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Lack of association of chromosome 9p21.3 genotype with cardiovascular structure and function in persons with stable coronary artery disease: The Heart and Soul Study

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ABSTRACT

Objective: Recent large-scale genome-wide association studies have identified a novel susceptibility locus on chromosome 9p21.3 that contributes a significant attributable risk for myocardial infarction. The phenotypic significance of this locus in patients with established coronary artery disease is unknown. We sought to compare cardiovascular structure and function in carriers and non-carriers of the risk haplotype in a cross-sectional study.
Methods: We genotyped the rs1333049 single-nucleotide polymorphism in 593 Caucasian individuals with stable coronary artery disease recruited in the Heart and Soul Study. All study subjects underwent resting and stress echocardiography. Linear and logistic regression models were used to examine the association between the rs1333049 polymorphism and echocardiographic parameters of cardiovascular structure and function.
Results: There was no association between rs1333049 genotype and echocardiographic phenotype (left ventricular hypertrophy, systolic dysfunction, diastolic dysfunction, inducible ischemia, exercise capacity, mitral annular calcification, and aortic plaque).
Conclusions: In a cross-sectional study of individuals with stable coronary artery disease, there was no association of chromosome 9p21.3 genotype with cardiovascular structure and function.

1. Text

Coronary artery disease affects over 13 million Americans and results in approximately one adult death per minute in the United States [1]. Lifestyle and environmental risk factors for coronary artery disease have been well characterized and intensively studied [2]. However, coronary artery disease also exhibits clustering in families, suggesting a substantial genetic contribution [3]. Identification of underlying genetic risk factors may improve prognostication and uncover novel therapeutic targets [4]. Recent technological advances have enabled large-scale genome-wide association studies to identify single-nucleotide polymorphisms that are associated with increased risk of coronary artery disease and myocardial infarction [5–7]. In seven independent case–control study populations, these studies identified a single susceptibility locus of approximately 100 kbp on chromosome 9p21.3 that appears to contribute a significant attributable risk for MI in those of European descent [8]. Commercial DNA tests for this locus are already available and directly marketed to the general public [9].

Intriguingly, the chromosome 9p21.3 locus does not contain any known annotated genes, but lies adjacent to two important cell cycle regulatory genes, CDKN2A and CDKN2B [10]. To date, however, no definite functional significance has been determined for the 9p21.3 locus and thus detailed intermediate phenotyping may be critical in identifying possible mechanistic pathways.

Whether chromosome 9p21.3 genotype is associated with cardiovascular structure and function in persons with established coronary artery disease is unknown. We sought to investigate the phenotypic significance of the 9p21.3 locus in 593 patients with stable coronary artery disease.

2. Methods

2.1. Participants

The Heart and Soul Study is a prospective cohort study investigating the influence of psychosocial factors on cardiovascular events in outpatients with stable coronary artery disease. The enrolment process for the Heart and Soul Study has been previously

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described [11]. Eligible participants were recruited from outpatient clinics in the San Francisco Bay Area if they met at least one of the following inclusion criteria: (1) history of myocardial infarction, (2) angiographic evidence of at least 50% stenosis by area in at least 1 coronary artery, (3) evidence of exercise-induced ischemia by treadmill electrocardiogram or stress nuclear perfusion imaging, or (4) history of coronary revascularization. Individuals were excluded if they had a history of myocardial infarction in the past 6 months, deemed themselves unable to walk 1 block, or if they were planning to move out of the local area within 3 years.

The study protocol was approved by the following Institutional Review Boards: the University of California San Francisco Committee on Human Research, the Research and Development Committee at the San Francisco VA Medical Center, the Medical Human Subjects Committee at Stanford University, the Human Subjects Committee at the VA Palo Alto Health Care System, and the Data governance Board of the Community Health Network of San Francisco. All participants provided written informed consent. Between September 2000 and December 2002, a total of 1024 participants enrolled in the study. Of these, DNA samples were available for analysis in 982 individuals. There were no demographic differences between individuals who did and those who did not provide DNA samples for analysis. In order to prevent confounding by genetic ancestry (population stratification), we restricted subsequent analysis to Caucasians ($N = 593$), the largest ethnically homogenous subset within our cohort study.

