with diabetes mellitus (DM) have not been comprehensively investigated. Here, we examine diverse postmortem CVP outcomes, including permutations of single or mixed CVP, in 2163 older adults with or without DM who were followed in longitudinal studies of aging. Annual clinical evaluations included data to classify DM status by medical history (DM diagnosis), direct medication inspection (anti-diabetic therapy), and hemoglobin A1C level (≥6.5 %). Upon death, neuropathological examinations were performed and included evaluation for CVP (considering vessel pathologies and brain infarcts) and Alzheimer's disease neuropathologic change (AD-NC). Among all participants [mean age, 89.49 ± 6.89 years (SD)], single CVP were more common than mixed CVP. Logistic regression was used to analyze the association of DM with CVP permutations, controlling for age at death, sex, education, and AD-NC, and revealed increased odds of microinfarcts alone (odds ratio, 1.56 [95 %CI, 1.03–2.35]) and mixed microinfarcts and macroinfarcts (odds ratio, 1.90 [95 %CI, 1.16–3.13]). These associations remained after adjusting for demographic factors and cohort or vascular comorbidities including stroke, heart disease, hypertension, claudication, smoking, and systolic blood pressure. Furthermore, after controlling for demographic factors as well as AD-NC and APOE type, mixed microinfarcts and macroinfarcts were associated with approximate threefold increased risk of dementia (odds ratio, 2.95 [95 %CI, 1.13–7.70]) in participants with DM. Evidence suggests that older adults living with DM have higher odds of microinfarcts and mixed microinfarcts and macroinfarcts in the absence of intracranial vessel pathologies that cannot be explained by vascular comorbidities, and in this population mixed microinfarcts and macroinfarcts are associated with higher odds of dementia.

Dr. A Wallin

Introduction

Type 2 diabetes mellitus (DM) is associated with vascular diseases and Alzheimer's disease (AD) [3,24,43,44]. Fluorodeoxyglucose positron emission tomography (PET) studies show that individuals with AD dementia have abnormally low glucose metabolic rates in various brain regions during preclinical and prodromal stages of disease [39], with similar findings noted in persons with and without DM [4,18]. A recent study also reports alterations in AD plasma biomarkers due to acute food intake [20], while others link DM with cognitive decline, whole-brain and hippocampal atrophy, and mortality [3,4,18]. Additionally, cognitive deficits are associated with insulin signaling abnormalities [44] and DM, glucose levels, and insulin signaling are posited driving factors for the precipitation of AD neuropathologic change (AD-NC) and dementia [12,15–17,32]. Data also demonstrate a role of apolipoprotein E (APOE) in brain metabolic transport systems and suggest susceptibility of APOE ε4 carriers to metabolic impairments brought on by DM [23]. While glucose metabolic changes are linked to dementia and its severity has been reported to correlate with levels of cognitive impairment [17], the mechanisms involved are unclear.

Mixed pathologies and DM are common in old age [14,33]. DM is associated with higher insulin levels as a compensatory mechanism to maintain normal blood glucose [10]. Yet, various cerebrovascular pathologies (CVP) co-occur with and influence nutritive delivery and brain metabolism in persons with and without DM [2,19,48]. Moreover, due to many complexities, CVP permutations in persons with and without

Abbreviations: AD, Alzheimer's disease; AD-NC, Alzheimer's disease neuropathologic change; CVP, cerebrovascular pathologies; DM, diabetes mellitus, type 2; PET, positron emission tomography; DM, diabetes mellitus type 2 or peripheral insulin resistance.

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DM have not been comprehensively investigated. We therefore studied permutations of CVP in individuals with and without DM in community-based cohorts with high brain autopsy rates and examined the effects of APOE type and vascular comorbidities on relationships between DM and common CVP permutations. Additionally, we studied the associations of CVP permutations with cognitive decline.

Materials and methods

Sample, brain specimens, and definition of DM

Brain specimens for this study derived from one of five ongoing epidemiologic longitudinal clinical-pathologic cohort studies of aging at the Rush Alzheimer's Disease Center: The Religious Orders Study (ROS) [9], the Rush Memory and Aging Project (MAP) [9], the Minority Aging Research Study (MARS) [8], the African American Clinical Core (AACore) [41], and the Latino Core (LATC) [31]. ROS began enrolling Catholic clergy (nuns, priests, and brothers) in 1994 from over 40 monasteries or convents across the United States, whereas MAP began enrolling older laypersons from the Chicago region in 1997, MARS began recruiting minoritized seniors from a variety of community-based settings in the metropolitan Chicago area and outlying suburbs beginning in 2004, and AACore and LATC were initiated subsequently [8,9,31,41]. All studies were approved by the Institutional Review Board of the Rush University Medical Center. All participants are consented for autopsy as well as tissue donation for research. Inclusion for the studies include age 65 years and older, having no known dementia at baseline, and consenting to undergo annual clinical evaluations. Additional details regarding study designs and components have been published previously [9]. Harmonized methods are used for enrollment and procedures including data collection and summarization, allowing for combined datasets to be analyzed, as published previously [8,31,41]. From the time of inception, 1503 ROS, 2376 MAP, 858 MARS, 416 AA, or 282 LATC participants had complete baseline data. Of the 5435 total, 2916 died, 2222 underwent autopsy, and 2163 had completed autopsy reports available in the database at the time of this analysis and were thus eligible for this study.

