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A genome-wide association study reveals susceptibility loci for myocardial infarction/coronary artery disease in Saudi Arabs

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ABSTRACT

Background: Multiple loci have been identified for coronary artery disease (CAD) by genome-wide association studies (GWAS), but no such studies on CAD incidence has been reported yet for any Middle Eastern population.
Methods: In this study, we performed a GWAS for CAD and myocardial infarction (MI) incidence in 5668 Saudis of Arab descent using the Affymetrix Axiom Genotyping platform.
Results: We describe SNPs at 16 loci that showed significant ($P < 5 \times 10^{-8}$) or suggestive GWAS association ($P < 1 \times 10^{-5}$) with CAD or MI, in the ethnic Saudi Arab population. Among the four variants reaching GWAS significance in the present study, the rs10738607_G [0.78(0.71−0.85); p = 2.17E-08] in *CDNK2A/B* gene was associated with CAD. Two other SNPs on the same gene, rs10757274_G [0.79(0.73−0.86); p = 2.98E-08] and rs1333045_C [0.79(0.73−0.86); p = 1.15E-08] as well as the rs9982601_T [1.38(1.23−1.55); p = 3.49E-08] on *KCNE2* were associated with MI. These variants have been previously described in other populations. Several SNPs, including the rs7421388 (*PLCL1*) and rs12541758 (*TRPA1*) displaying a suggestive GWAS association ($P < 1 \times 10^{-5}$) with CAD as well as rs41411047 (*RNF13*), rs32793 (*PDZD2*) and rs4739066 (*YTHDF3*), similarly showing weak association with MI, were confirmed in an independent dataset. Furthermore, our estimation of heritability of CAD and MI based on observed genome-wide sharing in unrelated Saudi Arabs was approximately 33% and 44%, respectively.
Conclusions: Our study has identified susceptibility variants for CAD/MI in ethnic Arabs. These findings provide further insights into pathways contributing to the susceptibility for CAD and will enable more comprehensive genetic studies of these diseases in Middle East populations.

1. Introduction

Coronary artery disease (CAD), a chronic process that includes the damaging of arterial endothelial cells and the deposition of lipid-rich atheroma in the sub-endothelial layer [1], is a leading cause of mortality and disability worldwide [2]. A number of risk factors contribute to the pathogenesis of the disease. These include smoking, diabetes mellitus, hypertension, hypercholesterolaemia, obesity and family history [3]. Recently, genome-wide association studies (GWAS) have identified multiple chromosomal regions, such as the *CDKN2A/2B/ANRIL* gene cluster on 9p21.3, and *PHACTR1* on chromosome 6p24.1, associated with CAD [4–8]. However, these studies have been performed primarily in populations of European descent, and the identified loci altogether explain only a small fraction of the risk for the disease. Furthermore, because of the well-acknowledged genetic differences between ethnic groups, the variants identified thus far might not explain the susceptibility to CAD in other populations. Therefore, GWASs for non-European populations are needed to identify such additional loci and to enhance our understanding of the mechanisms underlying individual susceptibility to the disease. In addition, replication of

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discovered GWAS variants in multiple populations will provide greater confidence in their role(s) in the pathophysiology of the disease.

Recently, a study of 17,232 subjects from the Saudi Arab general population found a 5.5% incidence of CAD [9]. The data also showed that other genetically determined factors such as body mass index (BMI), hypertension, diabetes mellitus, fasting blood glucose, fasting cholesterol and triglycerides were important predictors of CAD in this population. Here, in ongoing efforts to identify genes associated with the disease, we carried out a GWAS study in 5431 Saudi Arab subjects. We present two main scientific results from this study: first, we found both known and unfamiliar loci associated with CAD/myocardial infarction (MI), and second, estimates based on observed genome-wide sharing imply that approximately 47% of CAD heritability underlies the etiology of the disease in Saudi Arabs.