2.2. Clinical evaluation

Baseline demographics, age, sex, and self-reported ethnicity were recorded. Cardiovascular co-morbidities including hypertension, diabetes, hyperlipidemia, smoking status, and prior revascularization were determined by self-report of medical history. Recumbent blood pressure was measured after 5 min at rest. Medication use was determined by having participants bring bottles to the study appointment during which study personnel recorded all medications. Medications were categorized using Epocrates Rx (San Mateo, CA). Participants were weighed and measured without shoes. Body mass index (BMI) and waist-to-hip ratio were calculated using standard methods [12]. All participants were instructed to bring their medication bottles to the study appointment where study personnel recorded all current medications. Fasting serum chemistry samples were used to measure triglycerides, HDL cholesterol, LDL cholesterol, creatinine, and N-terminal-pro-BNP. High sensitivity C-reactive protein (CRP) was measured using the Roche Integra assay in approximately 20% of participants and, due to a change at the laboratory, the Beckman Extended Range high sensitivity CRP assay in the remaining 80% of participants. Results from these two assays were highly correlated ($r = 0.99$ in 185 participants).

2.3. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a salt modification method (Gentra Systems). The ABI PRISM 7900HT Sequence Detection System was used to perform genotyping of the rs1333049, rs10757274, and rs10757278 single-nucleotide polymorphisms using the 5′nuclease allelic discrimination assay (Taqman Assay, Applied Biosystems, Foster City, USA). The assay kit included the forward and reverse target-specific polymerase chain reaction primers, and the Taqman MGB probes labeled with dyes DFAM and VIC. The total volume of the sequencing reaction was 5 μl containing 2.5 μl of Taqman Universal PCR Master Mix, 0.083 μl of 40X Taqman MGB Assay Mix, 1.417 μl of ddH_2O, and 1 μl (5 ng) of genomic DNA. For quality control purposes, random duplication was performed on four samples per 96-well polymerase chain reaction assay. Reproducibility of single-nucleotide polymorphism genotyping was >99.99%. Investigators blinded to the clinical data performed the genotyping assays.

2.4. Cardiovascular structure and function

All patients underwent complete resting two-dimensional echocardiography and Doppler examination using an Acuson Sequoia ultrasound system (Siemens Medical Solutions, Mountain View, CA) with a 3.5-MHz transducer. Standard parasternal short axis and apical 2- and 4-chamber views were obtained and planimetered to determine end-diastolic and end-systolic volumes. The left ventricular ejection fraction (LVEF) was calculated as (end-diastolic volume − end-systolic volume)/end-diastolic volume. Systolic dysfunction was defined as LVEF <50%. Diastolic dysfunction was defined as the presence of one of the following: pseudonormal transmitral inflow pattern defined as a ratio of peak mitral early diastolic to atrial contraction velocity (E/A) of $0.75 < E/A < 1.5$ with diastolic dominant pulmonary vein flow; restrictive filling defined as an E/A of 1.5 or greater with diastolic dominant pulmonary vein flow. Left atrial volume index was measured at end-ventricular systole in the apical 2- and 4-chamber views using the biplane method of discs as previously described. Left ventricular mass was calculated using a truncated ellipsoid equation as previously validated [13]. Left ventricular hypertrophy was defined as LV mass index <90 g/m^2. Aortic arch diameter was measured in the parasternal long view. The abdominal aorta plaque score was obtained in the subcostal view and defined as: 0 = no visible plaque, 1 = plaque <3 mm thickness, 2 = plaque >3 mm. Resting wall motion score was calculated using a standard 16-segment model (2 = normal, 1 = dyskinetic, 0 = akinetic, −1 = dyskinetic) as previously described and validated [14]. Mitral annular calcification grade was evaluated in the parasternal long axis as follows: 0 = none; 1 = mild-to-moderate; 2 = severe).

