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No Association of 9p21 with Arterial Elasticity and Retinal Microvascular Findings

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Abstract

Objective—How 9p21 variation affects risk of cardiovascular disease is unclear, so we assessed whether 9p21 variants are associated with arterial elasticity or retinal microvascular findings.

Methods—In the prospective Multi-Ethnic Study of Atherosclerosis (MESA) we assessed 378 SNPs in the 9p21 locus. Within four ethnic groups, we used an additive genetic model to relate the 9p21 SNPs to five vascular phenotypes: small and large elasticity derived from radial diastolic pulse contour analysis; Young's elastic modulus from carotid artery ultrasound measurements; and the diameter of the central retinal arteries and veins.

Results—In neither ethnic-specific nor pooled data was there any statistically significant association between any of the 9p21 SNPs and any of the five vascular phenotypes.

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Conclusion—Our study does not support an association of 9p21 variation with arterial elasticity or retinal microvascular abnormalities.

Keywords

Prospective study; 9p21 SNP; retinal microvascular abnormalities; arterial elasticity

1. Introduction

Common genetic variants on 9p21 are consistently associated with risk of coronary heart disease (CHD), coronary artery calcium, and certain other cardiovascular diseases (CVD), including abdominal and intracranial aneurysms and ischemic strokes [1, 2]. The 9p21 variants associated with CHD are in a gene desert. Although genes and enhancer regions in or near this gene desert have been implicated as causative, for example *ANRIL*, the mechanisms by which genetic variants in 9p21 alter the risk of CHD are largely unknown [1, 3]. The CHD mechanisms are believed to involve atherosclerosis [4], but do not seem to involve elevated classical cardiovascular risk factors or increased carotid intima-media thickness [1]. Reduced expression of *CDKN2B*, leading to an increased smooth muscle cell apoptosis, may be a mechanism underlying aneurysmal disease [5].

We hypothesized that variation in 9p21 may influence certain functional or structural markers of the arterial system, such as arterial elasticity or microvascular abnormalities observable in the retina, which are themselves associated with increased risk of subsequent CVD [6–12]. Yet, very few studies have examined in detail the relation of arterial elasticity or retinal microvascular findings with 9p21 variants in apparently healthy adults. A small clinical study of 821 hypertensives reported no association of 384 SNPs on 9p with carotid-femoral pulse wave velocity [13]. Another small clinical study of 400 adults, 70–88 years old, found in men, but not women, a significant inverse – rather than positive – association of aortic stiffness with two CVD relevant single nucleotide polymorphisms (SNPs) in 9p21 (rs10757274 and rs2891168) [14]. A genome wide association study and a genome-wide linkage study of retinal microvascular changes did not report 9p21 variants related to retinal vascular diameters [15, 16].

We therefore examined whether there is any association of 9p21 variants with arterial elasticity and retinal arteriolar or venular findings in the Multi-Ethnic Study of Atherosclerosis (MESA).

2. Methods

The MESA study, described in detail previously [17], involves a cohort of 6814 adults, aged 45 to 84 years, of Caucasian, Hispanic, African American or Chinese ancestry, recruited in 2000–2002. MESA performed multiple examinations of the cohort and follow-up for CVD events.

We examined five subclinical vascular phenotypes, whose standardized methods in MESA have been published: (a) small and large elasticity derived from radial diastolic pulse contour analysis [6, 7, 18]; (b) Young's elastic modulus from the carotid artery ultrasound measurements [19], and (c) central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE), which are markers of the diameters of the retinal microvasculature [20, 21]. Lower elasticity values and higher Young's modulus values indicate greater arterial stiffness. Smaller CRAE values and larger CRVE values reflect a more adverse retinal microvasculature [22].

