Journal of the American Heart Association

ORIGINAL RESEARCH

Cerebral Hemodynamic Impairment and Cognitive Dysfunction in *APOE4* Carriers With Asymptomatic Carotid Artery Stenosis/Occlusion

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BACKGROUND: Our previous preclinical study demonstrated that *APOE4*-targeted replacement mice exhibit more severe cerebral hypoperfusion and cognitive impairment than *APOE3*-targeted replacement mice with carotid artery stenosis due to neurovascular dysfunction. Therefore, we clinically investigate whether *APOE4* contributes to cerebral hemodynamic and cognitive impairment in subjects with asymptomatic carotid artery stenosis or occlusion.

METHODS AND RESULTS: A cross-sectional observational study was conducted between January 2017 and March 2022. In a primary analysis, 91 subjects (114 affected cerebral hemispheres) with asymptomatic carotid artery stenosis or occlusion who underwent neuropsychological examinations and ^{15}O-gas positron emission tomography were included to examine associations of *APOE4* with cognitive impairment and cerebral hemodynamic impairment. A sensitivity analysis was performed with 161 subjects (201 affected cerebral hemispheres) who underwent ^{15}O-gas positron emission tomography scan. In the primary analysis, 20 (22.0%) subjects were *APOE4* carriers. *APOE4* was an independent risk factor of lower cerebral blood flow in the anterior circulation territory (β=−0.058 [95% CI, −0.098 to −0.018], *P*=0.005) and short-term memory impairment in Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (β=1.16 [95% CI, 0.009–2.30], *P*=0.048) in a multivariable linear regression analysis. In the sensitivity analysis, 31 (19.3%) subjects carried *APOE4*, which was an independent risk factor of lower cerebral blood flow (β=−0.048 [95% CI, −0.079 to −0.012], *P*=0.003) in the anterior circulation territory.

CONCLUSIONS: *APOE4* may confer an increased risk of decreased cerebral blood flow accompanied by memory impairment in asymptomatic carotid artery stenosis or occlusion consistent with our experimental study. *APOE* genotyping in such subjects may be useful for early detection of disease severity.

Key Words: *APOE4* ■ carotid artery occlusion ■ carotid artery stenosis ■ cerebral blood flow ■ memory

Extracranial atherosclerotic carotid artery stenosis or occlusion (CASO) is a major cause of cerebral infarction due to cerebral hemodynamic impairment.[1] Nuclear medicine, including positron emission tomography (PET) imaging, is essential for evaluating cerebral hemodynamic impairment and determining the indication for surgical revascularization.[2] This is because subjects with asymptomatic carotid stenosis of ≥60% receiving only best medical treatment are at a high risk of ischemic stroke.[3] Epidemiologically, men

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This article was sent to Neel S. Singhal, MD, PhD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.039210

For Sources of Funding and Disclosures, see page 12.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- *APOE4* carriers with carotid artery stenosis/occlusion are susceptible to more severe cerebral hemodynamic and memory impairment.

What Are the Clinical Implications?

- *APOE4* is a genetic risk factor of severe cerebral hemodynamics and memory impairment in carotid artery stenosis/occlusion as well as Alzheimer's disease.
- *APOE* genotyping may be useful for early detection of disease severity.

Nonstandard Abbreviations and Acronyms

ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale 13
CASO	carotid artery stenosis or occlusion
CBF	cerebral blood flow
$CMRO_2$	cerebral metabolic rate of oxygen
MoCA	Montreal Cognitive Assessment
NCVC	National Cerebral and Cardiovascular Center
OEF	oxygen extraction fraction
TR	targeted replacement

are more susceptible to asymptomatic extracranial atherosclerotic CASO; in subjects aged 75 to 79 years, the prevalence among men was 6.9%, whereas the prevalence among women was 4.3%.[3]

The restricted cerebral hemodynamics by extracranial atherosclerotic CASO leads to vascular cognitive impairment.[4,5] Asymptomatic CAS of ≥50% is associated with cognitive impairment, which is primarily driven by executive and visuospatial dysfunction and memory impairment and is independent of known vascular risk factors.[6,7] Thus, asymptomatic extracranial atherosclerotic CASO is no longer considered asymptomatic but symptomatic. However, the anatomical percentage of CAS is known not to correlate with the severity of functional consequences of stenosis.[2] Thereby, it is necessary to identify noninvasive markers that estimate cerebral hemodynamic impairment and cognitive dysfunction in subjects with asymptomatic extracranial atherosclerotic CASO. Our recent study demonstrated that midregional proadrenomedullin can be a biomarker to predict cerebral hemodynamic impairment evaluated by ^{15}O-gas PET in subjects with asymptomatic extracranial atherosclerotic CASO.[8] The current study is being proposed to explore genetic markers.

The apolipoprotein E4 (*APOE4*) allele is associated with alteration of cerebrovasculature and its function.[9] *APOE4* targeted-replacement (TR) mice were generated by replacing the mouse *ApoE* gene with the human *APOE4* allele. This replacement was achieved using E14TG2a embryonic stem cells, which were injected into blastocysts. The resulting chimeras were backcrossed to the C57BL/6 strain for further breeding.[10] Our recent preclinical study demonstrated that *APOE4*-TR mice models exhibit neurovascular dysfunction, such as impaired functional hyperemia and endothelial dysfunction, with excessive reactive oxygen species produced by their border-associated macrophages.[11,12] We also showed that *APOE4*-TR mice with bilateral CAS showed significant marked reduction in cerebral blood flow (CBF) accompanied by more severe cognitive impairment via neurovascular dysfunction than *APOE3*-TR and wild-type mice.[11,12] Clinically, the *APOE4* allele was shown to have no effect on common carotid artery intima-media thickness and plaques,[13] and it remains unclear whether *APOE4* carriers exhibit lower CBF in the asymptomatic stage of extracranial atherosclerotic CASO. Therefore, this study aimed to clinically determine whether *APOE4* is associated with worsening cerebral hemodynamic impairment, as assessed by ^{15}O-gas PET, and cognitive impairment in patients with asymptomatic extracranial atherosclerotic CASO. *APOE4* could be a genetic risk factor for cerebral hemodynamic disruption and dementia in asymptomatic extracranial atherosclerotic CASO.

METHODS

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design

This was a cross-sectional retrospective study performed at the National Cerebral and Cardiovascular Center (NCVC) in Japan. The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of NCVC (approval number: R20113). To investigate associations of *APOE4* with cerebral hemodynamic and cognitive impairment, the inclusion criteria of subjects who visited NCVC between January 2017 and March 2022 were as follows: (1) asymptomatic extracranial atherosclerotic CASO subjects showed a peak systolic velocity of ≥130 cm/s at the stenotic lesions or occlusion using carotid Doppler ultrasonography. A peak systolic

velocity of ≥130 and ≥200 cm/s at a stenotic lesion are comparable to angiographic stenosis of ≥50% and ≥70%, respectively.[14,15] Asymptomatic status is determined by a history of no stroke or transient ischemic attack due to the carotid lesions; (2) subjects who underwent both ^{15}O-gas PET to assess CBF, cerebral metabolic rate of oxygen ($CMRO_2$), and oxygen extraction fraction (OEF) and neuropsychological examinations, including the Montreal Cognitive Assessment (MoCA) and Alzheimer's Disease (AD) Assessment Scale-Cognitive Subscale 13 (ADAS-Cog); and (3) subjects who provided written informed consent for the NCVC biobank. Subjects were excluded if (1) subjects had been clinically diagnosed with dementia caused by other neurological diseases such as AD by neurologists or psychiatrists; or (2) the subjects were diagnosed with endogenous psychosis, such as depression and schizophrenia, by psychiatrists. The medical histories, blood tests, and medication were collected from the medical records of all subjects. In a sensitivity analysis, we enrolled subjects with asymptomatic extracranial atherosclerotic CASO who visited NCVC and underwent ^{15}O-gas PET between January 2017 to March 2022 to examine the association between *APOE4* and cerebral hemodynamics for a more in-depth study (Figure S1). Despite receiving best medical treatment alone, subjects with asymptomatic extracranial CAS of ≥60% are at a high risk of ischemic stroke.[16] That is, all the subjects in the study clinically underwent ^{15}O-gas PET to validate if surgical revascularization was indicated. The primary outcome was to determine if *APOE4* was associated with an increased rate of cerebral hemodynamic impairment. The secondary outcome was to determine if *APOE4* was associated with an increased rate of cognitive impairment.

