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Original Article

Association of *SERPINA9* gene variants with carotid artery atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study

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Abstract: The SNP rs11628722 in the *SERPINA9* gene was previously associated with incident ischemic stroke in the Atherosclerosis Risk in Communities (ARIC) study. Centerin, the protein encoded by *SERPINA9*, is involved in maturation and maintenance of naïve B cells, which play a role in atherogenesis. We investigated whether 21 tag SNPs in the *SERPINA9* gene are associated with features of carotid artery atherosclerotic plaque measured by magnetic resonance imaging (MRI). Carotid MRI data were obtained from 1,282 European Americans and 341 African Americans of the ARIC Carotid MRI study, which recruited participants from ARIC by a stratified sampling plan that over-sampled participants with carotid intima-media thickening. Five MRI measures, focused on carotid wall volume, wall thickness, and lipid core, were analyzed. Genetic associations between the MRI measurements and each of the 21 SNPs were analyzed in linear regression models with adjustment for sample weights and traditional risk factors. Rs11628722 was tested *a priori*. In African Americans, rs11628722 was significantly associated with carotid wall volume ($p < 0.05$). Among the other 20 SNPs, adjusted for multiple testing, rs4905204, which encodes an Ala to Val amino acid change, was significantly associated with maximum wall thickness ($p < 0.000625$) and suggestively associated with total wall volume ($p < 0.0026$) in European Americans. In conclusion, SNPs in the *SERPINA9* gene showed race-specific associations with characteristics of carotid atherosclerotic plaques. Replications in other populations are needed to validate findings of this study and to establish the *SERPINA9* gene as a candidate in the etiology of carotid atherosclerosis.

Keywords: SERPINA9 gene, carotid atherosclerosis, MRI, genetic association

Introduction

Vulnerable atherosclerotic plaques are plaques that have a high risk to rupture and consequently cause cardiovascular complications such as myocardial infarction or stroke [1]. Magnetic resonance imaging (MRI) of atherosclerosis allows direct visualization of diseased artery walls and characterization of plaque composition and morphology [1, 2], providing useful information for identifying vulnerable plaques. For example, in prospective studies, MRI identified the following plaque characteristics that predicted symptoms of cerebrovascular events: presence of a thin or ruptured fibrous cap, intraplaque hemorrhage, larger mean intraplaque hemorrhage area, larger maximum percentage lipid-rich/necrotic core, and larger maximum wall thickness [3, 4].

In the Atherosclerosis Risk in Communities (ARIC) study, the SNP rs11628722 in the *SERPINA9* gene, which results in an amino acid change from Val to Ala, was significantly associated with incident ischemic stroke in both African Americans and European Americans [5].

The hazard rate ratio of stroke associated with each copy of at-risk allele was 1.32 in European Americans and 1.27 in African Americans after adjusting for traditional risk factors ($p < 0.05$) [5]. The *SERPINA9* gene encodes a protein named SERPINA9, also known as centerin or GCET1 (germinal center (GC) B-cell-expressed transcript 1) [6, 7]. Centerin is involved in maturation and maintenance of naïve B cells [6]. Since B cells are involved in atherogenesis [8, 9], it is possible that the association of the *SERPINA9* gene variant and stroke is mediated via cerebrovascular atherosclerosis. In this study, we investigated the associations between *SERPINA9* gene variants, including rs11628722, and characteristics of carotid atherosclerotic plaques measured by MRI in 1,623 participants from the ARIC Carotid MRI study.

Materials and methods

Population

The ARIC study is a prospective investigation of atherosclerosis and its clinical events among 15,792 African American and European American adults aged 45 to 64 years at recruitment in 1987 through 1989, from Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland [10]. The Jackson sample comprised African Americans only; the other three samples represented the ethnic mix of their communities. The ARIC Carotid MRI study recruited 2,066 ARIC participants to undergo an examination in 2004-2005. A stratified sampling plan was used to over-sample participants with carotid intima-media thickening on previous ultrasound exams. The goal was to recruit 1,200 participants with thick carotid artery walls (> 85^{th} percentile intima-media thickness (IMT)) at their last ultrasound examination and 800 participants randomly sampled from the remainder of the carotid IMT distribution. The carotid IMT cutpoint was field center-specific based on the IMT distribution of that site. Participants who met the following criteria were excluded: 1) race groups other than African American or European American; 2) missing IMT measurements at their most recent examination in the 1990s; 3) standard contraindications to the MRI exam or to the contrast agent; and 4) carotid revascularization on either side for the low IMT group or on the side selected for imaging for the high IMT group. This study was approved by the institutional review board of corresponding centers and all participants provided informed consent.