MESA obtained consent for DNA extraction at the time of baseline enrollment, and consent for genome-wide studies at subsequent examinations. MESA typed SNPs in the 9p21 locus using two methods. The gene-centric ITMAT-Broad-CARe (IBC) array [23] directly assessed 80 SNPs in 9p21 (rs3731255 to rs1022243, spanning 191kb). The MESA SHARe project performed a genome-wide association scan using the Affymetric Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California, USA), consisting of approximately 1 million SNPs, with imputation HapMapII to achieve 2.5 million SNPs using IMPUTE v.2.1.0 software [24]. From the Affymetrix 6.0 genotyping, 67 SNPs in the 9p21 locus were directly genotyped and 231 SNPs were imputed (rs7041637 to rs10811667, spanning 198kb). Together, there were 378 SNPs, including 327 with minor allele frequency (MAF) greater than 5% in at least one ethnic group, in the 9p21 locus spanning 198 kb (rs7041637 to rs10811667).

The association of each 9p21 SNP with each of the five phenotypes was investigated assuming an additive genetic model, within each of MESA's four ethnic groups and by overall meta-analysis when there was no heterogeneity of SNP associations by ethnicity. The beta for the linear regression of each continuous phenotype per copy of each risk allele was calculated along with a confidence interval and p-value. We mainly focused on three specific SNPs, based on the work of Silander et al [25]. They investigated genetic diversity in the 9p21 region in 51 worldwide populations and determined that there is a common haplotype block in the CHD-associated region. They concluded that three SNPs— rs4977574, rs2891168, and rs10757278— each tag the haplotype, are highly correlated with each other in all four HapMap populations, and are the most appropriate for further analyses in non-Europeans. Of these three SNPs, we had genotyped the first two and imputed the third. Because LD was 1.0 between rs4977574 and rs2891168 and 0.89 between rs4977574 and rs10757278, and associations with the phenotypes were similar for three SNPS, we present results only for rs4977574 in Table 1, with data for the other two SNPs provided as Supplemental Tables. Given the number of SNPs (n = 327) and phenotypes (n = 5) tested, a most likely conservative, Bonferroni-corrected p-value of 3.1×10^{-4} was required to declare statistical significance.

3. Results

Among the four race-ethnic groups in MESA, the mean age ranged from 61.4 to 62.7 years and the percentage of male participants ranged from 46 to 50%. Mean ± SD values were 4.5 ± 2.8 mL/mmHg × 100 for small artery elasticity, 13.3 ± 5.5 mL/mmHg × 10 for small artery elasticity, 1296 ± 645 mmHg/mm for Young's elastic modulus, 144 ± 14 μM for CRAE, and 214 ± 22 μM for CRVE. The Spearman correlation among the five studied phenotypes was <0.20, except for the correlations between small and large artery elasticity (r= 0.40 to 0.46 within the four ethnic groups), between large artery elasticity and Young's elastic modulus (r= −0.18 to −0.28), and between CRAE and CRVE (r= 0.45 to 0.56). This suggests that the retinal microvascular, large artery elasticity, and small artery elasticity measures are three relatively independent entities.

Our hypotheses were that the 9p21 risk alleles would be associated with more adverse retinal microvascular and arterial elasticity phenotypes, in other words, associated inversely with CRAE and with small and large artery elasticity, and associated positively with Young's elastic modulus and CRVE unadjusted for CRAE. Yet, in neither ethnic-specific nor the pooled data was there any statistically significant association at 3.1×10^{-4} between any of the 327 9p21 SNPs and any of the five subclinical CVD phenotypes.

As Table 1 shows, the G risk allele for rs4977574, the primary tag SNP for the 9p21 CHD common haplotype, was half as common in African Americans than the other three ethnic

groups. Table 1 further demonstrates by ethnic group the lack of association of each phenotype with the tag SNP. Although rs4977574 had nonsignificant p-values between 0.01 and 0.05 for CRAE in whites and African Americans, the beta coefficients for the associations were in opposite directions for these two ethnic groups. In general, the directions of the beta coefficients for any phenotype were not consistent across ethnic groups. Similar findings were seen for the other two 9p21 tag SNPs (Supplemental Tables 1 and 2). Results were unchanged, remaining essentially null, with further adjustment for other cardiovascular risk factors (data not shown0.