^{15}O-Gas PET Measurements

All subjects underwent a series of ^{15}O-gas PET examinations to assess CBF, $CMRO_2$, and OEF (Figure 1A). The radioactive ^{15}O was produced by accelerating a deuteron (d) beam via the ^{14}N(d,n)^{15}O nuclear reaction using a cyclotron (CYPRIS HM-12, Sumitomo Heavy Industry, Tokyo, Japan). A 0.3% O_2 amount in the N_2 target gas was used to produce the ^{15}O-O_2 and ^{15}O-CO gases, and a 1.0% CO_2 amount in the N_2 target gas was used to produce the ^{15}O-CO_2 gas. The PET scanner was Biograph mCT (Siemens Healthinier, Erlangen, Germany). The PET scan was initiated at 3 minutes after the 2-minute inhalation of ^{15}O-CO for a 4-minute duration. An additional dynamic PET scan was performed for 8 minutes while ^{15}O-O_2 and ^{15}O-CO_2 gases were inhaled for 1 minute sequentially at a 4.5-minute interval. A 2-channel rapid gas chromatograph (Micro 990, Agilent Technologies, inc. Santa Clara, CA, USA) was used to verify radiochemical purity to be >99%

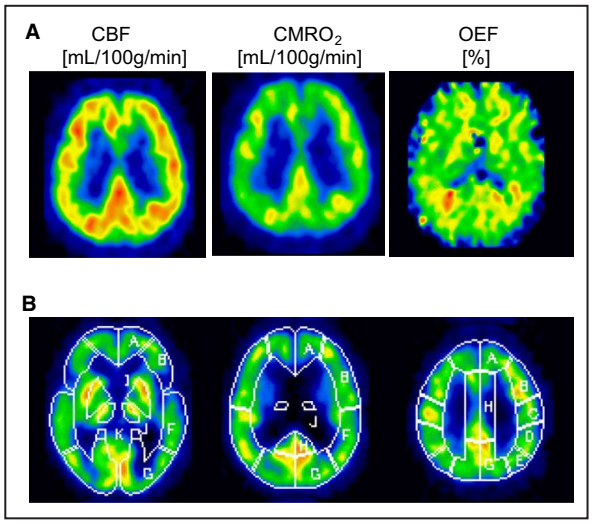

Figure 1. Representative images of ^{15}O-gas positron emission tomography and a 3-dimensional stereotaxic region-of-interest template software.
A, Representative images showing cerebral blood flow, cerebral metabolic rate of oxygen, and oxygen extraction fraction assessed by ^{15}O-gas PET in a patient with asymptomatic carotid artery stenosis. **B**, Region-of-interest segments in the 3-dimensional stereotaxic region-of-interest template software are shown as follows: A, prefrontal area; B, precentral artery area; C, central artery area; D, parietal artery area; E, angular artery area; F, temporal lobe; G, occipital lobe; H, pericallosal artery area; I, lenticular nucleus; J, thalamus; K, hippocampus. CBF indicates cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen; OEF, oxygen extraction fraction; and PET, positron emission tomography.

before each radio gas inhalation, as described in an earlier study.[17]

Functional images of CBF, $CMRO_2$, and OEF were processed by performing a previously validated technique, that is, dual-table autoradiography technique.[18] The arterial input function was obtained from a continuously-monitored radioactivity concentration in the arterial blood continuously withdrawn from the brachial artery.[19] The metabolized ^{15}O-water in the arterial blood, which is produced as a result of whole-body metabolism from ^{15}O-O_2, was estimated by modeling a physiological oxygen metabolism.[20] Vendor software was used to reconstruct PET images, with adequately-selected methodology that accounts for the presence of the gaseous ^{15}O-radioactivity surrounding the face during the inhalation period. The PET scanner used was the Biograph mCT (Siemens Medical Solutions, Knoxville, TN, USA), with a spatial resolution of 4.4 mm at the center of the field of view. It was operated in 3-dimensional mode with scatter correction, ensuring accurate PET image reconstruction, even with gaseous radioactivity surrounding the patient's face.[21] The PET scanner was operated in 3-dimensional mode with the scatter correction option that ensureed the accurate

PET image reconstruction when gaseous radioactivity surrounded the patient's face.

Neuropsychiatric Assessments

The MoCA evaluates the following cognitive domains: executive function, memory, attention, concentration, language, visuoconstructional skills, conceptual thinking, calculations, and orientation, with scores ranging from 0 to 30.[22] ADAS-Cog includes all ADAS-Cog-11 items, as well as tests for delayed word recall and tasks such as number cancellation or maze navigation, with scores ranging from 0 to 85.[23] All subjects clinically underwent neuropsychological examinations performed by 2 well-trained neuropsychologists (C.K. and M.Y.).

Evaluation of Brain Magnetic Resonance Imaging

Intracranial major artery stenosis was defined as ≥50% stenosis based on the brain magnetic resonance angiography (MRA) findings. The degree of stenosis was measured using the following equation: Stenosis (%)=[1−($D_{stenosis}$/D_{normal})×100], where $D_{stenosis}$ is the diameter of the artery at the site of the most severe grade of stenosis, and D_{normal} is the diameter of the proximal normal artery.[24] White matter hyperintensities, including periventricular hyperintensities and deep white matter hyperintensities were graded using a 0 to 3 scale based on the previously proposed Fazekas scale.[25] These findings were independently evaluated by 2 certified neurologists (Y.K. and Y.H.). To assess intrarater reliability, each neurologist reviewed all brain magnetic resonance imaging (MRI)/MRA findings on 2 separate occasions, with an interval of more than 2 months between evaluations.

APOE Genotyping

A fully automated gene analysis system (GTS-7000; Shimadzu Corporation, Kyoto, Japan) was used to genotype the *APOE* gene. The GTS-7000 directly detected single-nucleotide variants in 1 μL of whole-blood samples by polymerase chain reaction. We examined 2 single-nucleotide variants, rs429358 and rs7412, that determine the *APOE* ε allele, and rs429358-C and rs7412-C were used to determine *APOE4*. The primer sequences for rs429358 and rs7412 were 5′-CAAGGAGCTGCAGGCGG-3′ (forward), 5′-CAGCTCCTCGGTGCTCTG-3′ (reverse), and 5′- CGCAAGCTGCGTAAGCG-3′ (forward) and 5′-CGCGGATGGCGCTGAG-3′ (reverse), respectively. The probe sets were 5′-GGACGTGTGCGGCCG-3′ for rs429358-T, 5′- GGACGTGCGCGGCCG –3′ for rs429358-C, 5′-CTGCAGAAGCGCCTGGC-3′ for rs7412-C, and 5′-CTGCAGAAGTGCCTGGC-3′ for rs7412-T.