DM was defined (at baseline or any annual follow-up visit) by medical history (i.e., self-report or medical report of DM), direct medication inspection (i.e., use of insulin and/or oral hypoglycemic agent), and/or abnormal hemoglobin A1C level (AIC) of 6.5 %, or higher. The latter criteria (A1C$\geq$6.5 %) was used since many older adults with diabetes are undiagnosed [28]. Self-reports for DM included an affirmative response to three specific queries on a clinical questionnaire: (1) Have you ever been told by a doctor, nurse, or therapist, etc., that you had diabetes, or sugar in the urine, or high blood pressure; (2) Have you ever been told by a doctor, nurse, or therapist, etc., to take insulin or injections for your high blood sugar; and (3) Have you ever been told by a doctor, nurse, or therapist, etc., to take medicine by mouth for your high blood sugar? For medication inspection, participants were asked to supply all medications for direct visual inspection. Medications were documented and then coded using the Medi-Span Drug Data Base system. DM was present if a diabetes medication (i.e., insulin and/or oral hypoglycemic agent) was recorded in the database. Eligible study participants were categorized into two groups. The group without DM consisted of 1625 participants (75.13 %) and the group with DM consisted of 538 participants (24.87 %). Demographic, genetic, CVP, and other characteristics of total participants and each group are summarized in the Table. Briefly, participants died on average at the age of 89 years, were highly educated, and mostly women. The mean age at death was 89.86 $\pm$ 6.77 (SD) years for participants without DM and 88.38 $\pm$ 6.79 (SD) years for participants with DM (Table 1).

Table 1
Characteristics and distribution of participants (N = 2163).

	All Participants (n = 2163)	Participants without DM (n = 1625)	Participants with DM (n = 538)
Demographic Factor, Mean (SD) or Frequency (%)			
Age at death (years)	89.49 (6.81)	89.86 (6.77)	88.38 (6.79)
Education (years)	16.25 (3.60)	16.41 (3.55)	15.79 (3.74)
Female	1493 (69.02)	1158 (71.26)	335 (62.27)
APOE Genotype (Type), Frequency (%)			
Type 2 (ε2/ε2 or ε2/ε3)	266 (12.30)	192 (11.82)	74 (13.75)
Type 3 (ε3/ε3)	1251 (57.84)	917 (56.43)	334 (62.08)
Type 4 (ε3/ε4 or ε4/ε4)	487 (22.52)	399 (24.55)	88 (16.36)
Comorbid Factor, Mean (SD)			
Body mass index	25.37 (5.14)	24.74 (4.81)	27.37 (5.62)
Creatinine (mg/dL)	1.14 (0.60)	1.10 (0.53)	1.26 (0.75)
Comorbid Factor, Frequency (%)			
AD-NC	1408 (65.09)	1090 (67.08)	318 (59.11)
Claudication	615 (28.43)	434 (26.71)	181 (33.64)
Congestive heart failure	168 (7.77)	119 (7.32)	49 (9.11)
Heart disease	414 (19.14)	282 (17.35)	132 (24.54)
Hypertension	1478 (68.33)	1046 (64.37)	432 (80.30)
Stroke	428 (19.79)	303 (18.65)	125 (23.23)
Smoking	699 (32.32)	518 (31.88)	181 (33.64)
Cerebrovascular Pathology, Frequency (%)			
Atherosclerosis			
None/Mild	1478 (68.33)	1087 (66.89)	391 (72.68)
Moderate/Severe	676 (31.25)	530 (32.62)	146 (27.14)
Arteriolosclerosis			
None/Mild	1418 (65.56)	1045 (64.31)	373 (69.33)
Moderate/Severe	732 (33.84)	570 (35.08)	162 (30.11)
CAA			
None/Mild	1314 (60.75)	953 (58.65)	361 (67.10)
Moderate/Severe	811 (37.49)	638 (39.26)	173 (32.16)
Infarcts			
Chronic macroscopic	778 (35.97)	562 (34.58)	216 (40.15)
Chronic microscopic	687 (31.76)	490 (30.15)	197 (36.62)
Cognition, Frequency (%)			
No cognitive impairment	721 (33.33)	526 (32.37)	195 (36.25)
Mild cognitive impairment	493 (22.8)	379 (23.32)	114 (21.19)
Dementia	947 (43.78)	718 (44.18)	229 (42.57)