All participants then underwent a symptom-limited graded exercise treadmill test according to a standard Bruce protocol. To achieve maximal heart rate, participants who were unable to continue the standard Bruce protocol were switched to lower manual settings on the treadmill and encouraged to exercise for as long as possible [15]. Exercise testing was supervised by study support staff who were blinded to genotype and outcome data. Maximal exercise capacity in metabolic equivalents (Mets) was determined at peak exercise. Poor exercise capacity was defined as <5 Mets at peak exercise [16]. Inducible ischemia was defined as the presence of one or more new wall motion abnormalities occurring with exercise. Resting and stress echocardiograms were read by a single, experienced reader (N.B.S.) who was blinded to genotype and outcome data.

3. Statistical analysis

Differences in baseline characteristics between participants with distinct rs1333049 genotypes (GG, GC, and CC) were compared with the use of analysis of variance for continuous variables and the chi-squared test for dichotomous variables, as appropriate. Normally distributed continuous variables were determined as means ± standard deviation. Variables with a skewed distribution (CRP and NT-pro-BNP) were natural logarithm-transformed prior to statistical analysis.

We used linear regression models to determine the relationship, expressed as β-coefficients per “C” allele at rs1333039, between chromosome 9p21.3 genotype and echocardiographic parameters of cardiovascular structure and function (expressed as continuous dependent variables). In a parallel analysis, we performed

Table 1
Baseline characteristics according to rs1333049 genotype in 593 Caucasian participants.

Genotype at rs1333049	GG, $N = 137$	CG, $N = 273$	CC, $N = 183$	p value
Demographics				
Age ± S.D.	69 ± 11	67 ± 11	68 ± 11	0.20
Male (%)	119 (87)	226 (83)	157 (86)	0.45
BMI ± S.D.	28.1 ± 4.7	29.0 ± 5.5	28.2 ± 5.0	0.10
Waist-to-hip Ratio ± S.D.	0.97 ± 0.06	0.96 ± 0.07	0.97 ± 0.09	0.94
Co-morbidities (%)				
Current smoking	16 (12)	55 (20)	29 (16)	0.09
Hypertension	96 (70)	173 (63)	119 (65)	0.40
History of MI	82 (60)	144 (53)	103 (57)	0.35
History of CHF	26 (19)	46 (17)	33 (18)	0.84
History of stroke	19 (14)	39 (14)	18 (10)	0.36
History of diabetes	27 (20)	57 (21)	38 (21)	0.95
Revascularization	97 (71)	164 (60)	121 (66)	0.08
Medications (%)				
Statin use	99 (72)	166 (61)	123 (68)	0.06
Beta-blocker use	79 (58)	148 (54)	110 (60)	0.41
Aspirin use	111 (81)	211 (77)	140 (77)	0.63
ACE-I/ARB use	77 (56)	136 (50)	94 (52)	0.47
Other Measurements ± S.D.				
Systolic BP (mmHg)	131 ± 22	132 ± 21	131 ± 22	0.41
Diastolic BP (mmHg)	72 ± 11	74 ± 11	73 ± 11	0.15
Triglycerides (mg/dl)	141 ± 86	158 ± 73	132 ± 96	0.13
LDL cholesterol (mg/dl)	102 ± 31	106 ± 36	99 ± 31	0.08
HDL cholesterol (mg/dl)	46 ± 15	44 ± 14	47 ± 16	0.17
Log CRP	0.7 ± 1.3	0.8 ± 1.3	0.7 ± 1.3	0.58
Log NT-pro-BNP	5.4 ± 1.3	5.3 ± 1.3	5.4 ± 1.3	0.79
Creatinine (mg/dl)	1.1 ± 0.5	1.1 ± 0.3	1.1 ± 0.3	0.11
Hemoglobin (g/dl)	14.0 ± 1.4	14.1 ± 1.4	14.1 ± 1.3	0.96

logistic regression to determine the association of chromosome 9p21.3 genotype with abnormal cardiovascular structure and function (expressed as unadjusted odds ratios per "C" allele for each dichotomous outcome).

Based on a two-tailed alpha of 0.05, we had >80% power to detect the following differences in cardiovascular phenotype in risk allele carriers ($N = 137$) versus non-carriers ($N = 456$): left ventricular ejection fraction 3%; left ventricular mass index 7 g/m^2; exercise capacity 1.0 METS; left atrial volume index 3 ml/m^2; resting wall motion score 1.0; aortic arch diameter 0.3 cm; abdominal aorta plaque score 0.3; mitral annular calcification grade 0.3; proportion with inducible ischemia 11%.

