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Four SNPS on Chromosome 9p21 Confer Risk to Premature, Familial CAD and MI in an American Caucasian Population (GeneQuest)

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Summary

Genome-wide association studies have separately identified four single nucleotide polymorphisms (SNPs) on chromosome 9p21 that confer susceptibility to coronary artery disease (CAD) and myocardial infarction (MI). This study presents the first analysis of these SNPs (rs10757274, rs2383206, rs2383207, and rs10757278) in a premature, familial CAD/MI population (GeneQuest). We performed a case-control analysis of the GeneQuest Caucasian population with 310 cases with premature CAD and MI (average age at onset of 40.3 ± 5.1) and 560 non-CAD controls to determine if these SNPs are associated with risk of CAD using both the population-based and family-based association study designs. The four SNPs are significantly associated with premature and familial MI and CAD in the GeneQuest Caucasian population (allelic $P = 6.61 \times 10^{-7}$ to 1.87×10^{-8}). Sib-TDT analysis showed that three of the four SNPs could confer significant susceptibility to premature CAD and MI. These results indicate that the four SNPs on chromosome 9p21 are also associated with premature, familial CAD.

Keywords

coronary artery disease (CAD); myocardial infarction (MI); single nucleotide polymorphism (SNP); 9p21; association study; haplotype analysis

Introduction

Recently, several genomewide association studies have implicated chromosome region 9p21 as a novel locus that confers susceptibility to coronary artery disease (CAD) and myocardial infarction (MI) in multiple Caucasian cohorts. Two single nucleotide polymorphisms (SNPs)

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(rs1075724 and rs2383206) were initially identified by McPherson et al. (McPherson et al. 2007); two additional SNPs (rs2383207 and rs10757278) were located by Helgadottir et al. (Helgadottir et al. 2007), and were then identified in several other genomewide linkage scans for CAD (Samani et al. 2007, Wellcome Trust Case Control Consortium, 2007), making it the best replicated locus to date that confers susceptibility to CAD and MI. Recent work from our group has shown that the four SNPs in this region are able to confer cross-race susceptibility to CAD in an Asian population and we have also shown that they are associated with risk of CAD in a Mediterranean population (Shen et al. 2007a,b).

In this study, we conducted a case-control association study with unrelated Caucasian members of our well-characterized American GeneQuest population with premature and familial CAD and MI. We then utilized the pedigrees of each affected patient in the GeneQuest cohort to perform sibling trait disequilibrium testing (STDT) to determine if these SNPs were associated with risk of CAD by a family-based study design. Our findings reveal an association between premature and familial CAD and MI in the GeneQuest population with both population-based and family-based study designs.

Materials and Methods

Ascertainment of Patients

We utilized the American GeneQuest population to conduct both population-based and family-based association studies. Our GeneQuest cohort with premature and familial CAD and MI has been previously described (Shen et al. 2007c; Wang et al. 2004). Briefly, over a five year span, patients were recruited by cardiologists and data coordinators in the Department of Cardiovascular Medicine at Cleveland Clinic and 10 other collaborating institutions. Premature CAD or MI was defined as affected men and women aged 45 and evidence ≤45 ≤50, respectively, with prior or current of atherosclerotic coronary artery disease or MI. Diagnosis of premature CAD was performed by a panel of cardiologists on the existence of two or more of the following parameters: ECG patterns consistent with acute MI, chest pain of ≥30 min duration, history of cardiac intervention, or current treatment of angina pectoris. Patients were considered “unaffected” if they were women aged >50 years or men aged >40 and did not fit the aforementioned criteria for CAD or MI. For purposes of recruitment, each proband was required to have a living sibling that satisfied the same criteria for premature CAD or MI. The 560 normal controls used for the case-control study were selected from Cleveland GeneBank, comprising the persons who underwent coronary angiography in Cardiac Catheterization Laboratories at Cleveland Clinic; only Caucasian individuals without detectable atherosclerotic lesions by angiography were included. All participants completed a health questionnaire, had fasted blood drawn, and had anthropomorphic measurements taken. The clinical features of the GeneQuest cases with CAD/MI and controls are shown in Table 1.

The studies were approved by appropriate local institutional review boards on human subject research; written informed consent was obtained from all participants, and conformed to the guidelines set forth by the Declaration of Helsinki. Whole blood was drawn from each participant, and genomic DNA was isolated from blood using standard protocols using the DNA Isolation Kit for Mammalian Blood (Roche Pharmaceuticals, Basel, Switzerland).