Assessment of cognitive function

Cognitive performance was assessed at each visit using a comprehensive battery of 19 neuropsychological tests, as previously published [45] The battery tested a spectrum of cognitive abilities, including a composite measure of global cognition as well as five specific cognitive domains, with higher scores indicative of better cognitive performance. The battery included tests of working memory (Digit Span forward and backward; Digit Ordering), semantic memory (Boston Naming; Verbal Fluency; Reading Test), episodic memory (Word List Memory, Recall and Recognition; immediate and delayed recall of the East Boston Story; immediate and delayed recall of Story A of the Wechsler Memory Scale-Revised), four of perceptual speed (Symbol Digit Modalities Test; Number Comparison; two indices from a modified version of the Stroop Test), and visuospatial abilities (Line Orientation; Progressive Matrices). Raw scores were standardized into z-scores using the baseline mean and standard deviation of the entire cohort. Composite scores for each domain were calculated by averaging the z-scores for all tests within that domain. A composite score of global cognitive function was derived by averaging z-scores across all 19 tests to determine presence or absence of dementia (binary score), as well as presence or absence of mild cognitive impairment or dementia (ordinal score).

Assessment of comorbidities

Participant weight and height measurements were collected

annually during life by a trained technician who was blinded to other clinical data and body mass index (BMI) was calculated using the median weight (kilograms) divided by the median height (meters^2). The presence of general vascular comorbidities including smoking, heart disease, hypertension, claudication, and stroke was reported by participants at baseline and on each follow up visits and was also determined on clinical interviews, diagnostic records and/or neurological examination. Last valid cumulative summaries were used to determined presence or absence of each specific comorbidity. The overall vascular disease burden including presence of claudication, stroke, heart condition(s) (e.g., history of heart attack, coronary thrombosis, coronary occlusion, or myocardial infarction), and/or congestive heart failure was also determined on each visit by self-reports and the last valid scores were used to compute a cumulative score (binary scores of 0 or 1 were used for presence of each vascular disease type, with a possible cumulative score of 0 to 4). Creatinine level (mg/dL) was measured as part of a basic metabolic panel performed by Quest Diagnostics, and the last valid creatinine level was used for analysis. Blood pressure (mmHg) was measured by a trained research assistant at each visit and the mean systolic blood pressure across visits and systolic blood pressure slope were used for analyses.

Brain autopsy, tissue processing, and neuropathological assessment

Brain autopsies were performed by staff blinded to premortem clinical data. As previously described, a standardized protocol was used for the autopsy procedure [9,37]. In brief, brains of deceased participants were extracted, weighed, and one cerebral hemisphere was frozen whereas the other cerebral hemisphere was sliced into 1 cm coronal slabs that were fixed in 4 % paraformaldehyde for 3 to 21 days. Subsequently, the fixed brain slabs were examined to evaluate for macroscopic infarcts and diagnostic tissue blocks were dissected from standardized brain regions (i.e., at least nine brain regions in total that encompass middle frontal, middle temporal, amygdala, hippocampus, inferior parietal, occipital, basal ganglia, thalamus, and midbrain) as well as any possible area(s) of macroscopic infarct(s), hemorrhage, discoloration, and/or other lesions. Collected tissue blocks were paraffin embedded and serially sliced into 6 μm thick sections that were mounted on glass slides. Then hematoxylin and eosin (H&E)-stained sections were prepared from each block and modified Bielschowsky silver stain and anti-β-amyloid immunohistochemistry slide preparations were made from designated blocks, according to consensus protocols [21,37].

