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Effect of 9p21 genetic variation on coronary heart disease is not modified by other risk markers. The Atherosclerosis Risk in Communities (ARIC) Study

Aaron R. Folsom, MD[a], **Vijay Nambi, MD, PhD**[b], **James S. Pankow, PhD**[a], **Weihong Tang, MD, PhD**[a], **Kian Farbakhsh, MS**[a], **Kazumasa Yamagishi, MD, PhD**[c], and **Eric Boerwinkle, PhD**[d]

[a] Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, 1300 South 2nd Street, Suite 300, Minneapolis, MN 55454, USA

[b] Division of Atherosclerosis and Vascular Medicine, Department of Medicine, Baylor College of Medicine, and the Center for Cardiovascular Prevention, Methodist DeBakey Heart and Vascular Center, 6565 Fannin St., Suite STE B 160, Room MS-A601, Houston, TX 77030, USA

[c] Department of Public Health Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8575, Japan

[d]Human Genetics Center, University of Texas Health Science Center, School of Public Health, 1200 Herman Pressler Drive, Houston, TX 77030, USA

Abstract

Objective—To determine whether the 9p21 SNP association with coronary heart disease is modified by other classical or novel risk markers.

Methods—The 9p21 SNP (rs10757274) and multiple risk markers were measured in the Atherosclerosis Risk in Communities Study, and incident coronary disease events were ascertained. Effect modification (interaction) of the 9p21 SNP with risk markers was tested in Cox proportional hazard regression models.

Results—The incidence rates of coronary heart disease per 1000 person-years were 14.4, 17.0, and 18.7 for AA, AG, and GG genotypes, yielding hazard ratios of 1.0, 1.20 (95% CI = 1.07-1.36), and 1.34 (95% CI = 1.16-1.53). There was no meaningful evidence of an interaction (all p-interaction > 0.04) between 9p21 SNP and any of 14 other risk markers for coronary heart disease. These included novel markers not previously explored for 9p21 interaction (e.g., cardiac troponin T and N-terminal pro-brain natriuretic peptide).

Conclusion—Our study extends evidence that the 9p21 SNP association with coronary heart disease is not modified by classical or novel risk markers. Our findings therefore rule out additional plausible pathways by which 9p21 might have increased coronary heart disease risk.

Address for correspondence: Aaron R. Folsom, MD, Division of Epidemiology & Community Health, School of Public Health, University of Minnesota, 1300 South 2nd Street, Suite 300, Minneapolis, MN 55454 USA. Phone: + 1 612-626-8862; Fax: +1 612-624-0315; folso001@umn.edu.

Disclosures None.

Keywords

coronary disease; prospective study; 9p21 SNP

1. Introduction

Recent genome-wide association studies have consistently documented that genetic variation in chromosome 9p21 influences risk of coronary heart disease (CHD) [1-6] and other arterial disorders such as aneurysms [7] and peripheral artery disease [8]. Mechanisms for 9p21 associations with atherosclerotic disease are unclear, but 9p21 variants influence expression of *ANRIL*, which has a role in regulating expression of *CDKN2A* and *CDKN2B*, which may influence vascular integrity and atherogenesis [9,10]. The association of 9p21 variation with CHD is not explained by classical CHD risk factors (e.g., lipids, hypertension, diabetes), which are uncorrelated with 9p21 variation [1,9].

Both genes and "environment" contribute to CHD. Yet, studies have not consistently demonstrated interactions ("effect modification") of common gene variants with risk factors. Single research studies have suggested that 9p21 single nucleotide polymorphisms (SNPs) might interact with age [11,12]; fruit and vegetable intake [13]; poor glycemic control [14]; abdominal obesity [15]; alcohol, smoking, and hypertension [16]. However, these single study findings have not been replicated, and other studies reported no interactions between 9p21 SNPs and CHD risk factors [17-20].

We used data on whites in the prospective, population-based Atherosclerosis Risk in Communities (ARIC) Study cohort to examine in one of the largest cohorts to date whether interactions exist between a 9p21 SNP (rs10757274) and other risk markers in relation to CHD incidence. In other words, we sought to see whether or not the 9p21 SNP association with CHD is modified by other risk markers. Our battery of potential interacting factors includes several "novel" plasma biomarkers not previously examined for 9p21 interactions.