2. Materials and methods

2.1. Study populations

The study subjects consisted of 5668 Saudi Arab individuals (2668 cases versus 3000 controls) who underwent cardiac catheterization at our Institutional Catheterization Centre. These individuals visited the Cardiology Clinic of our Institution from all five regions of the country. The ethnicity of the individuals was ascertained through both a questionnaire and consulting the Institutional medical records. All candidates underwent coronary angiography and echocardiography (ECG) which were independently reviewed by two experienced interventional cardiologists. The CAD cases consisted of individuals with angiographically confirmed narrowing of the coronary vessels by at least 50%, and at least one-vessel disease (Data in Brief, Table 1). The evidence for MI was ascertained through ECG abnormalities indicative of the presence of ischemia as per recommendations of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction [10]. Accordingly, these patients harboured unstable angina, non-ST or ST-elevation MI. In addition to ECG manifestations, other supportive clinical variables, including the changes in the biomarkers myoglobin, cardiac troponin T, pro-brain natriuretic peptide and pro-calcitonin were also provided as routine through the Institutional Clinical Chemistry Laboratory. Exclusion criteria for the disease cases were major cardiac rhythm disturbances, incapacitating or life-threatening illness, major psychiatric illness or substance abuse, history of cerebral vascular disease, neurological disorder, and administration of psychotropic medication. The controls (CON) for CAD were a group of individuals undergoing surgery for heart valvular diseases, and those who may have reported with chest pain, but were established to have no significant coronary stenosis by angiography. Exclusion criteria for this group were, among others, diseases such as cancer, autoimmune disease, or any other disorders likely to interfere with variables under investigation. Ten mL of blood sample was collected from the arterial access site of patients who provided a written consent for the whole study that included blood collection and genetic analysis. Trained healthcare professionals collected further data on the socio-demographic background of all patients. Annotations were coded from medical charts for additional data such as laboratory tests, prescribed medications, and presence of other diseases and conditions. Genomic DNA was extracted using a standard phenol extraction procedure. The study protocol was approved by the Institutional Review Board (IRB) at the King Faisal Specialist Hospital and Research Centre.

2.2. Genotyping and SNP quality control

Genotyping was performed using Affymetrix Axiom Genome-Wide ASI Array (Asian population) that provides high genetic coverage containing a total of 598,000 SNPs including those in genic as well as conserved regions, coding SNPs and X chromosome SNPs. For the assay, total genomic (200 ng) DNA was amplified and randomly portioned into 25–125 bp fragments, which were in turn purified, re-suspended and hybridized to Axiom Genome Wide Human Array Plates. Following hybridization, the bound target was washed under stringent conditions to remove non-specific background to minimize noise resulting from random ligation events. Each polymorphic nucleotide was queried via a multi-colour ligation event carried out on the array surface. After ligation, the arrays were stained and imaged on the Gene Titan MC Instrument (Affymetrix, Santa Cruz, CA). Image files were automatically processed for allele calling and quality control with the Axiom GT1 algorithm available through the Affymetrix Power Tools Software v.1.16 to generate genotyping data. The call rate for each array was obtained to determine the quality of the chip genotyping data. Quality control filtering of the GWAS data was performed as follows: Samples

Table 1
Genome-wide association test for SNPs with coronary artery disease/myocardial infarction in Saudi Arabs.

SNP_Risk allele	Region	P	OR (95% CI)	Frequency	Gene
Coronary artery disease					
rs7421388_G	2q33.1	4.31E-06	0.77(0.69–0.85)	0.79	*PLCL1*
rs13082914_T	3q25.31	3.64E-06	1.21(1.09–1.34)	0.22	*KCNAB1*
rs9985766_G	4q31.23	3.36E-06	1.35(1.2–1.52)	0.16	*DCLK2*
rs17775862_T	6q25.3	5.33E-06	1.55(1.3–1.85)	0.06	*Intergenic*
rs12541758_T	8q13.3	3.87E-07	1.25(1.15–1.36)	0.40	*TRPA1*
rs10738607_G**	9p21.3	2.17E-08*	0.78(0.71–0.85)	0.64	*CDKN2A/B*
rs10981012_A	9q31.3	5.43E-06	1.34(1.17–1.52)	0.13	*C9orf84*
rs1746049_C	10q11.21	2.44E-06	0.80(0.73–0.88)	0.72	*CXCL12***
Myocardial infarction					
rs41411047_A	3q25.1	1.07E-07	1.51(1.3–1.76)	0.09	*RNF13*
rs32793_G	5p13.3	6.13E-06	1.25(1.14–1.37)	0.28	*PDZD2*
rs16880442_G	5q11.2	8.56E-06	0.72(0.61–0.83)	0.93	*ITGA1*
rs4739066_A	8q12.3	2.47E-06	0.73(0.65–0.82)	0.86	*YTHDF3*
rs10757274_G**	9p21.3	2.98E-08*	0.79(0.73–0.86)	0.63	*CDKN2A/B*
rs1333045_C**	9p21.3	1.15E-08*	0.79(0.73–0.86)	0.54	*CDKN2A/B*
rs7211079_A	17q25.3	4.81E-06	0.77(0.7–0.85)	0.78	*EIF4A3*
rs9982601_T**	21q22.11	3.49E-08*	1.38(1.23–1.55)	0.16	*KCNE2*

The table lists the SNPs showing significant ($P < 5 \times 10^{-8}$; marked with asterisks) and suggestive ($P < 10^{-5}$) association with coronary artery disease (CAD)/myocardial infarction (MI). **SNP/gene previously associated with CAD/MI in European South Asian populations. Region, Chromosomal region; OR, odds ratio; P, P-value from the association test; Frequency: frequency of risk allele; Gene: Candidate nearby gene.