Statistical Analysis

Continuous variables are expressed as mean±SD, ordinal valuables as median (interquartile range), and categorical variables as frequencies with percentages. The chi-square test was performed to assess differences between *APOE4* carriers and noncarriers in the categorical variables, and the Mann–Whitney *U* test was performed for differences in the ordinal variables. The Mann–Whitney *U* test or Student's *t* test, as appropriate, were performed to assess differences in continuous variables between the groups. To identify *APOE4* as an independent risk factor for cerebral hemodynamic or cognitive impairment in the subjects with extracranial asymptomatic atherosclerotic CASO, multivariable linear regression analyses were performed after adjusted for age, sex, hypertension, diabetes, dyslipidemia, coronary artery disease, smoking, and alcohol drinking. Hypertension was defined based on the use of an antihypertensive agent, systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Diabetes was defined based on hemoglobin A1c ≥6.5% or a treatment history for diabetes. Dyslipidemia was defined based on the use of a lipid-lowering drug, a low-density lipoprotein cholesterol level ≥140 mg/dL, a triglyceride level ≥150 mg/dL or a high-density lipoprotein cholesterol level <40 mg/dL. Pearson's correlation analysis was performed to examine the relationship between CBF in the anterior circulation territory and the delayed word recall scores on the ADAS-Cog. All ^{15}O-gas PET images were analyzed using a 3-dimensional stereotaxic region-of-interest template on anatomically standardized PET images to objectively estimate the CBF where 12 regions of interest are set on the lateral hemispheres (PDRadiopharma, Tokyo, Japan).[8,26] Twelve region-of-interest segments, grouped according to the arterial supply, were examined in each hemisphere: the pericallosal, precentral, central, parietal, and angular artery territories; prefrontal area; temporal lobe; occipital lobe; hippocampus; lenticular nucleus; thalamus; and cerebellum (Figure 1B). The anterior circulation territory included the precentral, central, parietal, angular, and pericallosal artery territories as well as the prefrontal area, temporal lobe, and lenticular nucleus.[8,27] CBF, $CMRO_2$, and OEF values in the anterior circulation territory of the hemisphere supplied by asymptomatic carotid arteries with ≥50% stenosis or occlusion were presented after cerebellar normalization.[26] All statistical tests were 2 sided, and P<0.05 was accepted as indicative of statistical significance. Bonferroni correction was applied to evaluate differences in mean CBF between *APOE4* carriers and noncarriers. Significance was defined as a P value <0.006 (0.05/8), reflecting the 8 regions of interest within the anterior circulation territory.

Cohen's kappa coefficients were calculated to evaluate the consistency of intrarater and interrater assessments in brain MRI/MRA imaging. The interpretation of these coefficients was as follows: values ≥0.81 indicated excellent agreement, 0.61 to 0.80 substantial agreement, 0.41 to 0.60 moderate agreement, 0.21 to 0.40 fair agreement, and ≤0.20 poor agreement.

SPSS software version 27 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) and GraphPad PRISM (GraphPad Software, Boston, MA, USA) were used to perform all statistical analyses.

RESULTS

Baseline Characteristics of the Subjects

This study enrolled a total of 91 subjects who underwent both ^{15}O-gas PET and neuropsychological examinations, such as MoCA and ADAS-Cog. The numbers of *APOE4* noncarriers and carriers were 71 (78.0%) and 20 (22.0%), respectively. The number of men was 73 (80.2%) in all the subjects. The numbers of subjects with ≤12 years of education were proportionally comparable between *APOE4* noncarriers and carriers (noncarriers, 42 [59.2%] versus carriers, 13 [65.0%]; *P*=0.78). There were no significant differences in the levels of conventional vascular risk factors, including triglycerides (*P*=0.95), low-density lipoprotein cholesterol (*P*=0.38) and high-density lipoprotein cholesterol (*P*=0.47). The prevalence of right CASO, left CASO, or bilateral CASO significantly differed between the 2 groups (*P*=0.019), with *APOE4* noncarriers showing a higher prevalence of left and bilateral CASO and *APOE4* carriers showing higher prevalence of right CASO. Prevalences of intracranial major artery stenosis on brain MRA did not differ between *APOE4* noncarriers and carriers (5 [7.0%] versus 3 [14.3%], *P*=0.27). Fazekas grades of white matter hyperintensities on brain MRI were comparable between *APOE4* noncarriers and carriers (periventricular hyperintensities: 1 [0–3] versus 1.5 [0.5–3.5], *P*=0.33; deep white matter hyperintensities: 1 [0–2] versus 1 [0–3], *P*=0.57). The mean total MoCA scores (23.5 versus 22.9; *P*=0.50) and total ADAS-Cog scores (15.6 versus 17.5; *P*=0.26) were similar between *APOE4* noncarriers and carriers. The mean time intervals between ^{15}O-gas PET and the cognitive assessments were comparable between *APOE4* noncarriers and carriers (44.7 days versus 37.9 days; *P*=0.85) (Table). No subjects had been clinically diagnosed with dementia caused by other neurological diseases and endogenous psychosis. The reproducibility of brain MRI/MRA findings, as assessed by intra- and interobserver evaluations, ranged from substantial to excellent (Table S1).

APOE4 Was Significantly Associated With Lower CBF in the Anterior Circulation Territory

The extent of cerebral hemodynamic impairment was assessed by ^{15}O-gas PET between the 2 groups. Among a total of 91 subjects, *APOE4* noncarriers had 91 affected hemispheres and *APOE4* carriers had 23 affected hemispheres. CBF in the *APOE4* carriers was significantly lower in the anterior circulation territory than that in noncarriers (0.75±0.073, 0.69±0.11; *P*=0.01). In the anterior circulation territory, *APOE4* carriers showed significantly lower CBF in the territories of precentral (*P*=0.024), central (*P*=0.006), parietal (*P*=0.005), pericallosal (*P*<0.001) arteries, prefrontal area (*P*=0.012), and temporal lobe (*P*=0.013) lobe compared with noncarriers (*P*<0.05). Following Bonferroni correction (*P*<0.006), significant differences were retained for the central (*P*=0.006), parietal (*P*=0.005), and pericallosal (*P*<0.001) artery territories (Figure 2). A multivariable linear regression analysis revealed that *APOE4* carriage was an independent risk factor for lower CBF in subjects with asymptomatic CASO in the anterior circulation territory (adjusted β [mean difference in CBF between *APOE4* carriers and noncarriers as reference]=−0.058 [95% CI, −0.098 to −0.018], *P*=0.005), and in the territories of central (adjusted β=−0.061 [95% CI, −0.10 to −0.019]; *P*=0.004), parietal (adjusted β=−0.054 [95% CI, −0.097 to −0.011]; *P*=0.014) and pericallosal (adjusted β=−0.077 [95% CI, −0.12 to −0.030]; *P*=0.001) arteries (Figure 3). The severity of carotid stenosis or occlusion did not alter CBF (Figure S2). $CMRO_2$ and OEF in the anterior circulation territory did not differ (Figure S3).In the posterior circulation territory (hippocampus, thalamus, and occipital lobe), *APOE4* carriers exhibited significantly lower CBF than noncarriers (0.79±0.086 versus 0.74±0.12; *P*=0.032). Among these regions, CBF in the only occipital lobe was significantly reduced in *APOE4* carriers (*P*=0.020; Figure S4). $CMRO_2$ and OEF levels showed no significant differences between the 2 groups (Figure S5).