2. Methods

2.1. Study population

The ARIC study cohort comprised a sample of 15,792 individuals aged 45-64 years old at baseline when recruited between 1987-1989 from four communities: Forsyth County, North Carolina; Jackson, Mississippi (African Americans only); Washington County, Maryland; and the northwestern suburbs of Minneapolis, Minnesota [21]. Three subsequent examinations were also conducted between 1990 and 1998, with a fifth examination in 2011-2013. Participants also were telephoned annually to help ascertain events, and the telephone response rate was over 96% in earlier follow-up and remained over 90% through 2008. The relevant human subjects review boards approved ARIC, and all participants gave written informed consent.

2.2. Baseline assessments

Many of the risk markers tested for interaction with 9p21 variation were measured at the ARIC baseline examination in 1987-89, but certain biomarkers were obtained at later visits. ARIC used standardized methods to assess baseline risk factors [21]. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or taking antihypertensive medication. Diabetes was defined as a fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, a physician diagnosis of diabetes, or pharmacologic treatment for diabetes. Obesity was defined as BMI ≥30 kg/m^2. Hypercholesterolemia was

defined as plasma total cholesterol ≥200 mg/dL or self-reported use of lipid lowering medication. At baseline, review of medication containers indicated that only 3 percent of the ARIC cohort was taking lipid lowering medications (20% statins). Ten-year risk of CHD was estimated from the Framingham risk score [22] and was dichotomized at <5% or ≥5% for the analyses presented here.

High resolution B-mode ultrasound (Biosound 2000 II SA; Biosound, Indianapolis, IN, USA) was used to measure intima-media thickness (IMT) bilaterally in the extracranial carotid arteries in the areas of the common carotid artery (1 cm proximal to the dilatation of the carotid bulb), the carotid bifurcation (1 cm proximal to the flow divider), and the internal carotid artery (1 cm distal to the flow divider). The mean IMT values at the six carotid sites were combined to produce an overall mean IMT. Correlations between scans at different visits 7 to 10 days apart performed by different sonographers and read by different readers were 0.77, 0.73, and 0.70 for the bifurcation, internal, and common carotid, respectively. For analysis, IMT was dichotomized at the sex-specific 75 percentiles. The presence of atherosclerotic plaque at any of the 6 segments was recorded by ultrasound readers as wall-thickness in excess of 1.5 mm or the presence of lumen encroachment or irregular internal surface and/or image characteristics indicative of structural heterogeneity of the arterial wall [23].

Genotyping for rs10757274 (9p21 alleles) was performed using TaqMan assays (Applied Biosystems, Foster City, CA, USA). The agreement between blind replicate pairs of independently collected and handled samples was 98.2% [4].

2.3. Assessments at later ARIC visits

Using -70°C frozen whole-blood samples from ARIC visit 2, glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography [24].

Several assays were performed on visit 4 serum or plasma samples stored at -70° C. Cardiac troponin T levels were determined using the Elecys Troponin T, a novel high sensitivity assay (Roche Diagnostics, Indianapolis, IN). The lower limit of detection is 0.003 ug/L. The reliability coefficient for blinded quality control replicate measurements (n=418 pairs) of troponin T from single blood draws was 0.98. Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured on a Cobas e411 analyzer using the Elecys proBNP II immunoassay (Roche Diagnostics, Indianapolis, IN). The range of detection was 5-35,000 pg/ml. The reliability coefficient for blind replicate measurements (n=418 pairs) was 0.99. C-reactive protein (CRP) levels were assessed by the immunoturbidimetric CRP-Latex (II) high sensitivity assay from Denka Seiken (Tokyo, Japan) using a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis). The blind replicate reliability coefficient for blind replicate measurements (n=55) was 0.99. Lipoprotein-associated phospholipase A2 (Lp-PLA2) levels were measured by a dual monoclonal antibody immunoassay standardized to recombinant Lp-PLA2 (PLAC; diaDexus Inc, South San Francisco, CA). The blind replicate reliability coefficient for Lp-PLA2 measurement (n=419) was 0.92. Cystatin C was measured by a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Inc., Deerfield, IL) with a BNII nephelometer (Dade Behring, Inc., Deerfield, IL). The reliability coefficient for blinded quality control replicates of cystatin C (n=421) was 0.65, but after removing 10 replicate pair outliers (>3 SD), the reliability was 0.94.