4. Discussion

Although the 9p21 locus is consistently related to several cardiovascular outcomes (e.g., CHD and aneurysms), 9p21 variation does not seem to relate to classical risk factors or carotid intima-media thickness [1, 3]. Our novel results extend prior evidence by showing that 9p21 is also not significantly associated with small or large artery elasticity or retinal microvascular diameters, which are functional measures of pre-clinical vascular changes predictive of clinical CVD [6–12]. Thus, these phenotypes do not provide a mechanistic link between 9p21 variation and CVD. Our findings for radial and carotid artery elasticity seem to parallel a small study that reported 9p21 variants were not associated with carotid-femoral pulse wave velocity [13] and another small study that found 9p21 variants were not associated with aortic stiffness in women [14]. This latter study, in contrast, also reported that in elderly men 9p21 SNPs were significantly associated with reduced aortic stiffness [14]. Why a 9p21 association would exist for men but not women seems unclear. Reasons for the contrasting findings among studies may relate to differences in age, prevalences of CVD, or other confounding factors.

In a null study, it is important to consider what was the minimum effect size that we could detect given the MESA sample size. Minimum detectable effect sizes were evaluated using Quanto (http://hydra.usc.edu/gxe/). Calculations were performed for rs4977574, which was associated with multiple CVD phenotypes in populations of European ancestry [25]. With a continuous outcome, additive genetic model, two-sided alpha=0.00025, and race/ethnicity-specific allele frequencies, there was 80% power to detect an association if the genetic variant accounts for 1.1% in whites, 1.7% in blacks, 1.9% in Hispanics, 3.6% in Chinese, and 0.5% in race/ethnicity-combined analyses. Thus, our study was able to rule out fairly small effects of 9p21 on these phenotypes, particularly in whites.

One drawback of our study is the single static measurement of the five vascular phenotypes, which may not be sufficient to characterize individuals' vascular status reliably. Yet, single measures have been reliable enough to associate the phenotypes prospectively with clinical CVD [6–12]. Retinal microvascular findings have not been conclusively linked to coronary atherosclerosis or to recurrent CHD events in persons with pre-existing CHD, and retinal microvascular abnormalities therefore may be a weak surrogate for CHD. Yet, retinal microvascular findings have been associated with reduced coronary perfusion reserve [26] and with CHD mortality in the general population [27].

In conclusion, our study does not support an association of 9p21 variation with arterial elasticity or retinal microvascular abnormalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Linear regression of arterial elasticity and retinal vascular phenotypes on 9p21 tag SNP (rs4977574), MESA

	White (*n*=2343)	African American (*n*=1501)	Hispanic (*n*=1344)	Chinese (*n*=695)
Risk Allele (G) Frequency	0.51	0.19	0.42	0.46
Small Artery Elasticity (mL/mmHg × 100)				
Beta *	0.00	−0.09	0.00	0.15
95% CI	(−0.15, 0.15)	(−0.30, 0.12)	(−0.18, 0.19)	(−0.10, 0.40)
P value	0.98	0.40	0.95	0.25
Large Artery Elasticity (mL/mmHg × 10)				
Beta	0.06	−0.23	−0.12	0.37
95% CI	(−0.28, 0.35)	(−0.71, 0.25)	(−0.46, 0.22)	(−0.12, 0.87)
P value	0.67	0.35	0.48	0.14
Young's Modulus (mmHg/mm)				
Beta	4.0	16.2	35.9	−50.6
95% CI	(−23.9, 31.9)	(−50.1, 82.4)	(−16.7, 88.5)	(−118, 16.7)
P value	0.78	0.63	0.18	0.14
Retinal Arteriole Equivalent (μM)				
Beta	0.89	−1.61	−0.12	0.57
95% CI	(0.07, 1.71)	(−2.94, −0.27)	(−1.22, 0.98)	(−0.96, 2.09)
P value	0.03	0.02	0.83	0.47
Central Retinal Venule Equivalent (μM)				
Beta	0.49	−1.94	−0.55	−1.16
95% CI	(−0.73, 1.71)	(−4.02, 0.13)	(−2.24, 1.14)	(−3.33, 1.01)
P value	0.43	0.07	0.52	0.30

* Each beta was derived from a race-specific linear regression model for a single vascular phenotype and represents the estimated mean difference in the phenotype per G risk allele vs. the comparison A allele.