APOE4 Was Significantly Associated With a Worse Score of Delayed Word Recall in the ADAS-Cog

There was no significant difference in the MoCA scores between the 2 groups (Table S2), but the ADAS-Cog scores showed a significant worse score only in the delayed word recall in the *APOE4* carriers (4.5±2.3 versus 5.9±2.3, *P*=0.049) (Table S3). Lower

Table. Baseline Characteristics of the Subjects

	All	*APOE4* noncarriers	*APOE4* carriers	*P* value
Number of subjects	91	71	20	
Number of affected hemispheres	114	91	23	
Age, y	76.9 (±7.3)	77.2 (±6.8)	76.0 (±8.8)	0.087
Men	73 (80.2)	53 (74.6)	20 (100.0)	0.007
Smoking	35 (38.5)	25 (35.2)	10 (50.0)	0.23
Alcohol	45 (49.5)	32 (45.1)	13 (65.0)	0.12
Education <12 y	55 (60.4)	42 (59.2)	13 (65.0)	0.78
Hypertension	80 (87.9)	63 (88.7)	17 (85.0)	0.45
Diabetes	29 (31.9)	22 (31.0)	7 (35.0)	0.73
Dyslipidemia	83 (91.2)	65 (91.5)	18 (90.0)	0.56
Coronary artery disease	25 (27.5)	19 (26.8)	6 (30.0)	0.77
Atrial fibrillation	13 (14.3)	10 (14.1)	3 (15.0)	0.58
Systolic blood pressure, mm Hg	138.3 (±20.1)	139.1 (±20.7)	135.4 (±17.5)	0.47
Diastolic blood pressure, mm Hg	70.4 (±13.7)	70.2 (±14.0)	71.0 (±12.2)	0.83
Blood glucose, mg/dL	118.6 (±36.5)	119.7 (±38.6)	114.3 (±26.7)	0.57
Hemoglobin A1c, %	6.2 (±0.7)	6.2 (±0.7)	6.1 (±0.5)	0.64
Triglyceride, mg/dL	138.1 (±78.8)	138.4 (±80.5)	137.1 (±72.5)	0.95
Low-density lipoprotein cholesterol, mg/dL	81.8 (±25.4)	80.5 (±26.6)	86.3 (±20.1)	0.38
High-density lipoprotein cholesterol, mg/dL	52.1 (±13.4)	51.6 (±13.7)	54.1 (±11.9)	0.47
C-reactive protein, mg/dL	0.2 (±0.3)	0.2 (±0.4)	0.1 (±0.1)	0.21
Side of lesions				0.019
Right	27 (29.7)	16 (22.5)	11 (55.0)	
Left	41 (45.1)	35 (49.3)	6 (30.0)	
Bilateral	23 (25.3)	20 (28.2)	3 (15.0)	
Stenosis grade				0.12
50% ≤stenosis <70%	22 (24.2)	17 (23.9)	5 (25.0)	
70% ≤stenosis	39 (42.9)	34 (47.9)	5 (25.0)	
Occlusion	30 (33.0)	20 (28.2)	10 (50.0)	
Brain magnetic resonance imaging				
Intracranial major artery stenosis	8 (8.8)	5 (7.0)	3 (14.3)	0.27
Periventricular hyperintensities	1 (0–3)	1 (0–3)	1.5 (0–3.5)	0.33
Deep white matter hyperintensities	1 (0–2)	1 (0–2)	1 (0–3)	0.57
Total score of Montreal Cognitive Assessment	23.4 (±3.7)	23.5 (±3.8)	22.9 (±3.6)	0.50
Total score of Alzheimer's Disease Assessment Scale-Cognitive Subscale 13	16.0 (±6.8)	15.6 (±7.0)	17.5 (±5.7)	0.26
Time interval between ^{15}O-gas PET and neuropsychiatric examinations, d	43.2 (±139.6)	44.7 (±152.0)	37.9 (±85.0)	0.85
Time interval between ^{15}O-gas PET and brain MRI, d	33.9 (±50.6)	34.4 (±53.8)	32.3 (±37.2)	0.88
Time interval between brain MRI and neuropsychiatric examinations, d	64.7 (±148.5)	65.1 (±160.3)	62.8 (±92.8)	0.95

Categorical variables are presented as number (%), continuous variables are presented as the mean (±SD), or ordinal variables are presented as median (interquartile range). *APOE4* indicates apolipoprotein E4; MRI, magnetic resonance imaging; and PET, positron emission tomography.

MoCA scores or higher ADAS-Cog scores indicate more severe cognitive impairment. Multivariable linear regression analysis with delayed word recall as the dependent variable showed that *APOE4* carriage was an independent risk factor of short-term memory impairment (adjusted β [mean difference in the delayed word recall score between *APOE4* carriers and noncarriers as reference]=1.16 [95% CI, 0.009–2.30], *P*=0.048) (Figure 4). To examine the relationship between CBF and short-term memory, we performed Pearson's correlation analysis between CBF in the anterior circulation territory and delayed word recall score of ADAS-Cog. For *APOE4* noncarriers, all absolute values of the correlation coefficients were <0.4, whereas in *APOE4* carriers, the absolute values of the correlation coefficients

