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Apolipoprotein E genotypes were not associated with intracranial atherosclerosis: a population-based autopsy study☆

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Abstract

Background: Apolipoprotein E gene (APOE) ε4 allele is associated with a higher risk of carotid atherosclerosis, but less is known about the association of APOE with intracranial atherosclerotic disease (IAD). We aimed to investigate the association of APOE alleles with IAD in a cross-sectional autopsy study.

Methods: We measured the stenosis in the 12 arteries of the Circle of Willis using postmortem morphometric measurements. The APOE polymorphism was determined by real-time polymerase chain reaction. We assessed the association between APOE polymorphism and IAD using regression models adjusted for sociodemographic and clinical variables. We also verified the modifier effect of age, sex, and race on this association. We stratified the analysis by age group to investigate the possibility of attrition bias.

Results: In 400 participants (mean age=73.2±12.3 years old, 51% female, and 64% White), IAD was evaluated in 4,504 artery segments. APOE-ε4 was not associated with IAD nor with the number of artery stenosis compared to non-APOE-ε4 carriers. Sociodemographic variables did not modify this relationship. Among participants older than 70 years, there was a trend towards an association between APOE allele ε4 and a lower stenosis index in the middle cerebral artery, suggesting attrition bias related to the APOE-ε4 effect on mortality.

☆Disclosures: None.

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Conflicts of interest

none

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpath.2022.107479.

Conclusions: APOE alleles were not associated with IAD in this population-based autopsy study. Lower stenosis in older participants suggests the possibility of attrition bias.

Keywords

Apolipoprotein E; Atherosclerosis; Cerebral arteries; Epidemiology; Risk factors

1. Introduction

The leading cause of cerebrovascular diseases is ischaemic stroke, which has intracranial atherosclerosis as the most common pathologic cause [1,2]. Atherosclerosis pathophysiology is not entirely explained by traditional vascular risk factors and has a genetic contribution [3,4].

The apolipoprotein E gene (APOE) has three main alleles: *ε2*, *ε3,* and *ε*4, which can be combined to form six possible genotypes (*ε2/ε2*, *ε2/ε3*, *ε2/ε*4, *ε3/ε3*, *ε3/ε*4, and *ε*4/*ε*4) [5]. Some studies have found an association between APOE-*ε*4 and carotid atherosclerosis [6–10]. However, studies regarding the association between APOE and intracranial atherosclerotic disease (IAD) are still scarce. Although Chutinet et al. found an association of having at least one APOE-*ε*4 allele with extracranial carotid artery stenosis in 308 Thai participants, this association was not observed in patients with IAD (n=83) [10]. Recently, Liu et al. also investigated the relationship between seven genetic variants, including APOE, and intracranial large artery stenosis in the Northern Manhattan Study (NOMAS) (n=1,109) [11]. However, they did not find any significant association of APOE polymorphisms with IAD.

The associations between atherosclerosis and APOE have been evaluated using mainly imaging methods, which allow for indirect measurements of atherosclerosis. However, in autopsy studies, we can directly measure atherosclerotic plaques in all intracranial artery branches, even in arteries that are not easily assessed in imaging studies, such as portions of the internal carotid artery and distal arterial branches [12]. Evidence from an autopsy study showed that APOE-*ε*4 carriers presented a higher intracranial atherosclerosis score (calculated from nine branches of the circle of Willis arteries), but only among White men (n=400) [10]. Therefore, we aimed to evaluate the association between APOE genotypes and IAD using direct morphometric measurements of atherosclerosis in a population-based autopsy study with an admixed sample.

2. Methods

2.1. Participants

We used data from the Biobank for Aging Studies of the University of Sao Paulo Medical School [13]. Subjects dying from non-traumatic death of an unknown cause in Sao Paulo are submitted to autopsy exams. Autopsies were performed at the Sao Paulo Autopsy Service. The next of kin (NOK) signed an informed consent allowing the material collection and provided the sociodemographic and clinical information about the deceased. The local ethics committee approved this study [12,14]. From an initial sample composed of 1,195 subjects

aged 50 years or older at the time of death and with complete APOE data, we excluded individuals whose intracranial arteries were not collected (n=748); NOK who did not see the deceased at least weekly in the 6 months before death to ensure data accuracy (n=19); and incomplete data regarding sociodemographic and clinical variables (n=28).