Clinical examination and laboratory measurements

Questionnaires and in-person interviews were used to obtain information on medical history, prescription medication use, and lifestyle risk factors. Cigarette-smoking status was categorized as current, former, or never. Seated, resting blood pressure was measured three times with a random-zero sphygmomanometer and the mean of last two measurements was used. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertensive medications during the previous 2 weeks. Diabetes was defined as a fasting glucose level ≥ 126 mg/dL, a non-fasting glucose level ≥ 200 mg/dL, or a history of or treatment for diabetes. Body mass index (BMI; kg/m^2) was calculated using measured weight and height. Total and high-density lipoprotein (HDL) cholesterols were assayed using conventional techniques.

MRI protocol

Contrast enhanced MRI exams, guided by a standard protocol, were performed on a 1.5 T whole body scanner (Excite platform, GE Medical Systems, Symphony with Maestro upgrade, or Siemens Medical Solutions) equipped with a bilateral 4-element phased array carotid coil (Machnet, the Netherlands). A 3-dimensional time-of-flight MR angiogram (MRA) was acquired through both extracranial carotid bifurcations. Black blood MRI (BBMRI) images of the thickest 1.6 cm segment were then acquired through the carotid bifurcation known to have a thicker maximum wall by the most recent ultrasound study or the contralateral carotid bifurcation wall that appeared thicker on the MRA to the technologist, using a cardiac-gated, two-dimensional double inversion recovery fast spin echo sequence with the inversion time set to suppress the signal from flowing blood.

Sixteen transverse T1-weighted, fat-suppressing BBMRI slices (acquired resolution, 0.51 × 0.58 × 2 mm^3; total longitudinal coverage, 3.2 cm), centered at the thickest part of the internal or common carotid artery wall, were acquired 5 minutes after intravenous injection of

gadodiamide (Omniscan, GE Amersham), 0.1 mmol/kg body weight, with a power injector.

Image analysis

Eight readers were trained to interpret the MRI images using specialized software (VesselMASS, Leiden University Medical Center, Netherlands). The readers were blinded to the characteristics of the study population. All exams were assigned quality scores by the readers based on quality of image and protocol adherence, and exams that failed were not analyzed. Contours were drawn by each reader to delineate the lumen, outer wall, lipid core, and calcification. The fibrous cap contour was automatically generated to approximate the lumen and lipid core contours. Only eight slices centering around the thickest part of the plaque with matching precontrast and postcontrast images were analyzed.

Using custom analysis software, vessel walls were divided into 12 radial segments and mean minimum and maximum thickness values were generated for each segment. The fibrous cap was divided into radial segments at 15° increments, with mean minimum and maximum thicknesses generated for each segment. Area measurements were calculated for the lipid core and calcification contours. Volumetric data were computed by integrating area measurements over all slices examined.

To assess the reliability of MRI measures, 130 scans were re-read by the same or a different reader and 52 participants were re-scanned within two months. Reliability of lipid core and cap measurements was based on persons with lipid core: 40 repeat readings and 14 repeat scans. We included in this study four measures for carotid artery wall thickness and one measure for lipid core area that have acceptable consistency and reliability coefficients. The reliability coefficients for repeat readings and repeat scans of the measures are as follows: total wall volume (0.76, 0.79), maximum wall thickness (0.82, 0.77), and maximum lipid core area (0.72, 0.66) [11].