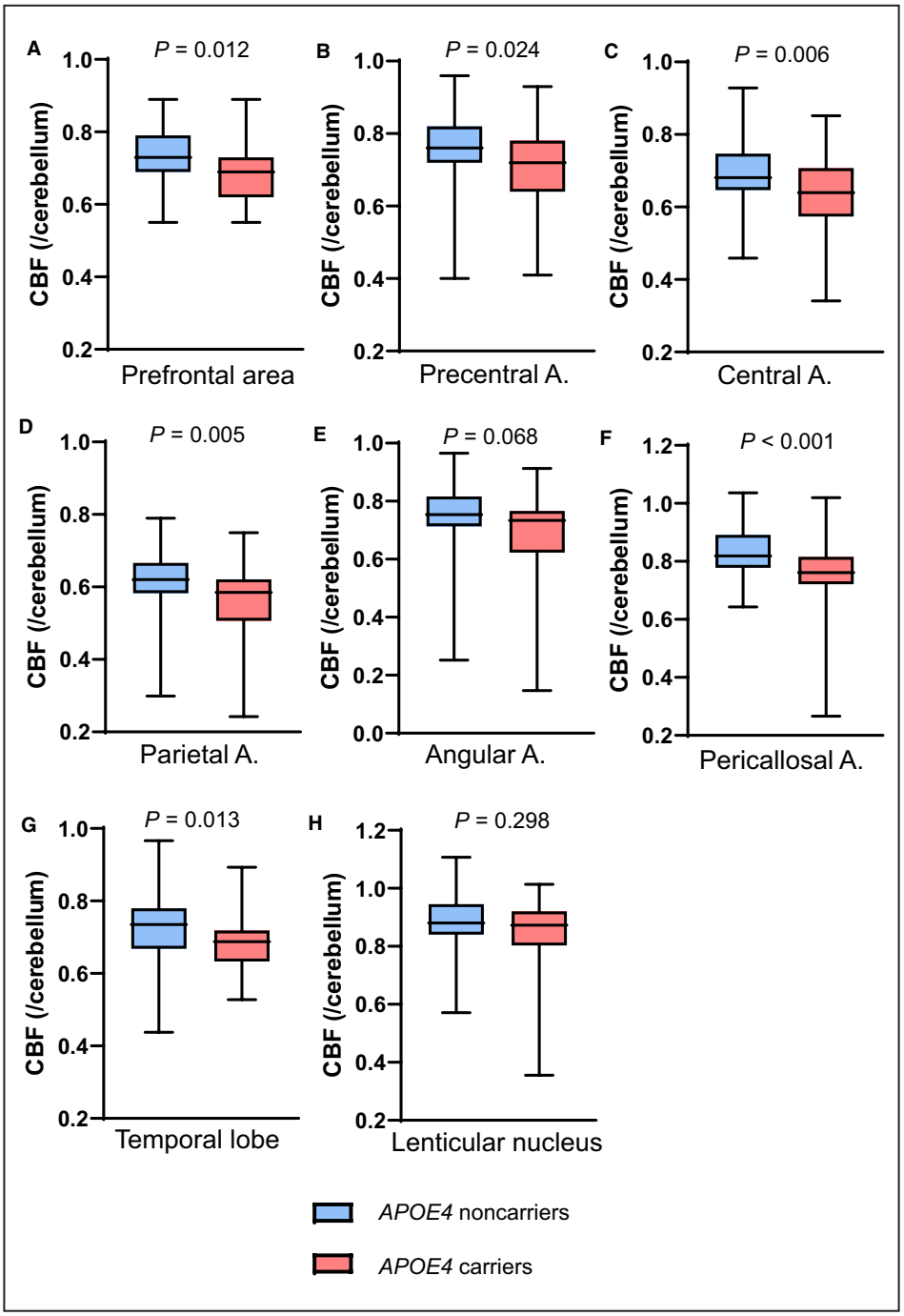

Figure 2. Comparison of the cerebral blood flow in the anterior circulation territory between *APOE4* carriers and noncarriers.
Bar graphs showing CBF in the anterior circulation territory of interest consisting of the prefrontal area (**A**), precentral (**B**), central (**C**), parietal (**D**), angular (**E**), and pericallosal (**F**) arteries; temporal lobe (**G**); and lenticular nucleus (**H**). Bonferroni's correction was applied for evaluating the differences in mean CBF between *APOE4* carriers and noncarriers. Significance was determined at a threshold of less than [0.05/8=0.006] *P* value after the correction as the anterior circulation territory consists of the 8 ROIs. A indicates artery; *APOE4*, apolipoprotein E4; CBF, cerebral blood flow; and ROI, region of interest.

of CBF in the prefrontal area and pericallosal artery territory were >0.4 and significant (prefrontal area: r=−0.47, P=0.024; pericallosal artery: r=−0.44, P=0.037) (Table S4). The severity of carotid stenosis or occlusion did not alter severity of the cognitive impairment (Figure S6).

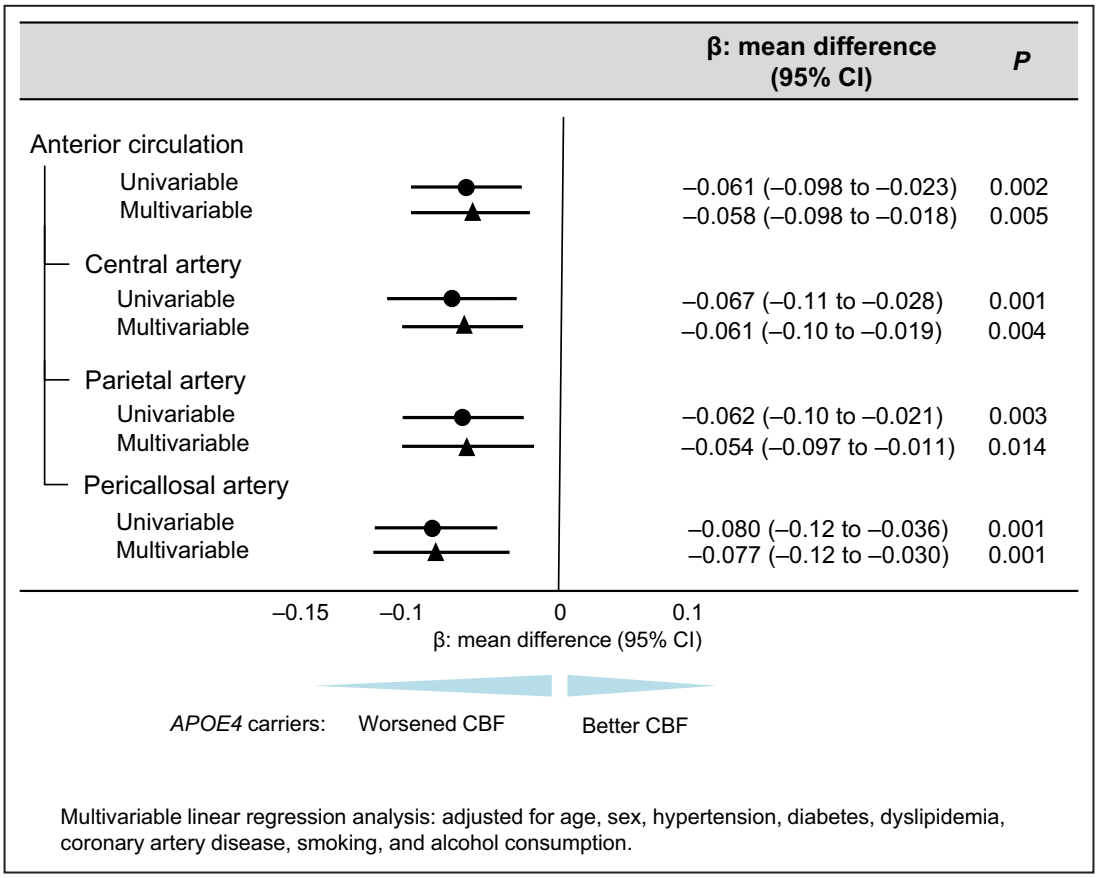

Figure 3. Linear regression analyses estimating the association of *APOE4* carriage with the cerebral blood flow in the anterior circulation territory examined by ^{15}O-gas positron emission tomography.
APOE4 carriage was significantly associated with lower CBF in the anterior circulation territory, and the central, parietal, and pericallosal artery territories. *APOE4*, apolipoprotein E4; and CBF, cerebral blood flow.

Baseline Characteristics of the Subjects in the Sensitivity Analysis

In addition to the 91 subjects who underwent ^{15}O-gas PET and neuropsychological examinations, we examined the data for total of 161 subjects with asymptomatic extracranial atherosclerotic CASO who had undergone ^{15}O-gas PET performed from January 2017 and March 2022. Of the total 161 subjects, the numbers of *APOE4* noncarriers and carriers were 130 (80.7%) and 31 (19.3%), respectively. The numbers of *APOE4* noncarriers and carriers were 130 (80.7%) and 31 (19.3%), respectively. The number of men was 130 (81.0%) in all the subjects. The left CASO or bilateral CASO differed between the 2 groups (P=0.033). *APOE4* noncarriers had a higher prevalence of left and bilateral CASO, whereas the *APOE4* carriers had a higher prevalence of right CASO. Prevalences of intracranial major artery stenosis on brain MRA did not differ between *APOE4* noncarriers and carriers (8 [6.2%] versus 3 [9.7%], P=0.49). Fazekas grades of white matter hyperintensities on brain MRI were comparable between *APOE4* noncarriers and carriers (periventricular hyperintensities: 1 [0–2] versus 1 [0.5–2], P=0.31; deep white matter hyperintensities: 1 [1–2] versus 1 [1–2], P=0.85) (Table S5). The intrarater and interrater reproducibility of brain MRI/MRA findings ranged from substantial to excellent (Table S6).