To explore potential modifying effects of chromosome 9p21.3 genotype, we tested for statistical interactions between chromosome 9p21.3 genotype and age, smoking, diabetes, body mass index, and waist-to-hip ratio. Statistical analysis was performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC). The two-tailed significance threshold was set at the 0.05 level for genotype–phenotype associations, and at the 0.10 level for tests of interaction.

Table 2
Association of genotype at 9p21.3 with cardiovascular structure and function expressed as β-coefficients per "C" allele at rs1333049 in unadjusted linear regression models.

Outcome	β-Coefficient	p value
LV mass index (g/m^2)	−0.43	0.76
Left atrial volume index (ml/m^2)	−0.26	0.71
Resting wall motion score	−0.00005	1.0
Aortic arch diameter (cm)	−0.1	0.61
Abdominal aorta plaque score	−0.008	0.89
Mitral annular calcification grade	−0.01	0.72
LVEF (%)	−0.17	0.76
Exercise capacity (METS)	0.23	0.26

Table 3
Association of genotype at 9p21.3 with cardiovascular structure and function expressed as odds ratios per "C" allele at rs1333049 in unadjusted logistic regression models.

Outcome	Odds ratio (95% CI)	p value
Systolic dysfunction	1.0 (0.7–1.4)	0.96
Left ventricular hypertrophy	1.0 (0.8–1.2)	0.95
Diastolic dysfunction	1.1 (0.8–1.6)	0.56
Inducible ischemia	1.0 (0.8–1.3)	0.86
Poor exercise capacity	1.2 (0.9–1.5)	0.27
Mitral annular calcification	1.0 (0.7–1.3)	0.85
Abdominal aorta plaque	1.0 (0.8–1.4)	0.83

4. Results

We genotyped 593 Caucasian subjects in the Heart and Soul Study for the rs1333039, rs10757274, and rs10757278 SNPs, all located on chromosome 9p21 and identified as risk loci in genome-wide scans. An analysis of 9p21.3 SNP haplotype showed that all 3 SNPs were in tight linkage disequilibrium ($r^2 > 0.9$). Their associations with cardiovascular structure, function, and outcomes were identical and therefore only the data for the rs1333049 SNP is considered hereafter.

The genotype distribution of rs1333049 in the study cohort as a whole was GG (279), CG (443), CC (261). In Caucasian participants, the distribution was GG (137), CG (273), CC (183). The observed frequencies of the G and C alleles in Caucasians were 0.46 and 0.54 respectively. These findings were consistent with Hardy–Weinberg equilibrium ($p = 0.96$). The baseline characteristics of the study population categorized by genotype at rs1333049 are shown in Table 1. There were no significant associations between rs1333049 genotype and baseline demographics, medical history, smoking, medication use, blood pressure, lipids, or biomarkers of systemic inflammation and hemodynamic compromise (CRP and NT-pro-BNP respectively).

In unadjusted linear regression models, there was no significant association of rs1333049 genotype with any echocardiographic parameter of cardiovascular structure or function (Table 2). In unadjusted logistic regression models, we found no association of rs1333049 genotype with abnormalities in cardiovascular structure or function (Table 3).

We found no evidence that the lack of association of rs1333049 genotype with echocardiographic phenotype was modified by body mass index, waist-to-hip ratio, hypertension, diabetes, or smoking (all p values for interaction >0.1).

5. Discussion

Recent genome-wide case–control association studies have consistently demonstrated an association of chromosome 9p21.3 genotype with coronary artery disease and incident myocardial infarction in individuals of European descent [17,18]. Increased risk at this locus, defined by the C allele at SNP rs1333049 or equivalent risk alleles at other SNPs in strong linkage disequilibrium with it, has also been confirmed in several other ethnic populations [19–22]. The magnitude of this effect has been estimated as a relative risk of 1.25–1.40 per risk allele in autosomal additive models [23]. In the present study, we found no association of chromosome 9p21.3 genotype with cardiovascular structure and function in individuals with established coronary artery disease. Our findings suggest that although 9p21.3 genotype confers an increased risk of developing coronary artery disease among healthy individuals, it does not predict abnormalities in cardiovascular structure and function, once the diagnosis of coronary artery disease has been made.