Neuropathological diagnoses were rendered by a licensed and board-certified neuropathologist who was blinded to demographic and clinical data. For diagnosis of CVP, both macroscopic and microscopic infarcts were confirmed by histologic examination of H&E-stained tissue slides and the number, location(s), and age (acute, subacute, or chronic) of each infarct was recorded [6]. For this study, only chronic macroscopic or microscopic infarcts were analyzed. Following a visual inspection of the large cerebral arteries and the circle of Willis, atherosclerosis severity was graded in primary cerebral arteries based on its degree in arteries as well as the number of arteries involved, using a semi-quantitative scale demarcating no disease (0, or no atherosclerosis) to severe disease (6, severe atherosclerosis with all visualized large arteries affected or one artery completely occluded) [7]. Following a histologic examination, arteriolosclerosis severity was graded in the basal ganglia using a semi-quantitative scale demarcating no disease to severe disease (e.g., 0 demarcated no arteriolosclerosis whereas 1 (mild) demarcated arteriole walls that were minimally thickened; 3 (moderate) demarcated arteriole wall thicknesses increased up to 2 times the normal thickness; and 5 (severe) demarcated arteriole wall thicknesses greater than two times the normal thickness [7,30]. Similarly, cerebral amyloid angiopathy (CAA) severity was graded based upon the degree of immunohistochemical labeling with anti-β-amyloid antibody, using a semi-quantitative scale demarcating no disease to severe disease. For each brain region, meningeal and parenchymal vessels were assessed for amyloid deposition and scored from 0 to 4 wherein 0 demarcated no deposition; 1 demarcated scattered segmental but no circumferential deposition; 2 demarcated circumferential deposition in up to 10 vessels in a region; 3 demarcated circumferential deposition in up to 75 % of vessels in a region; and 4 demarcated circumferential deposition in over 75 % of vessels in a region. The score for each region was taken as the maximum of the meningeal and parenchymal scores and scores were averaged across regions [13]. Each semiquantitative CVP severity score was collapsed into 4 levels (none, mild, moderate, or severe). For diagnosis of AD-NC, a modified Bielschowsky silver stain was used to evaluate for neuritic and diffuse plaques and neurofibrillary tangles and consensus staging criteria were used to grade severity from 0 (no AD-NC pathology) to 3 (severe, or high likelihood AD-NC pathology) [21]. Dichotomized (i.e., none/mild or moderate/severe) variables were used for a summary of the presence or absence of CVP and/or AD-NC diagnoses.

APOE types

For determination of APOE genotypes, DNA was extracted from peripheral blood mononuclear cells or the brain, and genotyping was performed utilizing high-throughput sequencing of codon 112 (position 3937) and codon 158 (position 4075) of exon 4 of the APOE gene on chromosome 19 [46]. For analysis, the APOE genotypes were grouped as type 2 to reflect APOE ε2/2 or ε2/3, type 3 to reflect APOE ε3/3, and type 4 to reflect APOE ε3/4 or ε4/4. Given the reported inverse effects of the ε2 and ε4 alleles on brain pathologies, data from the few participants with APOE ε2/4 genotype were excluded from analyses.

Statistical analysis

In initial analyses, the distribution of demographic, genetic, and clinical variables and distribution of CVP permutations were examined. Logistic regression models were used to examine the association of individual CVP permutations with DM group and with cognitive status. Models were adjusted for age at death, sex, education, and AD-NC. For interpretation purposes, age at death and education were centered at the mean. The models were repeated with additional adjustment for cohort and APOE type, alone and in combination. In secondary analyses, logistic regression models were used to examine the association of CVP permutations with DM, controlling for demographic factors and various comorbidities (see Table). Ordinal models were also used to study the associations of CVP permutations with cognitive decline in total participants and by DM status. All analyses were conducted using SAS/STAT software, Version 9.4 of the SAS System for Linux (SAS Institute, Cary, NC), and a significance alpha of 0.05 was considered.

Data Availability

Information regarding the data used and the parent studies description can be accessed on the Rush Alzheimer's Disease Center Research Resource Sharing Hub at https://www.radc.rush.edu. Following review of a submitted data request, approved applicants with a Data Use Agreement can receive data.

Results

CVP permutations

The overall frequency of CVP and the number of participants without any CVP in the total group of 2163 older adults are depicted in the Table and in Fig. 1A (horizontal bars at lower left-hand side). Approximately one-third of the total participants had each of the vascular-related pathologies (i.e., chronic microscopic infarcts, chronic macroscopic infarcts, atherosclerosis, arteriolosclerosis, and CAA) (Table). As summarized in the UpSet plot (Fig. 1A), 32 CVP outcomes were identified and included no CVP (CVP permutation "0"), single CVP (CVP permutation 1–5), or mixed CVP (CVP permutation 6–31). The findings