Anterior Circulation Remained Significantly Impaired in the *APOE4* Carriers in the Sensitivity Analysis

Among a total of 161 subjects, *APOE4* noncarriers had 165 affected hemispheres and *APOE4* carriers had 36 affected hemispheres. ^{15}O-gas PET revealed significantly lower CBF in the *APOE4* carriers than in noncarriers in the anterior circulation territory (0.76±0.080 versus 0.71±0.10; P=0.005). In the anterior circulation territory, *APOE4* carriers showed significantly lower CBF in the territories of precentral (P=0.016), central (P=0.002), parietal (P=0.003), angular (P=0.016), pericallosal (P=0.001) arteries, prefrontal area (P=0.010), and temporal lobe (P=0.018) lobes compared with noncarriers (P<0.05). Following Bonferroni correction

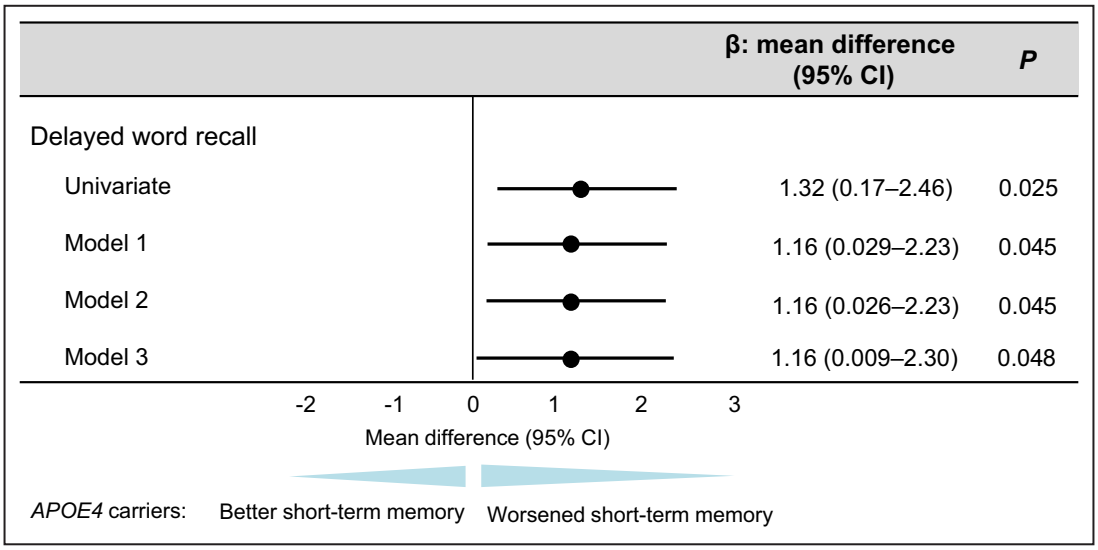

Figure 4. Linear regression analyses estimating the association of *APOE4* carriage with delayed word recall in the Alzheimer's Disease Assessment Scale-Cognitive Subscale 13.
APOE4 carriage was significantly associated with worsened score of delayed word recall, short-term memory impairment. Model 1: adjusted for age, hypertension, diabetes, dyslipidemia, smoking and alcohol; Model 2: further adjusted for coronary artery diseases; Model 3: further adjusted for education of <12 years. *APOE4*, apolipoprotein E4.

(*P*<0.006), significant differences were retained for the central (*P*=0.002), parietal (*P*=0.003), and pericallosal (*P*=0.001) artery territories (Figure 5). Multivariable linear regression analysis revealed that carriage of *APOE4* was an independent genetic risk factor of lower CBF in the anterior circulation territory (adjusted β [mean difference in CBF between *APOE4* carriers and noncarriers as reference]=−0.048 [95% CI, −0.079 to −0.017]; *P*=0.003), and in the central (adjusted β=−0.059 [95% CI, −0.093 to −0.025]; *P*<0.001), parietal (adjusted β=−0.048 [95% CI, −0.080 to −0.016]; *P*=0.003) and pericallosal (β=−0.060 [95% CI, −0.095 to −0.026]; *P*<0.001) artery territories (Figure 6).

DISCUSSION

This study demonstrated that *APOE4* carriers with asymptomatic extracranial atherosclerotic CASO were more vulnerable to severe cerebral hypoperfusion in the anterior circulation territory but not in the hippocampus. This hypoperfusion was associated with memory impairment and was not influenced by findings on brain MRI, such as intracranial major artery stenosis or white matter hyperintensities. $CMRO_2$ and OEF values were comparable between *APOE4* carriers and noncarriers.

APOE is essential for cholesterol metabolism. In particular, *APOE4* is associated with dyslipidemia and coronary artery disease.[28] Among healthy older adults, the prospective Framingham Offspring Cohort Study showed that carrying *APOE4* was associated with cognitive exacerbation with an increased midlife vascular risk burden.[29] Another study revealed that cardiovascular risk factors, including dyslipidemia, result in longitudinal preclinical memory decline in healthy *APOE4* homozygotes.[30] The current study showed that conventional vascular risk factors, such as blood pressure, blood glucose, hemoglobin A1c, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, were comparable between *APOE4* noncarriers and carriers and not in pathogenic levels (Table 1; Table S5). Therefore, significantly more severe cerebral hemodynamic disruption and memory impairment observed in the *APOE4* carriers with asymptomatic moderate or severe CASO could be attributed to other pathogeneses. We previously reported that *APOE4*-TR mice, which have targeted replacement of the mouse *ApoE* with human *APOE4*, with bilateral common CAS had more severely reduced CBF and impaired reference memory compared with those in *APOE3*-TR and wild-type mice.[11,12] These findings indicate a detrimental role of *APOE4* in inducing neurovascular uncoupling and endothelial dysfunction, most likely by excessive cerebrovascular oxidative stress produced by *APOE4*-positive border-associated macrophages, and subsequently leading to neurodegeneration.[11,12] No previous clinical reports have been published on the effects of *APOE4* and CASO on cerebral hemodynamics and cognitive performance. Therefore, targeting cerebrovascular oxidative stress, engendered by *APOE4*-positive border-associated