Genotyping

The SNP rs11628722 was genotyped by using PCR-based amplification of genomic DNA followed by an allele specific oligonucleotide ligation assay as previously described [5]. The other 20 SNPs in *SERPINA9* were tagSNPs supplemented by nonsynonymous SNPs with a minor allele frequency (MAF) > 0.05. The tagSNPs were selected based on genotype data from the HapMap European (CEU) and Yoruban (YRI) populations. The SNPs were selected in a race specific manner using Haploview's implementation of Tagger's multi-marker algorithm [12] at an $r^2 \geq 0.8$, LOD ≥ 2, and MAF ≥ 0.05. All tagSNPs selected for the CEU population were included in the SNP panel. TagSNPs that were not in blocks, or only tagged themselves in the YRI population were not included. A custom designed iSelect Infinium BeadChip with 7,600 bead types was used to generate the genotype data by Illumina's FastTrack Genoyping Services (San Diego, CA). SNPs that were monomorphic or had missing data > 20% were excluded. For *SERPINA9*, 23 SNPs were initially selected for genotyping and valid genotype data were obtained for 20 SNPs. For the 20 SNPs, an average concordance rate of 99.3% was achieved among 99-101 known replicate samples.

Statistical analysis

We excluded those not consenting to use of DNA (n=8), missing crucial MRI data (n=357), missing SNP data (n=50), or American Africans not from the Jackson center (n=28), resulting 1,623 for this study. Since the samples were collected by stratified random sampling, we used methods suitable for this sampling scheme in all the analyses. Specifically, we first calculated the sample fractions based on the pool actually screened for participation in the eight strata (4 communities by 2 IMT groups), then defined sample weights as the inverse of the sampling fractions, and finally used these weights to adjust the variance and confidence interval estimates of any quantity of interest. All the analyses using sample weights were carried out by SUDAAN (Research Triangle Park, NC).

For each SNP, the weighted genotype frequencies were estimated by SUDAAN CROSSTAB using sampling weights. Given the estimated genotype frequencies and the corresponding covariance matrix, Hardy-Weinberg (HW) equilibrium was tested using an asymptotic distribution of the tests statistics, which is estimated by the delta method. Departure from HW equilibrium was evaluated in both race groups but the results in African Americans should be interpreted with caution because population admixture in African Americans can result in

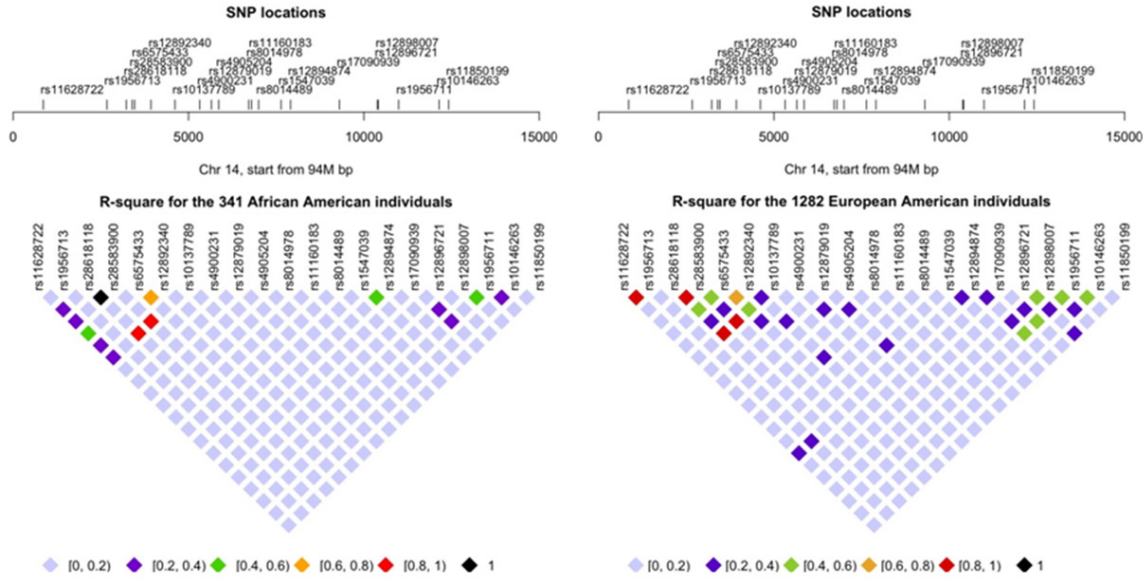

Figure 1. r^2 among the *SERPINA9* SNPs in African Americans and European Americans.

deviation of genotype frequencies from HW equilibrium [13, 14]. Significant threshold for HW test was based on $p < 0.002$, accounted for multiple testing by Bonferroni correction (ie, 21 SNPs).