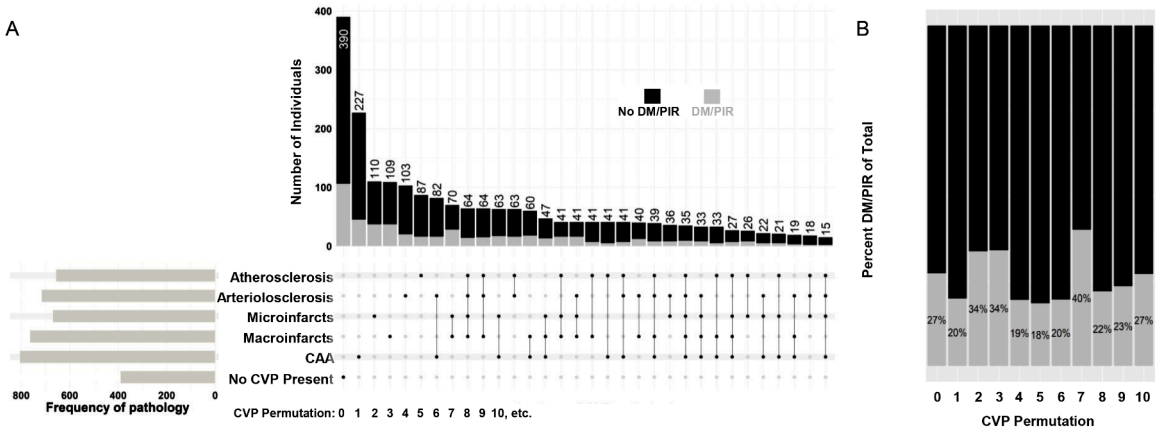

Fig. 1. CVP permutations in participants with and without DM. A) In total, 31 CVP permutations were identified. The UpSet plot highlights the heterogeneity of CVP in older persons with and without DM. the horizontal bars at the lower left-hand side depict the frequency of each CVP as well as the number of participants without any CVP. Black dots on the x-axis, in order from left to right, indicate the most frequent CVP permutations (dots/dots with connected lines represent individual CVP permutations). The frequencies of the CVP permutations are shown in the histogram. The height of each bar in the histogram corresponds to the number of participants with the indicated CVP permutation. The ten most common CVP permutations were tested in this study. B) The percentage of DM among total participants is depicted for no CVP (#0) and for the ten most common CVP permutations identified, i.e., #1–10 shown in (A). Gray bars summarize data from participants with DM (n = 538) and black bars summarize data from participants without DM (n = 1625).

highlight the heterogeneity of CVP among aging persons with and without DM. As shown, single CVP were more common than multiple/mixed CVP in this community-based sample of older research volunteers.

The ten most frequent CVP permutations (Fig. 1A and B) included: (1) CAA only, in 227 (10.49 %) participants; (2) microinfarcts only, in 110 (5.09 %) participants; (3) macroinfarcts only, in 109 (5.04 %) participants; (4) arteriolosclerosis only, in 103 (4.76 %) participants; (5) atherosclerosis only, in 87 (4.02 %) participants; (6) mixed CAA and arteriolosclerosis, in 82 (3.79 %) participants; (7) mixed microinfarcts and macroinfarcts, in 70 (3.24 %) participants; (8) mixed microinfarcts, macroinfarcts, arteriolosclerosis, and atherosclerosis, in 64 (2.96 %) participants; (9) mixed macroinfarcts, arteriolosclerosis, and atherosclerosis, in 64 (2.96 %) participants; and (10) mixed CAA and microinfarcts, in 63 (2.91 %) participants. Altogether, the 10 most common CVP permutations were found in 45.26 % of total brain samples. Of the total participants, 390 (18.03 %) exhibited no CVP (Fig. 1).

APOE types

APOE genotype was available in 2054 (94.96 %) participants in whom the following were recorded: ε2/2 (10 participants); ε2/3 (256 participants); ε2/4 (50 participants); ε3/3 (1251 participants); ε3/4 (448 participants); ε4/4 (39 participants). The frequency of each APOE type in participants with and without DM is summarized in the Table.

DM and CVP permutations

Participants with DM during life had over fifty percent increase in the odds of microinfarcts alone (i.e., permutation 2, odds ratio, 1.56 [95 % CI, 1.03–2.35]) and almost double the odds for mixed microscopic and macroscopic infarcts (i.e., permutation 7, odds ratio, 1.90 [95 % CI, 1.16–3.13) in logistic models adjusting for demographics and AD-NC (Fig. 2, black circles). The relationships between DM and permutations 2 and 7 remained after controlling for cohort (Fig. 2, gray circles) as well as AD-NC and cohort (Fig. 2, white circles), but there were no associations of these permutations when controlling for APOE type (data not illustrated in figures). The adjusted odds ratio was 1.56 [95 % CI, 1.04–2.36] for permutation 2 after controlling for AD-NC and cohort and 1.90 [95 % CI, 1.15–3.13] for permutation 7 after the same controls. There were no relationships between other CVP permutations and DM/PIR, among the 10 most common CVP permutations tested.