2.4. Coronary heart disease ascertainment and criteria

ARIC identified potential nonfatal and fatal CHD events through 2008 via (a) yearly phone interviews to ascertain hospitalizations and reported deaths, and (b) ongoing surveillance of local hospitals. Trained staff abstracted death certificates and hospital records to permit

event classification. Staff also sought next-of-kin interviews and physician questionnaires for out-of-hospital deaths. Physicians reviewed case materials and classified all potential incident CHD events using published criteria for definite fatal CHD, definite or probable myocardial infarction (MI), silent MI between examinations indicated by electrocardiograms, and coronary revascularization [25].

2.5. Data analysis

For analysis using baseline analytes, we excluded any of the 15,792 participants who at baseline reported a history of CHD (n=766), who were non-white (n=4142), who refused use of genetic material or had missing 9p21 information (n=370), or who had other missing variables (n=637), leaving 9,877 whites at risk of CHD. For analysis involving analytes from ARIC visit 2 or visit 4, additional exclusions were made for interim CHD and missing values on any of the analytes, leaving 9,024 whites for analysis after visit 2 and 6,985 after visit 4. Person-years of follow-up accrued from visit until CHD event, death, loss to follow-up, or else December 31, 2008.

Characteristics (prevalences or medians) were computed according to rs10757274 genotypes (AA, AG, GG). Using Cox proportional hazards regression, hazard ratios were computed (1) for each genotype and (2) per G allele assuming an additive genetic model. We tested for multiplicative interaction between the number of 9p21 G alleles and dichotomous risk markers using cross-product interaction terms in the proportional hazards models. We ran crude models first, and then models adjusted for other CHD risk factors from the same ARIC visit. Because crude and adjusted results were similar, we present only the adjusted hazard ratios.

3. Results

Among the 9,877 white participants free of CHD at baseline, the 9p21 variant frequencies were 26.5% for AA, 49.6% for AG, and 23.9% for GG. As shown in Table 1, there was no appreciable association between 9p21 genotype and other CHD risk markers.

Over a mean of 17.4 years of follow-up after baseline, 1653 CHD events occurred. The crude rates for 1000 person-years were 14.4, 17.0, and 18.7 for AA, AG, and GG genotypes, yielding hazard ratios of 1.0, 1.20 (95% CI = 1.07-1.36), and 1.34 (95% CI = 1.16-1.53), respectively.

As shown in stratified fashion in Table 2 and confirmed by interaction testing, no baseline risk factor interacted with the 9p21 SNP in a meaningful fashion. In other words, the association of 9p21 was virtually the same for all risk factor strata examined. The most significant interaction (p = 0.04, unadjusted for multiple comparisons) occurred between 9p21 and carotid IMT, but this was not meaningful because among those with carotid IMT above the sex-specific 75th percentile, the 9p21 association showed no monotonic increase in risk across the three genotypes.

Similarly for biomarkers measured at later ARIC visits (Table 3), there was no evidence of statistically significant interactions with the 9p21 variant in relation to CHD incidence. Across both tables, the RR per G allele was higher 11 of 15 times in the low risk factor stratum compared with the high risk factor stratum.

4. Discussion

In this large, community-based prospective study of incident CHD, we found no material gene-environment interaction between a 9p21 variant and any traditional or novel risk

markers studied. Although a few scattered interactions with 9p21 have been previously reported [11,13-16], many studies have reported no interactions [17-20]. Our findings specifically did not confirm most previously reported interactions of non-dietary factors and 9p21. Furthermore, as in virtually every previous 9p21 epidemiological study, there was no relation of 9p21 genotype with risk factors (Table 1). Thus, our study has ruled out additional potential pathways by which the 9p21 variant might increase risk of CHD.

In the U.S., patients sometimes now find out via commercial genotyping that they have 9p21 risk alleles and ask their physicians what they should do to reduce their risk. Many physicians are uncertain how to interpret or use genotype information. Our data suggest that such patients' risk is modestly increased by their 9p21 risk alleles regardless of other risk factors. Conversely, the absence of interactions implies that these patients' risk factors carry similar risk regardless of the 9p21 genotype. If anything, our findings suggested the RR per G allele tended to be higher in low risk patients, compared with high risk ones.