All genetic association analyses were assessed by linear regression in African Americans and European Americans separately and weighted by sampling fractions in SUDAAN. The SNP genotypes were coded as categorical variables and all three genotypes for one SNP were analyzed with a 2 degree of freedom test. If there were less than 10 observations in one homozygote group, we combined it with the heterozygote group. Two sets of covariate adjustments, basic and multivariate, were performed. The basic adjustment included age, gender, and center as covariates. The center variable was not needed for analyses within African Americans because they were all from the Jackson center. The multivariate model additionally adjusted for total cholesterol, BMI, current and former smoking, diabetes, and hypertension.

The SNP rs11628722 was tested *a priori* given its previously reported association with stroke in the ARIC cohort. Therefore, a $p < 0.05$ was used to judge significant associations between this SNP and the carotid MRI measurements. For the other 20 SNPs, we examined the extent of linkage disequilibrium (LD) among them (**Figure 1**) to determine the need for correction for multiple comparisons. Because these SNPs were selected to be TagSNPs, the extent of LD is relatively low, with only 5 SNPs in African Americans and 7 in European Americans having $r^2 > 0.60$ (**Figure 1**). At phenotype level, the correlations were high among the four wall thickness measures (0.71-0.99 in both race groups) and moderate between the wall thickness measures and maximum lipid core area (0.54-0.86 in both race groups). Therefore, the five measures were counted as two independent tests. Using a Bonferroni correction for the number of hypothesis tests, for the 20 SNPs we used a $p < 0.000625$ (ie, 0.05/(20*2*2)) as the criterion for significant associations and a $p < 0.0026$ (ie, 0.2/(20*2*2)) for suggestive associations. In addition, genetic associations were not tested for maximum lipid core area in African Americans due to limited participants with presence of lipid core ($n < 100$).

In a sensitivity analysis in a subset of 300 African Americans for whom percentage of African ancestry was estimated in an ancillary study [15], we repeated the analyses for significant associations with additional adjustment for the percentage of African ancestry.

Results

Table 1 presents descriptive statistics of the ARIC Carotid MRI Study participants stratified by race and gender. **Table 2** presents measures for carotid artery wall thickness and lipid core area. Statistically significant race-gender differ-

Table 1. Characteristics of ARIC Carotid MRI participants: mean (SE) or percentages

Characteristics	African American Female (N=198)	African American Male (N=143)	European American Female (N=626)	European American Male (N=656)
Age, years	69.5 (0.44)	68.8 (0.51)	70.2 (0.26)	71.0 (0.29)
LDL Cholesterol, mg/dL	128.2 (3.43)	120.5 (2.99)	118.9 (1.62)	105.4 (1.85)
HDL Cholesterol, mg/dL	55.4 (1.35)	45.1 (1.03)	55.2 (0.79)	43.7 (0.52)
Total Cholesterol, mg/dL	208.5 (3.87)	190.9 (3.41)	204.5 (1.88)	178.9 (2.11)
BMI, kg/m^2	30.1 (0.41)	28.5 (0.41)	28.1 (0.26)	28.1 (0.20)
*Diabetes, %	30.3	39.1	17.7	22.0
Current Smoker, %	6.9	14.2	7.4	6.9
Former Smoker, %	28.3	43.3	33.2	58.6
**Hypertensive, %	78.4	68.9	61.5	57.0

*Diabetes defined as fasting glucose ≥ 126 mg/dL, nonfasting glucose ≥ 200 mg/dL, self-reported physician diagnosis of diabetes, or treatment for diabetes; **Hypertension was defined based on systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or treatment for hypertension.