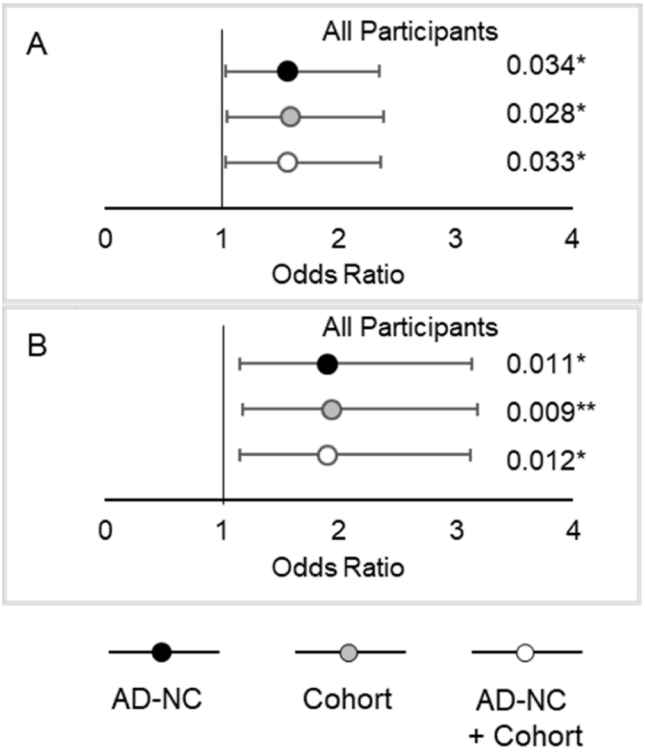

Fig. 2. Effect of DM on CVP permutations. The forest plots depict the logistic regression results (odds ratios) for (A) CVP permutation 2 (microinfarcts alone); and (B) CVP permutation 7 (mixed microinfarcts and macroinfarcts) in total participants. Models are adjusted for demographics (age at death, sex, and years of education) and are controlled for AD-NC (black circles); cohort (gray circles); or both (white circles). Error bars represent 95 % confidence intervals; p values are shown on the right-hand side.

Other Clinical, Pathologic or Demographic Factors Additional analyses were performed to determine if the relationships between DM and CVP permutations 2 and 7 were influenced by other clinical, pathologic or demographic factors, apart from age, sex, and education (independently of AD-NC), and to understand if these permutations increased the odds of cognitive decline after controlling for AD-NC.

Since systemic factors such as obesity [38], renal function [25], and general vascular diseases [5] influence brain infarct development in persons with and without DM, we studied whether BMI, creatinine, or history of stroke, heart disease, hypertension, or claudication confound the association of DM with CVP permutation 2 or 7. None of the covariates were found to alter the association of CVP permutation 7 with DM when adjusting for demographcs. The relationship of permutation 2 with DM also remained after adjusting for demographics and stroke, heart disease, hypertension, or claudication history. To further understand the role of general vascular diseases and vascular risk factors, we examined whether CVP permutation 2 or 7 remained after adjusting for combinations of comorbidities, including a cumulative vascular disease burden score (i.e., presence of claudication, stroke, heart condition(s), and/or congestive heart failure), systolic blood pressure (mean and slope over the lifespan), and smoking (binary measure). The association remained with permutation 7 after adjusting for demographics and each of these factors, and with permutation 2 after adjusting for demographics and mean systolic blood pressure, systolic blood pressure slope over the lifespan, or smoking.

Next, we studied the relationship of CVP permutations 2 and 7 with cognition in total participants and in subsets with and without DM. Permutation 2 was not associated with increased odds of dementia in total participants, or in subsets with or without DM, even after controlling for cohort or AD-NC with or without APOE genotype and/or cohort. Additionally, we found no relationship of permutation 7 with dementia in total participants, or in participants without DM, including after the same adjustments. Similarly, there was no relationship of permutation 7 with dementia in participants with DM after adjusting for demographics, cohort, or AD-NC with or without cohort. However, in participants with DM, permutation 7 was associated with an approximate three-fold increased risk of dementia after controlling for AD-NC and APOE genotype (odds ratio, 2.95 [95 % CI, 1.13–7.70]), or all covariates together with a term for cohort (odds ratio, 3.15 [95 % CI, 1.20–8.29]) (Fig. 3).

Finally, in logistic and ordinal models using dementia (i.e., presence or absence) or cognitive impairment (i.e., no cognitive impairment, mild cognitive impairment, or dementia), respectively, as the outcomes, no interactions were identified among permutation 2 and DM, even in subset analyses by APOE type. However, an interaction was identified with permutation 7 (i.e., mixed microinfarcts and macroinfarcts) and DM when considering total participants and evaluating cognition as an ordinal outcome ($p = 0.007$). In subset analyses, an interaction was also identified with permutation 7 and DM when evaluating cognition as an ordinal outcome in participants with APOE type 3 ($p = 0.009$), but no interaction was detected in subsets of participants with APOE type 2 or APOE type 4.