SNP Genotyping and Statistical Analysis

SNP genotyping was carried out using the 5′ nuclease allelic discrimination assay (TaqMan Assay, Appled Biosystems, Foster City, USA) as previously described (Shen et al. 2007a,b,c). Genotyping data were analyzed using statistical methods as previously described (Shen et al. 2007a,b,c). For all data, pointwise statistical significance was taken as $P < 0.05$.

Power Analysis

We carried out a statistical power analysis to ensure that our sample size was sufficient to identify associations. Utilizing the population parameter settings of an odds-ratio of 1.25 and allelic frequency of 0.45 from previously published reports, our study with 310 cases and 560 controls can provide statistical power of 88% at a Type I error rate of 0.05 (Helgadottir et al. 2007; McPherson et al. 2007), suggesting that our GeneQuest sample size is sufficient to identify associations between these SNPs and their susceptibility to CAD and MI.

Results

Association of Four SNPs on 9p21 and Familial, Premature CAD and MI Using a Population-Based Design

We found significant allelic association between the 4 SNPs on chromosome 9p21 and the phenotype of premature CAD and MI (*P*-obs [nominal p-value] = from 6.61×10^{-7} to 1.87×10^{-8}, OR 1.67 to 1.78; Table 2). After adjustment and multivariate logistic regression, we found that adjusted probabilities were still significant (*P*-adj [adjusted p-value] from <1.0 × = program, 10^{-6} to 7.0×10^{-4}; Table 2). Using the CLUMP we performed 100,000 Monte Carlo simulations to determine empirical P-values, and again all results were significant (for all SNPs, *P*-emp = $<1.0 \times 10^{-5}$).

Sib-TDT Analysis

Using the family based analysis of genotyping data from the GeneQuest population, we performed a sibling trait disequilibrium transmission test in 310 families (Table 3). We found that 3 of the SNPs were significant for association with CAD (rs10757274 $P = 0.014$, rs2383206 $P = 0.036$, rs10757278 $P = 0.0038$).

Analysis of Genotypic Association Suggests Dominant and Additive Models of Inheritance

We also found statistically significant genotypic association between the 4 SNPs and premature CAD and MI (Table S1). Nominal P-values (for all SNPs, *P*-obs $<1.0 \times 10^{-5}$), adjusted P-values (*P*-adj = from 0.013 to 1.0×10^{-6}), and empirical P-values (for all SNPs, *P*-obs = $<1.0 \times 10^{-5}$) were significant (Table S1).

We performed a genotypic association analysis assuming dominant, recessive, and additive models of inheritance (Table S2). Results of the analysis of the 4 SNPs were consistent in suggesting that the dominant model of inheritance was significant before adjustment for common covariates (for all SNPs, *P*-obs = $<1.0 \times 10^{-6}$, OR 2.22 to 2.61) and after (*P*-adj from 7.0×10^{-4},to 5.0×10^{-6}, OR 2.23 to 2.89) (Table S2). = For two SNPs, the recessive model remained marginally significant after adjustment (r2383207 *P*-adj 0.0378, OR 1.73 and rs10757278 *P*-adj 0.023, OR 1.77), although only SNP rs10757278 was significant both before and after adjustment for covariates. The additive model was also significant before adjustment (for all SNPs, *P*-obs $<1.0 \times 10^{-6}$, OR 1.62 to 1.76) and after (*P*-adj = from 1.0×10^{-4}, $<1.0 \times 10^{-6}$, OR 1.67 to 1.78). These results suggest that both dominant and additive models of inheritance confer susceptibility to premature CAD and MI.

Discussion

Here we report significant genetic association between four 9p21 SNPs and premature, familial CAD in the GeneQuest population. Both population-based and family-based study designs were employed. The four 9p21 SNPs demonstrated both allelic and genotypic association with familial, premature CAD in a population-based case control association study. The 9p21 SNPs also showed association with CAD in a family-based study by Sib-TDT analysis. It is notable that the *P* values for association reached 4.11×10^{-8} (Table 2), which may be due to the very

young age at onset and strong family history; the unique features of GeneQuest. The age at onset of CAD and MI in our GeneQuest population is the most restrictive to date, with cutoffs for males and females at ≤45 and ≤50 respectively and an average of 40.3 ± 5.1 years. Each proband in the GeneQuest family must have ± another affected sib to be included in the study. The necessity of a restrictive phenotype has been noted as a key determinant in identifying significant genetic loci for complex traits and confirming genetic, rather than environmental contributions (Van Eerdewegh et al. 2002;Wessman et al. 2002).