2.2. Morphometric atherosclerosis evaluation

We considered the following arteries of the Circle of Willis (CW): the basilar and anterior communicating arteries and the right and left side of anterior cerebral, middle cerebral, internal carotid (close to the CW), posterior communicating, and posterior cerebral arteries [12]. The cross-sectional fragment with the largest lumen obstruction in each artery was selected and photographed using a stereomicroscope (Nikon SMZ 1000; Nikon Inst., New York, USA). The area delineated by the outer arterial wall and lumen was measured using image processing software (ImageJ®). A stenosis index was calculated for each of the 12 arteries of the CW by subtracting the lumen area from the outer area, dividing this difference by the outer area, and multiplying it by 100 (Fig. 1) [12].

2.3. APOE genotyping

DNA samples of the individuals were obtained from blood or brain tissue, and the APOE polymorphism was genotyped using either Illumina OmniExpress 700k microarray or Illumina BeadXpress custom genotyping panel. APOE common alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$) were genotyped directly by real-time polymerase chain reaction (PCR) using allele-specific amplification or after imputation of rs429358 to compose haplotypes [15].

2.4. Other variables

Possible confounding variables considered in the association between APOE genotypes and IAD were age at death, sex, race (White, Black, and Asian), education, a previous diagnosis of diabetes, hypertension, dyslipidemia, heart disease, smoking (never, current, or past smoking), alcohol use (never, current, or past use), and physical activity. The deceased age and sex were obtained from government-issued documents. The NOK reported the other sociodemographic and clinical variables, except body mass index (BMI), which was calculated using the measured height and weight before the autopsy exam.

2.5. Statistical analysis

Data were presented as mean and standard deviation or relative frequencies. Non-normally distributed variables were described with median and interquartile ranges. The participants were initially classified into APOE-$\varepsilon 4$ carriers and non-carriers according to the presence of at least one $\varepsilon 4$ allele. Then, additional analysis was conducted with the sample divided into three groups determined by the APOE genotypes (Group $\varepsilon 2$=$\varepsilon 2/\varepsilon 2$ and $\varepsilon 2/\varepsilon 3$; group $\varepsilon 3$=$\varepsilon 3/\varepsilon 3$; and group $\varepsilon 4$=$\varepsilon 3/\varepsilon 4$ and $\varepsilon 4/\varepsilon 4$). In this analysis, we excluded individuals with the $\varepsilon 2/\varepsilon 4$ genotype because of the potential opposing biological effects of the $\varepsilon 2$ and $\varepsilon 4$ alleles. We used the unpaired t-test or the Mann-Whitney U test for continuous variables with normal or non-normal distribution for the baseline two-group analyses. One-way ANOVA or the Kruskal-Wallis test was used for comparison among the APOE alleles

groups (ε2, ε3, and ε4). To compare the categorical variables across the groups, we used the Chi-square test or Fisher's exact test.

We first investigated the association between APOE-ε4 carriers (categorical variable) and IAD (continuous variable) using linear regression models with cluster-robust standard errors due to 12 artery measurements in the same individual. In addition, we considered as additional dependent variables the maximum stenosis value between the left and right sides of the middle, anterior, posterior, and internal carotid arteries, as well as the basilar artery stenosis index. We used the natural log transformation of the stenosis index values + 1 to deal with several measures equal to 0 [ln(Stenosis index + 1)] [16]. We adjusted all analyses for age at death, sex, race, education, diabetes, hypertension, dyslipidemia, heart disease, smoking, alcohol use, physical activity, and BMI. In sensitivity analyses, we examined if APOE-ε4 carrier status was related to the presence of stenosis defined by an obstruction equal to or greater than 50% in any of the 12 CW arteries using adjusted logistic regression models [12]. We also assessed if APOE-ε4 was associated with the number of intracranial stenoses (e.g.,≥ 50% of obstruction) by applying Poisson regression models adjusted for the set of confounders described above. Additionally, all analyses were repeated using APOE as an independent variable with three categories (ε2, ε3, and ε4). APOE ε3 allele was used as the reference category.