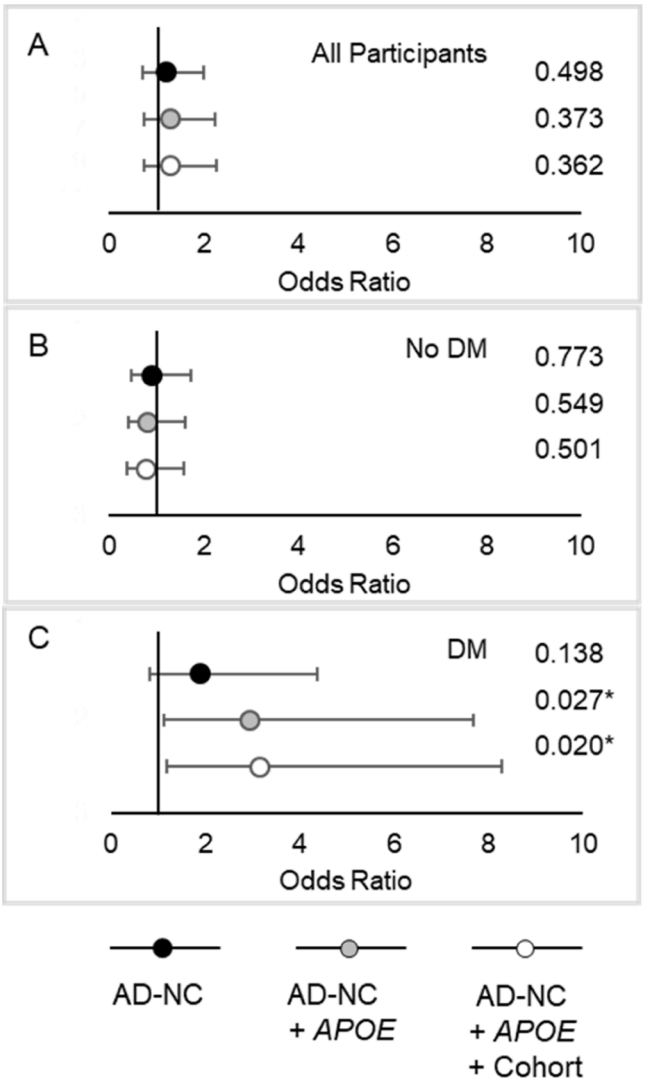

Fig. 3. Effect of CVP permutation 7 on dementia status. The forest plots depict the logistic regression results (odds ratios) for CVP permutation 7 (mixed microinfarcts and macroinfarcts) in (A) all participants, (B) participants without DM, and (C) participants with DM. All models are adjusted for demographics (age at death, sex, and years of education) and are controlled for AD-NC (black circles); AD-NC and APOE type (gray circles); or AD-NC, APOE type, and cohort (white circles). Error bars represent 95 % confidence intervals; p values are shown on the right-hand side.

Discussion

This study examined clinical and neuropathologic data from over 2000 older adults in the community and identified an association between DM and two specific CVP permutations: microinfarcts alone and mixed microinfarcts and macroinfarcts. Specifically, DM was associated with higher odds of microinfarcts and combined microinfarcts and macroinfarcts, with an approximate doubling of the odds of the latter CVP permutation, when controlling for demographic factors and AD-NC. No other associations were observed among the 10 most common CVP permutations analyzed. Given that APOE ε4 and DM are both significant risk factors for cerebrovascular disease as well as late-onset AD dementia [23], we tested the hypotheses that APOE type alone, or in combination with AD-NC, may be an important covariate that influences the odds of these specific CVP permutations, but did not observe an association of microinfarcts alone or combined microinfarcts and macroinfarcts with DM when adjusting for APOE type. Importantly, in this analysis DM was not independently associated with increased odds of cerebrovascular atherosclerosis, cerebrovascular arteriolosclerosis, or CAA alone.

Prior reports demonstrate clear associations between DM and brain infarcts. Studies by Abner et al. [2] (which incorporates analyses of ROSMAP data) and Ahtiluoto et al. [3] previously showed that DM increases the odds of brain infarcts, but not AD-NC in older adults. Moreover, Gerstein et al. [19] showed that an association between DM and brain infarcts persists even after adjusting for nonlacunar infarcts, clinical stroke history, and other cerebrovascular disease risk factors such as carotid wall volume, smoking, and hypertension, suggesting that adverse effects of DM may result from small blood vessel changes. Moreover, peripheral insulin resistance is often observed in patients with acute ischemic stroke [26,27], is known to negatively impact the outcome of acute ischemic infarction in persons with or without DM [24, 43], and is associated with early neurological deterioration [35], increased stroke recurrence, and death [22]. In a recent analysis, our team also demonstrated that A1C level is associated with higher odds of macroscopic subcortical infarcts [11]. Interestingly, this study also found that A1C mean and variability across the lifespan inversely associate with AD-NC, irrespective of DM status [11], consistent with

findings in some prior studies [40,42]. Overall, mixed CVD has also been reported to associate with cognitive decline more commonly than single CVD [29]. Despite this knowledge, the associations of DM with specific CVP permutations have not previously been comprehensively studied in large cohorts.