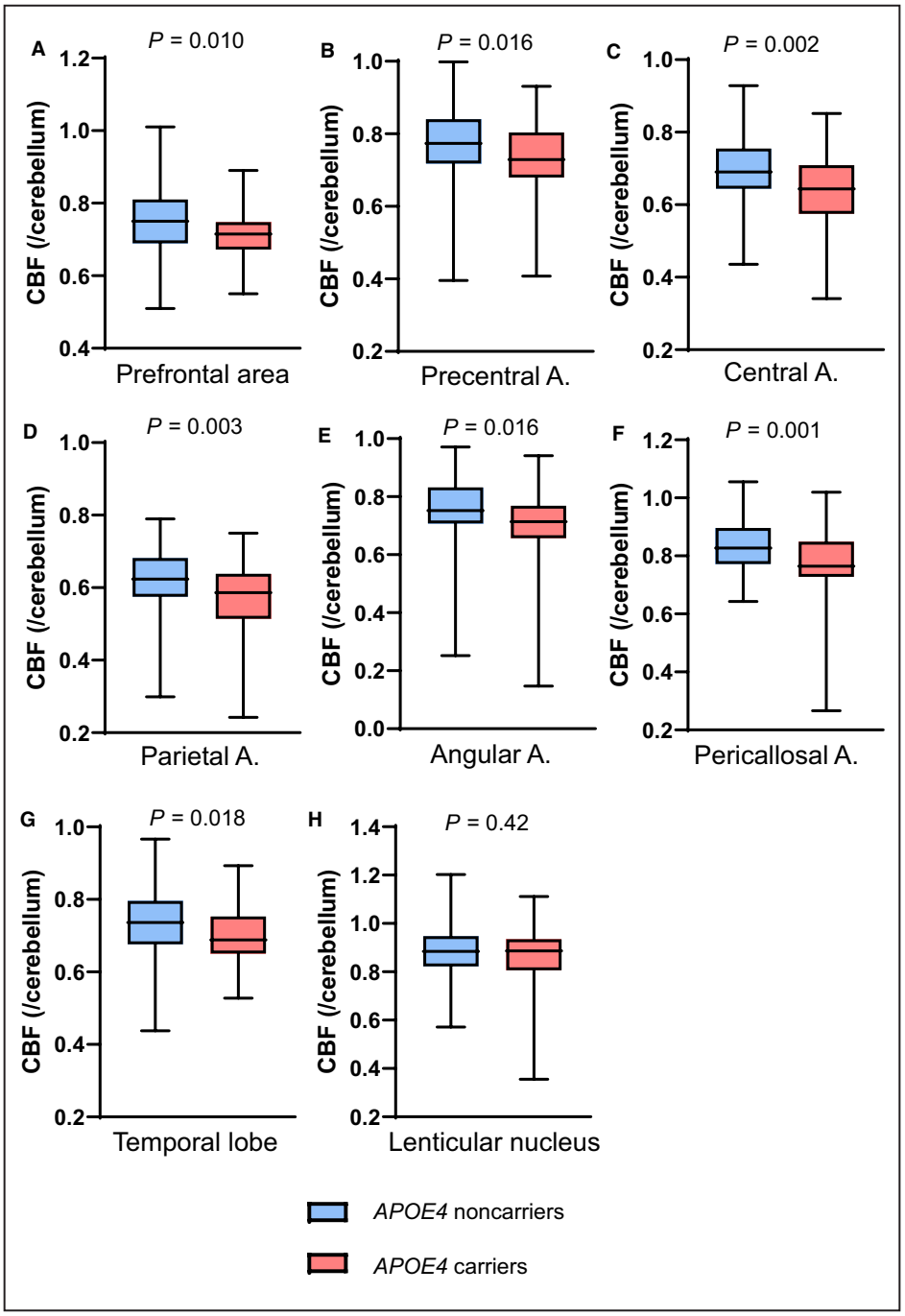

Figure 5. Comparison of the CBF in the anterior circulation territory between *APOE4* carriers and noncarriers in the sensitivity analysis.
Bar graphs showing the CBF in the anterior circulation territory of interest consisting of the prefrontal area (**A**), precentral (**B**), central (**C**), parietal (**D**), angular (**E**), and pericallosal (**F**) arteries; temporal lobe (**G**); and lenticular nucleus (**H**). Bonferroni's correction was applied for evaluating the differences in mean CBF between *APOE4* carriers and noncarriers. Significance was determined at a threshold of less than [0.05/8=0.006] *P* value after the correction as the anterior circulation territory consists of the 8 ROIs. A indicates artery; *APOE4*, apolipoprotein E4; CBF, cerebral blood flow; and ROI, region of interest.

macrophages might help ameliorate cerebral hemodynamic and cognitive impairment in subjects with asymptomatic extracranial atherosclerotic CASO.

In this study, significantly lower CBF in the anterior circulation territory and worse score for delayed word recall in the ADAS-Cog was observed in the *APOE4*

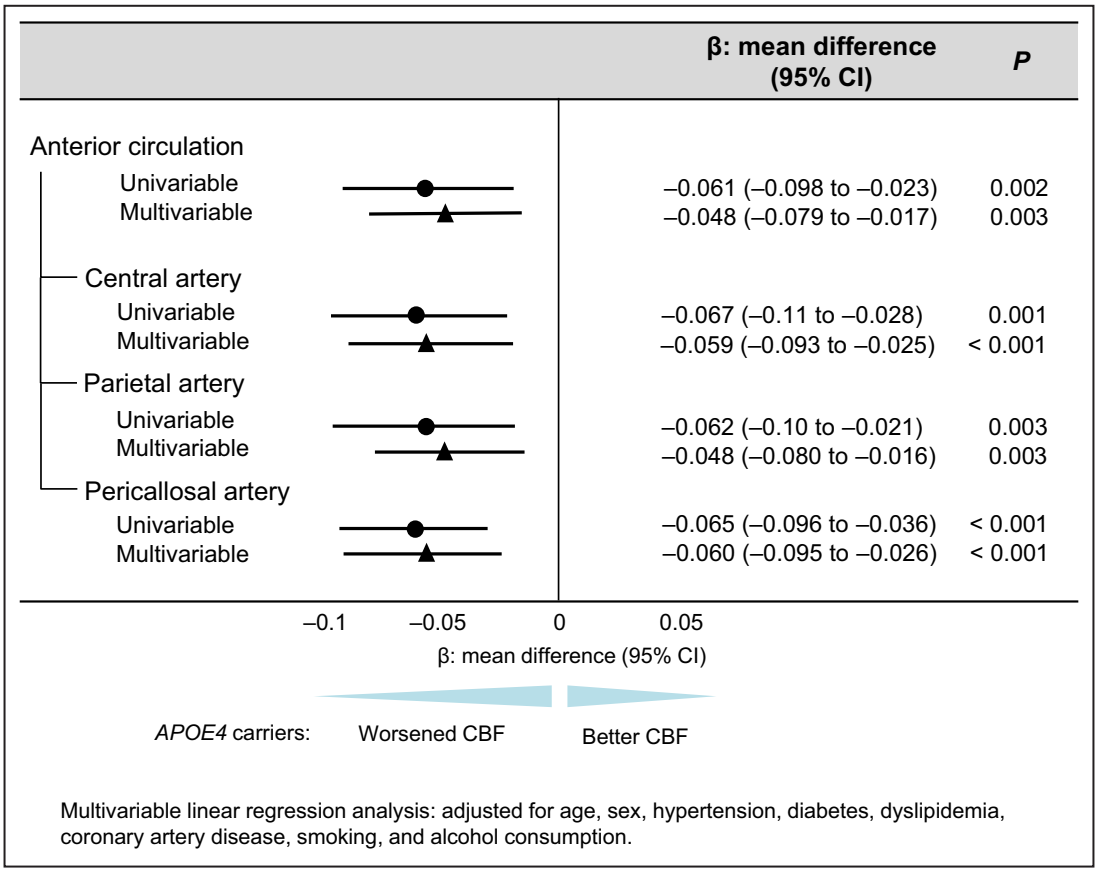

Figure 6. Linear regression analyses estimating the association of *APOE4* carriage with the cerebral blood flow in the anterior circulation territory examined by ^{15}O-gas positron emission tomography in the sensitivity analysis.
APOE4 carriage was significantly associated with lower CBF in the anterior circulation territory, and the central, parietal, and pericallosal artery territories. *APOE4* indicates apolipoprotein E4.