Our study's strengths were its large sample size, careful endpoint ascertainment, and extensive battery of risk markers. A few weaknesses are worth noting. Firstly, we did not study interactions of the 9p21 SNP with dietary factors, because dietary assessment in ARIC was limited. Secondly, for ease of presentation we dichotomized risk markers for interaction tests, but use of continuous risk markers would likely not alter our conclusions. Thirdly, detection of small gene-environment interaction requires very large sample sizes, so it is possible that we missed small interactions despite being one of the largest studies of this topic. Using the QUANTO program [26], we estimated our power to detect interactions. Assuming an additive genetic model, 1653 available events, 2-sided test, and significance level of 0.05, we had at least 80% power to detect an interaction RR ≤ 0.78 or ≥ 1.30 for all risk factors with the exception of diabetes. These minimally detectable interaction effects are similar in magnitude to significant interactions that have been reported between 9p21 variants and poor glycemic control (interaction RR: 1.24) [14] and prudent diet (RR: 1.27-1.29) [13]. Thus, this study was adequately powered to detect moderate interactions similar in size to those observed in other study populations. Fourthly, the narrow age range (20 years) may have obscured age by 9p21 interactions; Schunkert et. al. reported 9p21 odds ratios of 1.41 (1.34-1.48) for CHD onset <50 years and 1.24 (1.20-1.28) for CHD onset ≥50 years [12]. Finally, these observational data showing no interactions do not mean that the effects of 9p21 will not be modified by either pharmacologic or lifestyle interventions.

In conclusion, our prospective study suggests that the association of the 9p21 genetic variant with incidence of CHD is not modified by numerous classical or novel risk markers.

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Table 1

Baseline characteristics of white participants free of coronary heart disease, according to 9p21 genotype, ARIC

	9p21 rs10757274		
Characteristic	**AA**	**AG**	**GG**
Baseline (1987-89) measures	*n=2612*	*n=4900*	*n=2365*
Male, %	45.7	44.7	44.5
Age 45-54 y, %	53.1	52.1	52.7
55-64 y, %	46.9	47.9	47.3
Diabetes, %	7.9	8.1	8.2
Obese (BMI ≥30), %	23.2	20.9	20.7
Current cigarette smoker, %	25.5	25.0	24.6
Hypertension, %	26.6	25.5	25.2
Hypercholesterolemia, %	64.2	63.4	63.0
Framingham 10-year CHD risk ≥5%, %	28.2	27.4	26.1
Carotid Intima Media Thickness (mm), median	0.70	0.70	0.69
Carotid plaque, %	33.6	33.7	34.3
Visit 2 (1990-92) measures	*n=2401*	*n=4467*	*n=2156*
HbA1c (%), median	5.4	5.4	5.4
Visit 4 (1996-98) measures	*n=1878*	*n=3444*	*n=1663*
C-reactive protein (mg/L), median	2.2	2.1	2.2
Troponin T (μg/L), median	0.004	0.004	0.004
NT-proBNP (pg/mL), median	71.3	70.7	68.8
$LpPLA_2$ (μg/L), median	233	233	230
Cystatin C (mg/L), median	0.80	0.79	0.79

Table 2

Adjusted* hazard ratios (HR) and 95% confidence intervals (CI) of incident coronary heart disease according to 9p21 genotype, stratified by baseline risk factors, ARIC, 1987-2008≠