Table 2. Carotid artery wall thickness and lipid core measures: sample size and mean (SE)

	African American		African American		European American		European American		*P*-value*
Carotid MRI Measures	N	Female	N	Male	N	Female	N	Male	
Carotid Artery Wall Thickness Measures									
Total Wall Volume (mm^3)	198	0.349 (0.010)	143	0.419 (0.013)	626	0.375 (0.007)	655	0.480 (0.008)	< 0.0001
Total Wall Volume adjusted (mm^3)	198	0.361 (0.009)	143	0.435 (0.013)	626	0.387 (0.007)	655	0.495 (0.009)	< 0.0001
Maximum Wall Thick (mm)	198	1.741 (0.071)	143	1.945 (0.088)	626	1.945 (0.043)	653	2.426 (0.063)	< 0.0001
Maximum Wall Thickness (mm) (segment)	198	1.803 (0.082)	143	1.977 (0.090)	626	1.981 (0.045)	655	2.503 (0.065)	< 0.0001
Maximum Lipid Core Area (mm^2)**,†	46	0.077 (0.012)	40	0.084 (0.011)	185	0.090 (0.008)	272	0.131 (0.011)	0.0022

*p-value for test of differences in means across the four groups; **Includes only participants with lipid core present; †Maximum lipid core area at slice with the largest lipid core area.

ences were observed for all four carotid artery wall thickness measures and maximum lipid core area.

Information on the 21 *SERPINA9* SNPs are presented in **Table 3**. According to the p < 0.002 criterion, genotype distribution for rs4905204 in European Americans was not in accordance with HW equilibrium expectations. Among the 21 SNPs, the following code for amino acid changes in the *SERPINA9* gene: rs11628722, rs12879019, rs28583900, rs28618118, and rs4905204. Allele frequency for rs11628722, the prioritized SNP, was disparate between European Americans and African Americans with opposite minor alleles between the two groups. This discrepancy is also observed between samples of European and African ancestries in the HapMap data (CEU: 0.85, YRI: 0.35).

Among the five carotid MRI measures, rs11628722 was associated with total wall volume in African Americans (**Table 4**). The G allele of rs11628722 appeared to have a recessive effect in that G homozygotes had significantly greater total wall volume than AA and AG groups (**Table 4**). There were no significant associations between rs11628722 and the carotid MRI measures in European Americans (p > 0.05, data not shown).

Among the other 20 *SERPINA9* SNPs, according to the criteria for significant and suggestive associations after correcting for multiple testing, rs4905204 in European Americans was significantly associated with two maximum wall thickness measures in multivariate adjustment and suggestively associated with total wall volume (adjusted) in the multivariate adjustment model (**Table 5**). The minor allele homozygote AA appeared to have a negative association with the wall thickness and volume measures compared to AG and GG groups (**Table 5**). In African Americans, this SNP was rarer and there were no AA homozygotes, thus preventing replication of the associations in African Americans. There were no other associations

Table 3. Marker summary statistics for *SERPINA9* SNPs

		African American			European American		
Marker	Function	A1/A2	MAF*	HWE_p	A1/A2	MAF*	HWE_p
rs11628722	cns	G/A	0.40	0.72	A/G	0.17	0.14
rs10137789	intron	T/C	0.15	0.03	T/C	0.28	0.61
rs10146263	intron	A/G	0.09	0.83	A/G	0.13	0.08
rs11160183	intron	A/G	0.09	0.34	A/G	0.21	0.09
rs11850199	mrna-utr	A/C	0.30	0.86	A/C	0.27	0.25
rs12879019	cns	C/G	0.03	0.03	C/G	0.20	0.17
rs12892340	intron	A/G	0.18	0.40	A/G	0.40	0.07
rs12894874	intron	A/G	0.10	0.02	A/G	0.25	0.95
rs12896721	intron	T/C	0.10	0.19	T/C	0.44	0.62
rs12898007	intron	T/C	0.34	0.77	T/C	0.37	0.95
rs1547039	intron	G/T	0.14	0.0001	G/T	0.32	0.09
rs17090939	intron	A/G	0.08	0.81	A/G	0.12	0.37
rs1956711	intron	T/C	0.21	0.53	T/C	0.26	0.98
rs1956713	intron	C/T	0.11	0.86	C/T	0.15	0.28
rs28583900	cns	T/A	0.16	0.03	T/A	0.31	0.10
rs28618118	cns	A/C	0.16	0.03	A/C	0.31	0.12
rs4900231	cs	A/G	0.03	0.04	A/G	0.20	0.18
rs4905204	cns	A/G	0.02	0.16	A/G	0.08	0.0004
rs6575433	cs	T/C	0.23	0.63	C/T	0.48	0.22
rs8014489	intron	A/G	0.05	0.01	A/G	0.12	0.95
rs8014978	intron	C/T	0.47	0.68	C/T	0.44	0.11