We also verified if the association between APOE and IAD could be modified by age, sex, and race by adding interaction terms between these sociodemographic variables and APOE-ε4 to regression models adjusted for the confounding factors. To investigate potential attrition bias in the relationship between APOE alleles and IAD, we stratified the sample by age groups using a cut-off equal to 70 years old. We adopted this approach to verify if those who survived to older age possibly represented the group of APOE-ε4 carriers with a less severe IAD.[17] We considered an alpha level of 5% in two-tailed tests. The analyses were performed using R-4.0.3.

3. Results

Among the 400 participants included in the analysis, 109 were APOE-ε4 carriers. The mean percentage of stenosis was 27±30% in 4,504 artery segments. There was no difference in age at death, education, sex, race distribution, cardiovascular risk factors (Table 1), or stenosis extension and severity in the CW arteries comparing the two groups (Fig. 2). Similar findings were present when we compared the participants divided into carriers of allele ε2 (n=42), ε3 (n=249), and ε4 (n=103) (Supplementary Table 1).

APOE-ε4 was not associated with stenosis in the overall CW measures nor in the middle, anterior, posterior, internal carotid, or basilar arteries (Table 2). APOE alleles ε2 and ε4 were also not associated with stenosis in the intracranial arteries (Supplementary Table 2).

In sensitivity analysis using intracranial stenosis greater than 50% as the outcome, APOE allele ε4 status was also not associated with IAD (Table 3). Furthermore, APOE alleles were not related to the number of brain arteries with arterial obstructions of 50% or greater

(Table 3). Age, sex, and race did not modify the associations between APOE-ε4 and IAD (Supplementary Table 3).

Among participants older than 70 years, the APOE-ε4 allele was borderline associated with a lower stenosis index in the middle cerebral artery (β=−0.55, 95% CI=−1.11;0.01, P=.06). On the other hand, we did not find any association among participants younger than 70 years old (Table 4).

4. Discussion

In this cross-sectional study with morphometric measures of atherosclerosis in the 12 intracranial arteries, we did not find an association between APOE polymorphisms and arterial stenosis. The APOE-ε4 allele was borderline associated with a lower stenosis index in the middle cerebral artery in participants older than 70 years, suggesting the possibility of attrition bias in older participants probably due to the deleterious effect of the APOE-ε4 allele on mortality risk [17,18].

Our findings of no association between APOE genotypes and IAD agree with previous studies that used imaging techniques to assess intracranial atherosclerosis [10,11]. Chutinet et al. determined the degree of stenosis in the middle cerebral artery, anterior cerebral, internal carotid, posterior cerebral, basilar, and vertebral arteries in 308 Thai adults aged >45 years using transcranial Doppler ultrasonography. IAD was defined as artery stenosis equal to or greater than 50% without significant extracranial stenosis. They did not observe an association between APOE and IAD in this sample [10]. Recently, similar results were found in the NOMAS, which comprises a multiethnic population with a large Hispanic representation (70% of the sample). To evaluate the association between genetic determinants of IAD, they included 1,109 participants with a mean age of 70±9 years old. Only 7% had IAD, defined as focal narrowing >50% in any of the main large cerebral arteries (i.e., the middle, anterior, posterior, vertebral, or basilar arteries) using magnetic resonance imaging. Similar to our results, no significant relationships were observed between APOE alleles and IAD [11].

On the other hand, a previous study reported an association of APOE with intracranial atherosclerosis, but only in men [19]. In this study, the authors included material from two Finnish cross-sectional population-based autopsy studies (n=1,004), where atherosclerosis was evaluated in nine CW branches. Instead of considering the percentage of the artery obstruction, the IAD was defined based on a semi-quantitatively score ranging from zero to three, in which three represented a stenosis degree >50% by macroscopic evaluation besides other criteria, such as the arterial plaque calcification degree. APOE-ε4 carriers tended to have higher intracranial atherosclerosis scores compared to non-carriers, but this association was significant only among men (mean age=54 years) [19]. The authors suggest that APOE-ε4 can have a gender-specific role in developing IAD. However, we did not find an interaction between APOE-ε4 and sex in our sample.

Although APOE alleles frequency differs across races and ethnicities [20,21], the race did not modify the relationship between APOE alleles and IAD in our admixed sample. APOE

has also been independently associated with all-cause mortality and reduced lifespan [18]. However, we did not find significant interactions of APOE with age.