carrier group, whereas CBF in the hippocampus was comparable between the 2 groups. The association between anterior circulation disruption and worse score for delayed word recall requires further inquiry. There are 3 plausible explanations for this finding. First, the anterior circulation territory defined in the study included the basal forebrain. The basal forebrain is 1 of the 3 major brain circuits involved in episodic memory formation together with the hippocampus and diencephalon, and includes various structures, such as the septal nucleus, diagonal band of Broca and Meynert's basal ganglia.[31] Basal forebrain impairment is characterized by short-term memory impairment.[32] Second, the worsened delayed word recall could be derived from impaired working memory. Working memory requires maintaining information temporally so that its executive component can manipulate such information and operate with it.[33,34] In other words, short-term memory is a critical component of working memory.[34] Brain regions primarily related to working memory are the posterior parietal lobe perfused by parietal and central arteries, and the prefrontal cortex.[33–35] Finally, the *APOE4* carriers in this study showed decreased CBF in the pericallosal artery area, including the posterior cingulate gyrus and precuneus, which have close anatomic fiber connections with the entorhinal, perirhinal and posterior parahippocampal cortex. These areas typically show pathological changes associated with AD.[36] Subjects with AD from the AD Neuroimaging Initiative showed a higher regional CBF in the frontal cortex compared with normal controls evaluated by 3-dimensional background suppressed pseudo-continuous arterial spin labeling,[37] suggesting that the association between short-term memory impairment and lower CBF in the anterior circulation territory in *APOE4* carriers can be a characteristic feature in subjects with CASO. These 3 explanations may underlie the link between the lower CBF in the anterior circulation territory and the worse score of delayed word recall in the ADAS-Cog. Although the occipital lobe demonstrated reduced CBF in *APOE4* carriers, it is not typically associated with short-term memory.

Right-sided stenosis was more common in *APOE4* carriers, and *APOE4* was significantly associated with

poorer delayed word recall scores in the ADAS-Cog during the primary analysis. Previous studies have highlighted interhemispheric asymmetry. In Chinese patients with stroke, the association between high-grade stenosis of the right carotid artery and cognitive impairment was notably strong, whereas the association between high-grade stenosis of the left carotid artery and cognitive impairment was not statistically significant.[38] Furthermore, patients with moyamoya disease, characterized by occlusion of bilateral intracranial internal carotid arteries, also demonstrated significant correlations between cognitive impairments and CBF decline localized to the right hemisphere.[39] These findings suggest an asymmetrical relationship between the right and left carotid arteries concerning brain hemodynamics and cognitive function, although the underlying mechanisms remain unclear.

Chronic cerebral hypoperfusion induced by extracranial CASO may exacerbate AD phenotypes and pathologies in in vivo studies. Rodent models of extracranial CASO exhibit increased accumulation of brain β-amyloid in the anterior circulation territory.[40,41] The pathological changes may be attributed to the impaired intramural periarterial drainage pathway system.[42] This phenomenon is also associated with carotid stiffness in subjects with mild cognitive impairment.[43] Thus, the augmented AD phenotypes and pathologies should partially contribute to cognitive impairment in subjects with extracranial CASO.

CBF, $CMRO_2$, and OEF values were presented after cerebellar normalization in this study. Previous studies have shown that CBF normalized to the cerebellum was employed in subjects with AD, moyamoya disease, and CASO,[26,44–46] which are not supposed to affect the cerebellar perfusion. Furthermore, 3-dimensional stereotactic surface projection, which enables quantitative data extraction and provides reliable localization of abnormalities by means of stereotaxic coordinates using the cerebellar cortex as a reference region, is widely used to improve the diagnostic performance of PET.[47] Thus, CBF, $CMRO_2$, and OEF values after cerebellar normalization were justified in this study.

Despite the strengths of our study, this study had several limitations. First, due to a retrospective observational study, selection biases could originate at the time of enrolling the subjects with asymptomatic extracranial atherosclerotic CASO in this study. This is because all the subjects clinically underwent ^{15}O-gas PET to consider the indication of surgical revascularization, although this study investigated the relationship of *APOE4* with cerebral hemodynamic impairment and cognitive impairment. Second, several variables that have a relatively important effect on atherosclerotic disease, such as patient height, weight, and body mass index, were not measured, so these parameters should be included in future prospective studies. Third, extracranial atherosclerotic CASO may cause frontal or basal lobe dysfunction due to impairment of frontal-lobe subcortical circuits because of reduced CBF mainly in the anterior circulation territory. Specific tests, such as the frontal assessment battery, trail-making test, and Stroop test, may be more desirable to evaluate frontal-lobe function and frontal-lobe subcortical circuits. Fourth, subjects potentially suffering from AD could be included in the study, although none had been clinically diagnosed with AD by neurologists or psychiatrists. However, as mentioned, decreased CBF in the anterior circulation territory in subjects with CASO is not characteristic of subjects clinically diagnosed with AD based on the AD Neuroimaging Initiative study. Fifth, the sample size in this study may have been insufficient. To enhance statistical power and validity, future studies should consider larger cohorts.

Conclusions

In conclusion, subjects with asymptomatic extracranial atherosclerotic CASO who carry *APOE4* may be at a higher risk for severe alteration in cerebral circulation in the anterior circulation territory and more severe memory impairment. These findings are consistent with our experimental study using *APOE4*-TR mice. Consequently, *APOE* genotyping in asymptomatic extracranial atherosclerotic CASO subjects could serve as a clinically valuable noninvasive indicator of heightened cerebral hemodynamic dysfunction and memory impairment. This study highlights the urgent need to develop new therapies targeting neurovascular dysfunction induced by *APOE4*, bridging the gap between clinical practice and research.

ARTICLE INFORMATION

Received October 2, 2024; accepted January 30, 2025.

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Acknowledgments

We thank Ms Chikage Kakuta and Ms Miho Yamauchi for the neuropsychological testing, and Ms Natsuki Hanada for genotyping *APOE4*.

Author contributions: Yorito Hattori contributed to conception and design of the study. Yorito Hattori and Yoshinori Kakino contributed to collecting subjects, and acquisition and analysis of data. Yoshinori Kakino, Soshiro Ogata, Yuriko Nakaoku, and Kunihiro Nishimura contributed to analysis of data. Hidehiro Iida contributed to running ^{15}O-gas PET. Hattori and Yoshinori Kakino drafted a significant portion of the article and figures. Masafumi Ihara contributed to supervising the study and edited the article. All authors reviewed the article.

Sources of Funding

This study was supported by the Terumo Life Science Foundation (Yorito Hattori), Japan Cardiovascular Research Foundation (Yorito Hattori), Research Foundation of Dementia of Osaka (Yorito Hattori), Daiwa Securities Group (Yorito Hattori), Japan Geriatric Society (Yorito Hattori), and Honjo International Scholarship Foundation (Yorito Hattori).

Disclosures

None.

Supplemental Material

Data S1

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