*Allele frequencies were calculated using weighted counts; A1=minor allele, A2=major allele, MAF=minor allele frequency, HWE=Hardy-Weinberg equilibrium test, cns=coding-nonsynonymous, mrna-utr=within an exon, but not translated, cs=coding-synonymous.

Table 4. Association between rs11628722 and total wall volume (mm^3) in African Americans

	rs11628722						
	AA		AG		GG		
Model	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	*p*-value*
Basic Adjustments**	116	0.381 (0.0123)	152	0.363 (0.0103)	55	0.426 (0.0295)	0.099
Multivariate Adjustments†	111	0.384 (0.0122)	150	0.361 (0.0098)	50	0.435 (0.0315)	0.041

**p*-value for test of differences in means across the three genotype groups; **Adjusted for age and gender; †Additionally adjusted for total cholesterol, BMI, current and former smoking, diabetes, and hypertension.

between these 20 SNPs and MRI measures that were at least suggestive at p < 0.0026 in either race group (data not shown).

In the sensitivity analysis of the 300 African Americans for whom percentage of African ancestry was available, additional adjustment for the percentage of African ancestry did not materially change the significant association for rs11628722 (data not shown).

Discussion

This cross-sectional study, conducted to follow-up previously reported associations between *SERPINA9* rs11628722 and stroke in the ARIC Study, investigated the association between MRI-measured characteristics of carotid atherosclerosis and 21 *SERPINA9* SNPs in the ARIC Carotid MRI study. In African Americans, the G allele of rs11628722 that was associated with increased risk of stroke, was significantly associated with increased total wall volume (p < 0.05). This SNP was not significantly associated with any measures of carotid artery atherosclerotic plaques in European Americans. Among the other 20 SNPs for which adjustment for multiple testing was considered, the SNP rs4905204, which encodes a Ala to Val amino acid change, was significantly associated with maximum wall thickness measures (p <

Table 5. Associations between rs4905204 and carotid wall measures in European Americans

	rs4905204							
	AA		AG		GG			
Carotid Wall Measures	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	*p*-value*	*p*-value**
Maximum Segmental Wall Thickness (mm)	33	1.616 (0.1464)	135	2.316 (0.1418)	1083	2.154 (0.0377)	0.0009	0.0001
Maximum Wall Thickness (mm) (segment)	33	1.671 (0.1711)	135	2.383 (0.1445)	1085	2.207 (0.0392)	0.0044	0.0003
Total Wall Volume (mm^3)	33	0.361 (0.0238)	135	0.428 (0.0170)	1085	0.423 (0.0056)	0.0379	0.0031
Total Wall Volume adjusted (mm^3)	33	0.367 (0.0237)	135	0.446 (0.0168)	1085	0.436 (0.0057)	0.0154	0.0015

*p-value for test of differences in means across the three genotype groups after adjusting for age, gender, and field center; **p-value for test of differences in means across the three genotype groups after additionally adjusting for total cholesterol, BMI, current and former smoking, diabetes, and hypertension.

0.000625) and suggestively associated with total wall volume measures ($p < 0.0026$) in European Americans.