		9p21 rs10757274							
		AA	AG vs AA		GG vs AA		Per G Allele		p-interaction†
Risk Factor	Stratum	HR	HR	95% CI	HR	95% CI		95% CI	
Age*	45-54 y	1.00	1.33	1.10-1.61	1.53	1.24-1.89	1.23	1.11-1.36	0.44
	55-64 y	1.00	1.21	1.03-1.42	1.36	1.13-1.63	1.17	1.07-1.27	
Sex*	Men	1.00	1.29	1.04-1.59	1.32	1.04-1.68	1.15	1.02-1.29	0.41
	Women	1.00	1.23	1.06-1.42	1.45	1.22-1.72	1.22	1.12-1.32	
Diabetes*	Yes	1.00	1.24	0.93-1.65	1.48	1.07-2.05	1.24	1.06-1.46	0.59
	No	1.00	1.27	1.11-1.45	1.41	1.21-1.64	1.18	1.10-1.27	
Obesity*	Yes	1.00	1.13	0.91-1.41	1.24	0.96-1.60	1.11	0.98-1.27	0.22
	No	1.00	1.32	1.14-1.53	1.52	1.29-1.79	1.22	1.13-1.33	
Current smoker*	Yes	1.00	1.23	0.99-1.52	1.22	0.95-1.57	1.10	0.98-1.25	0.14
	No	1.00	1.28	1.10-1.48	1.54	1.31-1.82	1.23	1.14-1.34	
Hypertension*	Yes	1.00	1.30	1.07-1.58	1.52	1.22-1.90	1.25	1.19-1.39	0.31
	No	1.00	1.23	1.05-1.43	1.35	1.13-1.61	1.16	1.06-1.26	
Hypercholesterolemia*	Yes	1.00	1.29	1.12-1.49	1.36	1.16-1.60	1.16	1.08-1.26	0.24
	No	1.00	1.16	0.91-1.48	1.60	1.23-2.08	1.28	1.12-1.45	
10-yr CHD risk ≥ 5%	Yes	1.00	1.22	1.03-1.45	1.37	1.13-1.67	1.17	1.07-1.29	0.86
	No	1.00	1.23	1.03-1.46	1.42	1.17-1.72	1.19	1.08-1.31	
Carotid IMT ≥ 75%ile*	Yes	1.00	1.35	1.11-1.64	1.18	0.94-1.49	1.09	0.98-1.21	0.04
	No	1.00	1.21	1.03-1.42	1.60	1.35-1.91	1.27	1.16-1.38	
Carotid plaque*	Yes	1.00	1.29	1.09-1.53	1.38	1.13-1.67	1.17	1.06-1.29	0.57
	No	1.00	1.23	1.03-1.47	1.50	1.23-1.82	1.22	1.10-1.34	

*Adjusted for the other risk factors in the table and gender, but not for 10-year CHD risk.

†P-value for difference in 9p21 association (per G allele) between risk factor strata.

[‡]Based on 1653 CHD events over 17.4 years of follow-up.

Table 3

Adjusted* hazard ratios (RR) and 95% confidence intervals (CI) of incident coronary heart disease according to 9p21 genotype, stratified by risk biomarkers, ARIC‡

		9p21 rs10757274							
		AA	AG vs AA		GG vs AA		Per G Allele		p-interaction†
Risk Factor	Stratum	HR	HR	95% CI	HR	95% CI	HR	95% CI	
HbA1c ≥ 75%ile	Yes	1.00	1.13	0.93-1.37	1.34	1.07-1.67	1.16	1.04-1.30	0.70
	No	1.00	1.24	1.03-1.48	1.45	1.19-1.78	1.20	1.08-1.32	
C-reactive protein ≥ 75%ile	Yes	1.00	1.06	0.78-1.45	1.06	0.74-1.51	1.03	0.86-1.23	0.17
	No	1.00	1.29	1.04-1.60	1.45	1.14-1.84	1.20	1.06-1.35	
Troponin T ≥ 75%ile	Yes	1.00	1.14	0.88-1.47	1.32	0.99-1.77	1.14	0.99-1.32	0.94
	No	1.00	1.28	1.00-1.63	1.35	1.02-1.77	1.15	1.01-1.32	
NT-proBNP ≥ 75%ile	Yes	1.00	1.06	0.78-1.45	1.26	0.88-1.80	1.11	0.93-1.33	0.70
	No	1.00	1.26	1.01-1.56	1.36	1.06-1.73	1.16	1.03-1.31	
Lp-PLA$_2$ ≥ 75%ile	Yes	1.00	1.44	1.08-1.91	1.47	1.05-2.06	1.22	1.04-1.43	0.35
	No	1.00	1.05	0.84-1.32	1.23	0.96-1.58	1.11	0.98-1.26	
Cystatin C ≥ 75%ile	Yes	1.00	1.14	0.85-1.52	1.09	0.78-1.53	1.04	0.88-1.23	0.19
	No	1.00	1.24	1.00-1.55	1.44	1.13-1.85	1.20	1.06-1.36	

*Adjusted for other baseline risk factors (Table 2) except carotid IMT. HbA1c is not adjusted for diabetes.

†P-value for difference in 9p21 association (per G allele) between risk factor strata.

‡RRs for HbA1c based on 1393 CHD events over 15.0 years of follow-up; other RRs based on 786 CHD events over 10.2 years of follow-up.