The APOE-ε4 allele is associated with higher levels of LDL [5], which is an important risk factor in the early development of atherosclerosis [22]. In addition, evidence from a postmortem study showed that the initial stages of IAD were noted to begin during the fourth decade of life [23]. Recent findings showed that more severe and complicated lesions consisting of calcifications and plaque rupture are more frequent after the fifth decade, primarily in the basilar, vertebral, and internal carotid arteries [24,25]. Therefore, two hypotheses should be considered to explain the lack of association between APOE-ε4 and IAD in our sample and previous studies. The first is that the ε4 allele could mainly affect the initial stages of IAD, and the advanced lesions would be related to other mechanisms. Second, the inclusion of participants aged 50 or more in part can mitigate this association since APOE-ε4 is related to high mortality rates [18], and the APOE-ε4 participants who died before being included in this study could also have more severe IAD [17]. Supporting this hypothesis, we found that among participants older than 70 years, the presence of the ε4 allele was borderline associated with a reduction in the percentage of stenosis in the middle cerebral artery.

Our results need to be considered in light of some limitations. First, this is a cross-sectional study that is not suitable for inferring causality. Moreover, as discussed, the lack of association between APOE alleles and IAD could be affected by selection bias since we only observed a borderline negative association between those older than 70 years, which was in the opposite direction of our hypothesis. In addition, the low frequency of the APOE-ε2 (n=42) in our sample did not allow us to assess the isolated effect of this allele on IAD properly. We also did not perform histological analyses of the intracranial arteries due to the small artery diameter and the difficulty in processing most intracranial arteries. In addition, previous studies have shown that although stenoses are frequently found in the Circle of Willis, histological alterations, such as calcifications, are rare [26,27]. Furthermore, we did not measure blood lipids (e.g., LDL- and HDL-cholesterol) for comparison across the groups. However, we adjusted all analyses for important clinical information, including the presence of dyslipidemia. On the other hand, our study has strengths. We used direct anatomic measurements in all the extensions of the 12 CW arteries. In this way, we measured certain regions of the CW that are particularly difficult to evaluate using traditional imaging methods, such as the posterior cerebral artery, communicating arteries, distal arterial branches, and the cerebral section of the internal carotid artery [12]. In addition, previous imaging studies focused mainly on the association of APOE genotypes with carotid atherosclerosis, while scarce data are available on IAD.

5. Conclusions

APOE polymorphisms were not associated with stenosis in intracranial arteries evaluated using morphometric measurements in a large autopsy study with an admixed sample. We did not find evidence of effect modification of age, sex, or race on the association between APOE and IAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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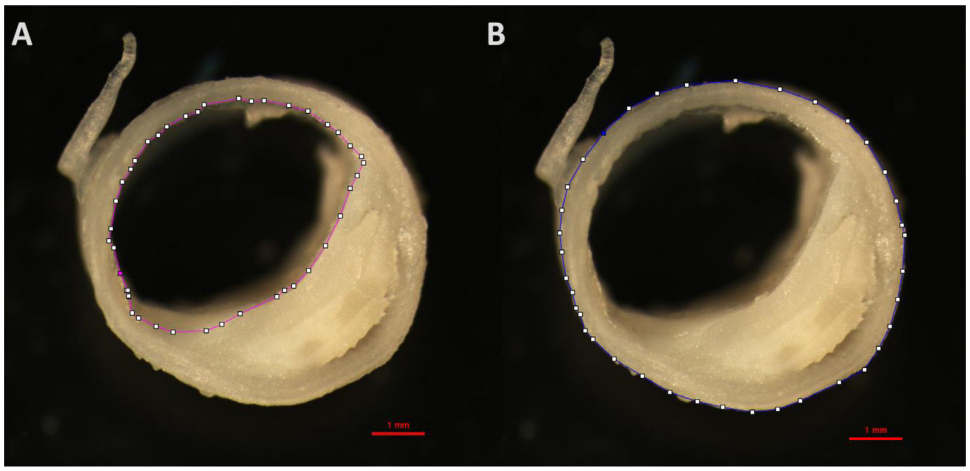

Fig. 1.

Sections of the largest arterial obstruction in the internal carotid artery used to calculate the stenosis index. A) Lumen area. B) Area delineated by the outer arterial wall of the vessel.