Here, we analyzed common CVP permutations present in older adults with and without DM and demonstrate higher odds of cerebral microinfarcts and mixed cerebral microinfarcts and macroinfarcts in persons with DM. These relationships remained after controlling for demographic factors and AD-NC, but did not remain when controlling for demographic factors and APOE type, with or without AD-NC or cohort. These data suggest that DM may be a driver for cerebral microinfarcts and mixed microinfarcts and macroinfarcts [3]. Given documented associations of DM with coronary artery disease, cerebrovascular disease, peripheral vascular disease, and hypertension [5], the observed lack of an association of DM with cerebrovascular atherosclerosis and/or cerebrovascular arteriolosclerosis was somewhat surprising. However, our results further underscore the importance of DM with brain infarcts and suggest the need for continued investigation into infarct causes and risks in distinct subsets of persons with DM [2,3,19].

Pre-clinical and clinical studies also highlight important relationships between DM and cognitive impairment, yet the specific mechanisms are unclear [2,3,36]. A growing body of literature suggests that DM may cause neurodegeneration and dementia primarily through non-AD-NC pathologies [2,3,36,40]. While DM was not associated with cerebrovascular atherosclerosis and cerebrovascular arteriolosclerosis in this study, intracerebral vasculature is known to be distinct from central and peripheral vasculature. Most notably, intracerebral vasculature possesses a blood-brain barrier that restricts the exchange of biomolecules and establishes a highly controlled interface between the brain and blood vascular compartment [1] and is surrounded by fluid [34]. Given the metabolic demand of the brain, which is significantly higher than that for any other organ, the efficiency of glucose, oxygen, and nutrient exchange and metabolism is especially critical in the brain parenchyma [47]. APOE is involved in the transport of cholesterol, lipids, and fat-soluble vitamins between the brain and blood, thus serving a critical role in brain metabolic transport systems [23]. Together with DM, APOE may have significant underrecognized metabolic and molecular consequences on brain that influence physiology and cognition [42]. In fact, in this study, mixed microinfarcts and macroinfarcts were associated with an approximate three-fold increased risk of dementia in persons with DM, when controlling for AD-NC and APOE type, but this association was not observed in individuals without DM. Moreover, an interaction was identified with permutation 7 (i.e., mixed microinfarcts and macroinfarcts) and DM when considering total participants and evaluating cognition as an ordinal outcome. Additionally, subset analyses showed an interaction effect with permutation 7 and DM when evaluating cognition as an ordinal outcome in participants with APOE type 3, but not APOE type 2 or APOE type 4.

This study has several limitations. Notably, the participants were predominantly highly educated non-Hispanic White individuals above the age of 65 years [9,41], which limits generalizability of the results. Additionally, tissue from only nine brain regions were systematically collected and the analysis was limited to community cohorts. The effects of medications, diabetic control (i.e., hemoglobin A1C levels or variability), sex, race, and other genetic, metabolic, hematologic, and/or lifestyle factors such as exercise or diet were not examined in this study. Given the cross-sectional nature of this analysis, cause and effect relationships cannot be drawn from this data. Moreover, while the definition of "DM" included persons with A1C$\geq$6.5 at any point during the study, there is complexity in the association of diabetes mellitus and A1C. For instance, five distinct populations can be defined by diabetes mellitus status and A1C level: (i) Individuals with well-controlled diabetes who do not exhibit A1C elevation; (ii) individuals with poorly-controlled diabetes who exhibit A1C elevation; (iii) normoglycemic individuals who do not have a history of A1C elevation; (iv) individuals without a diagnosis of diabetes who exhibit A1C elevation $\geq$6.5 (i.e., nondiagnosed individuals with diabetes); and (v) individuals with prediabetes (i.e., history of moderate A1C elevation in the 5.7 % to 6.4 % range). Thus, additional research in this area is needed. Despite these limitations, this study has many strengths. The analysis incorporates clinical and postmortem data from a community cohort, rather than a hospital autopsy series, and is therefore less prone to selection bias. A large number of well-characterized human brain specimens were included and were systematically harvested, processed, and investigated by a multidisciplinary team with experience in CVP. Moreover, the study controlled for a number of comorbidities including stroke, heart disease, hypertension, claudication, BMI, creatinine, systolic blood pressure (both mean and slope), smoking, as well as a cumulative vascular disease burden score. Yet, investigation using more granular stratification of CVP permutations in additional subsets of older persons with and without DM is needed to better elucidate the effects of complex combinations of covariates in diverse persons across a spectrum of age, demographic factors, APOE genotypes, and other comorbid conditions.

CRediT authorship contribution statement

Rupal I. Mehta: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Ana W. Capuano:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Roshni Biswas:** Writing – review & editing. **David A. Bennett:** Writing – review & editing. **Zoe Arvanitakis:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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