SERPINA9 is a member of a large serpins (serine protease inhibitors) family consisting of at least 16 clade subfamilies with divergent functions [16]. SERPINA9 belongs to the clade A subfamily and is located on chromosome 14q32 [17]. Two other members of the clade A, SERPINA8 (angiotensinogen) and SERPINA10 (protein Z-dependent protease inhibitor), have been associated with hypertension and venous thromboembolic disease, respectively [7]. Northern blot and gene expression analyses of human tissues showed that SERPINA9 was detected in immune system including lymph nodes [6] (www.genecards.org). In lymph nodes, transcription of centerin was highly expressed in GC B-cells and was up-regulated by CD40 signaling, which is fundamental for GC formation [6]. Therefore, it was speculated that centerin plays an important role in maturation and maintenance of naïve B cells [6]. Studies of transgenic mice given splenectomy or bone marrow transplantation showed that the involvement of B cells plays a protective role during atherogenesis [8, 9]. A recent report in the ARIC Carotid MRI study showed that blood cell activation is associated with the morphology of carotid atherosclerotic plaques [18]. While the mechanisms underlying the association between the *SERPINA9* gene variants and carotid artery atherosclerosis are unknown, it is possible that the association is mediated via the B cell pathway.

The G allele of rs11628722 was previously associated with increased risk of stroke in ARIC [5]. We observed a positive association of the GG homozygosity with total wall volume. This finding suggests that this allele might act through increased plaque burden to increase the risk of stroke, as larger maximum wall thickness, an index of plaque burden, similarly predicts symptoms of cerebrovascular events [3]. We searched the database of published genome-wide association studies (www.gwascentral.org) for the two associated SNPs (rs11628722 and rs4905204) with regard to their associations with atherosclerosis- or inflammation-related phenotypes. We found that rs11628722 was nominally associated with asthma in one study (p=0.015) [19] and rs4905204 associated with insulin resistance traits in another study (p=0.03-0.04) [20].

Strengths and limitations

The ARIC Carotid MRI study represents a large, population-based study to examine carotid atherosclerotic plaques by contrast-enhanced MRI. To the best of our knowledge, there have not been other reports of population-based carotid MRI studies of comparable size. On the other hand, the uniqueness of the data raised difficulty in finding replication studies to confirm our findings. Departure from HW equilibrium was observed for rs4905204, the SNP that showed significant associations with maximum wall thickness measures in European Americans. Several factors could cause this deviation, including genotyping errors, inbreeding, population stratification, selection, assortative mating, or chance. Among them, genotyping errors and population stratification and admixture would be the most important to the validity of our study. Genotyping errors are not likely, because strict quality control protocols were implemented for the genotyping. For rs4905204, the concordance rate of genotypes between 101 duplicate pairs was 100%. Population stratification is of less concern in European Americans than in African Americans; the analysis in European Americans was adjusted for field center, which should reduce the

influence of population stratification on the observed associations. Also, over-sampling participants with thick carotid artery walls in this study might contribute to the HW disequilibrium because thick carotid artery wall is associated with atherosclerosis; it has been shown that a HW test will result in higher false positive findings of population admixture when HW test is carried out in cases and controls together and the test variant is in LD with the disease locus [14]. Another limitation is that different SNP- atherosclerosis associations were observed for African Americans and European Americans. Differences in genetic characteristics between the two populations, such as allele frequency and magnitude of linkage disequilibrium, might contribute to the differences. For example, the minor allele homozygote of rs4905204 that was mostly responsible for the association between this variant and the wall thickness and volume measures in European Americans was not present in African Americans, making it difficult to replicate this association in African Americans. More importantly, the size of African American sample in our study was limited, reducing statistical power and increasing the possibility of chance finding in this sample. Therefore, the results in African Americans should be interpreted with more caution.

In summary, we found that the rs11628722 of *SERPINA9* previously associated with stroke in the ARIC study was associated with carotid wall atherosclerotic volume in African Americans. The protein-coding variant rs4905204 was associated with wall thickness and volume measures in European Americans. Nevertheless, results from this study need to be replicated in other populations in order to establish the *SERPINA9* gene as a candidate in the genetic etiology of carotid atherosclerosis.

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Disclosure of conflict of interest

The authors have no conflicts of interest to disclose.

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