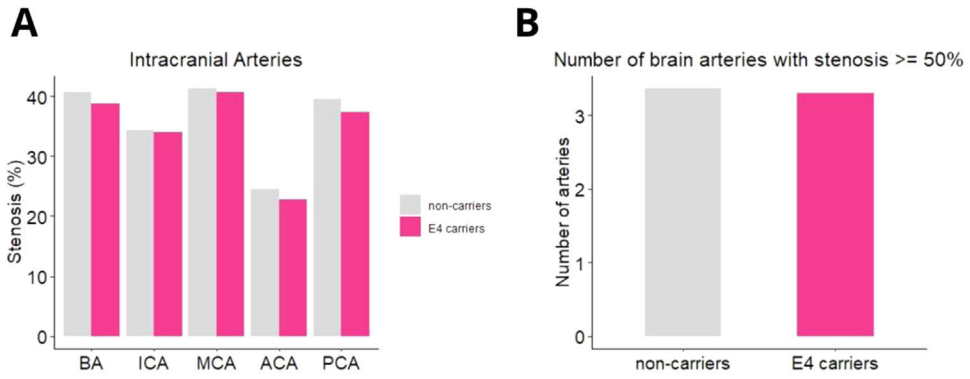

Fig. 2.

Extension and severity of the stenosis in the Circle of Willis (CW) arteries. A) Comparison of the mean stenosis index in each intracranial artery. BA: Basilar Artery, ICA: Internal Carotid Artery, MCA: Middle Cerebral Artery, ACA: Anterior Cerebral Artery, PCA: Posterior Cerebral Artery; and B) Comparison of the mean number of arteries with a stenosis index ≥50%. There was no significant difference between the groups in any of the comparisons.

Table 1

Comparison of baseline characteristics and major risk factors in apolipoprotein E allele ε4 carriers vs non-carriers.

Variable	ε4 carriers(n=109)	non-carriers(n=291)	*P*
Age (y), Mean ± SD[a]	72.5±12	73.4±12.5	.48
Education (y), Median (IQR)[b]	4 (2)	4 (4)	.45
Sex, n (%)[c]			.41
Female	52(47.7)	154(52.9)	
Race, n (%)[d]			.38
White	66(60.6)	192 (66.0)	
Black	42(38.5)	92 (31.6)	
Asian	1(0.92)	7(2.4)	
Hypertension, n (%)[c]	75(68.8)	200(68.7)	1.00
Diabetes, n (%)[c]	34(31.2)	104(35.7)	.46
Dyslipidemia, n (%)[c]	15(13.8)	27(9.3)	.26
Heart disease, n (%)[c]	31(28.4)	99(34.0)	.35
Stroke, n (%)[c]	17(15.6)	35(12.0)	.44
BMI (kg/m^2), Mean ± SD[a]	23.5±4.38	23.7±4.89	.66
Physical inactivity, n (%)[c]	66 (60.6)	164 (56.4)	.52
Smoking, n (%)[c]			.76
Never	53(48.6)	132(45.4)	
Current	27(24.8)	71(24.4)	
Former	29(26.6)	88(30.2)	
Alcohol drinking, n (%)[c]			.49
Never	65(59.6)	172(59.1)	
Current	29(26.6)	66(22.7)	
Former	15(13.8)	53(18.2)	

[a]Unpaired t-test.

[b] Mann-Whitney U test.

[c] Chi-square test.

[d] Fisher's exact test. SD: Standard deviation. IQR: Interquartile range.

Table 2

Association of apolipoprotein E allele ε4 carriers with stenosis in intracranial arteries (n = 400).

	Model 1		Model 2		Model 3	
	β (95% CI)	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Stenosis in CW arteries[a]	−0.88(−5.74; 3.99)	.72	−0.97(−5.86; 3.92)	.69	−0.96(−5.95; 4.03)	.71
Basilar artery[b]	−0.09(−0.47; 0.28)	.62	−0.09(−0.47; 0.28)	.63	−0.11(−0.49; 0.27)	.58
Posterior cerebral artery[b]	−0.21(−0.66; 0.24)	.35	−0.19(−0.65; 0.25)	.39	−0.17(−0.63; 0.28)	.45
Internal carotid artery[b]	−0.16(−0.59; 0.26)	.45	−0.17(0.60; 0.25)	.42	−0.18(−0.60; 0.25)	.42
Middle cerebral artery[b]	−0.08(−0.50; 0.34)	.70	−0.10(−0.52; 0.32)	.64	−0.13(−0.55; 0.29)	.55
Anterior cerebral artery[b]	−0.16(−0.60; 0.29)	.49	−0.16(−0.61; 0.28)	.47	−0.20(−0.66; 0.25)	.38

[a] Linear regression models with robust standard error estimators for clustered measurements in 12 arteries per individual (n = 4800); CW: Circle of the Willis.