In a previous report, we showed that the four 9p21 SNPs were associated with CAD and MI in a Mediterranean population (Shen et al. 2007b). Interestingly, we showed that the association was limited to those affected patients with apositive family history of CAD and MI (Shen et al. 2007b). Consistent with this finding, strong genetic association with impressive *P* values was identified between the 9p21 SNPs and CAD in the GeneQuest population with familial CAD and MI.

In conclusion, we have found associations between four SNPs on chromosome 9p21 and premature, familial CAD/MI in our American Caucasian GeneQuest population using both population-based and family-based association studies. This study is the first to examine the association between 9p21 SNPs and CAD using a family-based approach (Sib-TDT) in 310 well-characterized GeneQuest pedigrees.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical characteristics of the study population including patients and normal controls

	Gene Quest	
Characteristic	**Case (n = 310)**	**Control (n = 560)**
Gender (M/F)	201/109[b]	269/291
Age (years)[a]	40.3 ± 5.1[b]	53.5 ± 12.1
BMI (kg/m^2)	29.8 ± 5.6	29.2 ± 7.1
MI (%)	53.9	0
CABG (%)	15.4	0
PTCA (%)	12.2	0
Hypertension (%)	48.1	43.4
Diabetes (%)	13.5[c]	7.9
Smoking (%)[c]	80.0	47.2
Total cholesterol (mmol/L)	222.9 ± 60.6[c]	188.2 ± 43.2
LDL cholesterol (mmol/L)	136.4 ± 43.1[c]	116.6 ± 35.9
Triglyceride (mmol/L)	256.0 ± 290.6[c]	135.0 ± 82.5

Data are shown as mean ± SD, unless otherwise indicated.

MI = myocardial infarction; CABG = coronary artery bypass graft;

PTCA = Percutaneous transluminal coronary angioplasty.

[a] age-at-onset for cases and age-at-examination for controls.

[b] P < 0.001, compared with controls.

[c] P < 0.05, compared with controls.

Table 2

Allelic association of four SNPs on chromosome 9p21 with premature CAD and MI in the GeneQuest population

SNPs	Allele	Frequency (%)		OR (95% CI)[a]	P-value		
		Control	Case		Observed[b]	Adjusted[c]	Empirical[d]
rs10757274	G	0.469	0.611	1.78 (1.45-2.18)	1.87×10^{-8}	1.0×10^{-4}	$<1.0 \times 10^{-5}$
rs2383206	G	0.495	0.620	1.67 (1.36-2.03)	6.61×10^{-7}	7.0×10^{-4}	$<1.0 \times 10^{-5}$
rs2383207	G	0.509	0.642	1.72 (1.41-2.12)	1.39×10^{-7}	2.0×10^{-4}	$<1.0 \times 10^{-5}$
rs10757278	G	0.454	0.593	1.76 (1.43-2.14)	4.11×10^{-8}	$<1.0 \times 10^{-6}$	$<1.0 \times 10^{-5}$

[a] OR, odds ratio, CI, confidence interval.

[b] p-obs, uncorrected p-value.

[c] p-adj, p-value obtained after adjustment for gender, age of onset, BMI, hypertension, HDL, LDL, triglycerides and diabetes.

[d] p-emp, permutation p-value calculated using 100,000 Monte Carlo simulations.

Table 3

Sib-TDT analysis of four SNPs on chromosome 9p21 in the GeneQuest cohort

		Frequency†			
SNP	**Risk Allele**	**Affected**	**Unaffected**	**Z-Score**	**P-value**
rsl0757274	G	.58	.53	2.46	0.014
rs2383206	G	.60	.54	2.10	0.036
rs2383207	G	.63	.56	1.73	0.083
rs10757278	G	.55	.50	2.89	0.0038

† Frequency of risk allele among Sib-TDT cohort. The study comprised of 1,329 total family members. For the 310 families, there was a total of 710 affected family members, of which 400 were affected sibs (average age of onset of affected sibs = 41.9 ± 5.18 years), and 310 were probands (average age at onset = 40.3 ± 5.1 years). The average age of the unaffected family members was 59.2 ± 9.04 years.