An important feature of genome-wide association studies is freedom from pre-existing hypotheses incorporating established models of disease pathophysiology [24]. The region of association with coronary artery disease on chromosome 9p21.3 does not contain any known expressed gene products, but lies adjacent to CDKN2A and CDKN2B, two important cell cycle regulators. To date however, no known functional significance has been described for the 9p21.3 risk allele. In keeping with prior studies, we found no association between the 9p21.3 genetic variant and classical or novel cardiovascular risk factors including age, gender, smoking, diabetes, blood pressure, lipid levels, smoking, C-reactive protein, and N-terminal-pro-BNP. In addition, we performed "high density phenotyping" of all participants using rest and stress echocardiography and found no association of 9p21.3 genotype with parameters of cardiovascular structure and function including left ventricular mass, ejection fraction, diastolic dysfunction, inducible ischemia, aortic abdominal plaque, or exercise capacity.

To date, phenotypic studies of the 9p21.3 polymorphism have yielded conflicting results. In two large population-based cohorts, Samani et al. found no association of 9p21.3 genotype with either carotid-intima media thickness or flow-mediated dilatation [8]. They concluded that the 9p21.3 locus influences coronary artery disease risk through mechanisms independent of clinically silent intimal thickening or endothelial dysfunction. Ye et al. confirmed the lack of association of 9p21.3 genotype with carotid-intima thickening but found an association of the rs1333049 C allele with established carotid atherosclerosis and progression of atherosclerotic plaques by carotid duplex scanning [25]. This association was particularly strong in individuals with abdominal obesity. In the prospective Rotterdam Study of 7983 participants aged 55 years and older, no association was detected between 9p21.3 genotype and coronary heart disease or myocardial infarction [26]. Using a case–control study design, Anderson et al. found that the 9p21.3 locus predicted angiographic coronary disease prevalence but not the extent of disease [27]. These results underscore the substantial challenges of studying genotype–phenotype associations in a complex and clinically heterogeneous disease such as coronary atherosclerosis.

In the present study, we found no increased incidence of abdominal aortic plaque in riskallele carriers and no interaction between 9p21.3 genotype and abdominal obesity. Taken together with the results of prior studies, these findings are suggestive of differential risk according to vascular territory, co-morbidities, and stage of atherosclerotic disease. Further studies are warranted to clarify the relationships between 9p21.3 genotype, early atherogenesis, plaque progression, and abdominal obesity. The recent finding of an increased risk of abdominal aortic aneurysm and intracranial Berry aneurysm in 9p21.3 riskallele carriers raises the intriguing possibility that the observed risk is mediated through a fundamental abnormality of the vessel wall rather than the atherosclerotic process per se [28]. Meanwhile, our failure to detect any phenotypic association of 9p21.3 genotype with systolic function, diastolic function, or left ventricular hypertrophy appears to point away from a primary myocardial mechanism for the increased risk associated with this locus.

The main strength of the present study is the collection of detailed phenotypic information derived from resting and stress echocardiography in all participants [29]. However, several important limitations should be considered in the interpretation of our results. First, our study population was restricted to Caucasian individuals, predominantly men. Our results may not therefore extrapolate automatically to women or other racial groups. Second, we did not perform invasive coronary angiography or coronary CT angiography on all participants, which would determine the prevalence of significant coronary artery stenoses and provide further insights on disease extent and severity. Third, our sample size was inadequate to test the prognostic significance of the 9p21.3 polymorphism with respect to subsequent adverse cardiovascular events, or to investigate potential interactions with other genetic loci associated with abnormal cardiovascular phenotypes. Fourth, our findings are restricted to individuals with established coronary artery disease, and cannot be extrapolated to disease-free individuals or those with subclinical disease.

In conclusion, we found no association of chromosome 9p21.3 genotype with cardiovascular structure and function in a cross-sectional study of individuals with stable coronary artery disease. While 9p21.3 genotype confers increased risk of developing coronary artery disease in the general population, it does not appear to predict abnormalities in cardiovascular structure and function in patients with established coronary artery disease.

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Disclosures

No conflicts of interest declared.

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