[b] Linear regression models using the natural log transformation of the stenosis index. Model 1: univariate analysis. Model 2: adjusted for age, sex, race, and education. Model 3: adjusted for age, sex, race, education, diabetes, hypertension, dyslipidemia, heart disease, smoking, alcohol use, physical activity, and body mass index. Reference category: ε4 non-carriers.

Table 3

Association of apolipoprotein E allele ε4 carriers with stenosis (≥50%) in intracranial arteries (n = 400).

	Model 1		Model 2		Model 3	
	OR(95% CI)	***P***	**OR(95% CI)**	***P***	**OR(95% CI)**	***P***
Stenosis in CW arteries[a]	0.96(0.70; 1.31)	.78	0.95(0.69; 1.30)	.75	0.95(0.69; 1.30)	.74
Basilar artery[b]	0.95(0.61; 1.48)	.81	0.93(0.59; 1.47)	.77	0.94(0.58; 1.51)	.79
Posterior cerebral artery[b]	0.86(0.55; 1.33)	.49	0.86(0.54; 1.35)	.51	0.87(0.54; 1.40)	.56
Internal carotid artery[b]	1.16(0.74; 1.81)	.53	1.11(0.70; 1.75)	.66	1.11(0.69; 1.79)	.66
Middle cerebral artery[b]	0.92(0.59; 1.43)	.71	0.89(0.57; 1.41)	.63	0.86(0.53; 1.39)	.54
Anterior cerebral artery[b]	0.81(0.49; 1.34)	.41	0.81(0.48; 1.35)	.41	0.80(0.47; 1.35)	.40
Number of brain arteries with stenosis (≥50%)[c]	0.98(0.87; 1.10)	.77	0.98(0.96; 0.99)	.73	0.98(0.87; 1.11)	.73

OR: Odds ratio; CI: confidence interval.

Model 1: univariate analysis.

Model 2: adjusted for age, sex, race, and education.

Model 3: adjusted for age, sex, race, education, diabetes, hypertension, dyslipidemia, heart disease, smoking, alcohol use, physical activity, and body mass index.

Reference category: ε4 non-carriers.

[a] Logistic regression models with robust standard error estimators for clustered measurements in 12 arteries per individual (n = 4800).

[b] Logistic regression analysis.

[c] Incidence rate ratio obtained from Poisson regression analysis.

Table 4

Association of apolipoprotein E ε4 carriers with stenosis in intracranial arteries stratified by age (n = 400).

	Multivariate model		
	β	**95% CI**	***P***
Equal to or less than 70 years old (n=168)			
Stenosis in CW arteries[a]	−0.03	−7.63; 7.58	.99
Basilar artery[b]	−0.09	−0.69; 0.52	.78
Posterior cerebral artery[b]	0.06	−0.67; 0.80	.86
Internal carotid artery[b]	−0.09	−0.80; 0.61	.79
Middle cerebral artery[b]	0.38	−0.31; 1.08	.27
Anterior cerebral artery[b]	−0.13	−0.84; 0.59	.73
Greater than 70 years old (n=232)			
Stenosis in CW arteries[a]	−1.58	−8.59; 5.43	.66
Basilar artery[b]	−0.13	−0.65; 0.38	.61
Posterior cerebral artery[b]	−0.33	−0.95; 0.29	.30
Internal carotid artery[b]	−0.11	−0.68; 0.45	.69
Middle cerebral artery[b]	−0.55	−1.11; 0.01	.06
Anterior cerebral artery[b]	−0.31	−0.94; 0.32	.33

[a]Linear regression models with robust standard error estimators for clustered measurements in 12 arteries per individual (n = 4800); CW: Circle of the Willis.

[b]Linear regression models using the natural log transformation of the stenosis index. Model adjusted for age, sex, race, education, diabetes, hypertension, dyslipidemia, heart disease, smoking, alcohol use, physical activity, and body mass index. Reference category: ε4 non-carriers.