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Genomic variant in *CAV1* increases susceptibility to coronary artery disease and myocardial infarction

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ABSTRACT

Background: The *CAV1* gene encodes caveolin-1 expressed in cell types relevant to atherosclerosis. *Cav-1*-null mice showed a protective effect on atherosclerosis under the *ApoE*$^{-/-}$ background. However, it is unknown whether *CAV1* is linked to CAD and MI in humans. In this study we analyzed a tagSNP for *CAV1* in intron 2, rs3807989, for potential association with CAD.

Methods and Results: We performed case–control association studies in three independent Chinese Han populations from GeneID, including 1249 CAD cases and 841 controls in Population I, 1260 cases and 833 controls in Population II and 790 cases and 1212 controls in Population III (a total of 3299 cases and 2886 controls). We identified significant association between rs3807989 and CAD in three independent populations and in the combined population ($P_{adj} = 2.18 \times 10^{-5}$, OR = 1.19 for minor allele A). We also detected significant association between rs3807989 and MI ($P_{adj} = 5.43 \times 10^{-5}$, OR = 1.23 for allele A). Allele A of SNP rs3807989 was also associated with a decreased level of LDL cholesterol. Although rs3807989 is a tagSNP for both *CAV1* and nearby *CAV2*, allele A of SNP rs3807989 was associated with an increased expression level of *CAV1* (both mRNA and protein), but not *CAV2*.

Conclusions: The data in this study demonstrated that rs3807989 at the *CAV1*/*CAV2* locus was associated with significant risk of CAD and MI by increasing expression of *CAV1* (but not *CAV2*). Thus, *CAV1* becomes a strong candidate susceptibility gene for CAD/MI in humans.

1. Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality worldwide [1,2]. CAD is caused by stenosis of one of coronary arteries due to plaque formation. When the stenosis is severe or a plaque ruptures, blood flow through a coronary artery is blocked, which causes thrombosis, myocardial infarction (MI) and sudden death. Multiple factors can influence the development of CAD and MI, for example, the age, gender, smoking, alcohol intake, hypertension, obesity, diabetes mellitus (DM), and genetic factors as well as interactions between genetic factors and environmental factors [3]. Since 2007, large scale genome-wide association studies (GWAS) have identified more than 50 genomic variants or single nucleotide polymorphisms (SNPs) that either increase or decrease risk of CAD/MI. These SNPs include rs599839 on 1p13, rs17465637 on 1q41, rs2943634 on 2q36, rs1420101 on 2q12, rs12619285 on 2q13, rs4857855 on 3q21, rs6903956 on 6p24, rs4143832 on 5q31, rs6922269 on 6q25, rs1333049 on 9p21, rs501120 on 10q11, rs3184504 on 12q24, rs17228212 on 15q22 and the other loci such as 1p32, 2q33, 3q22, 7p22, 10p11, 10q23, 11q22, 15q25, 21q22, and 19p23 [4–17]. Most GWAS for CAD/MI were performed in European

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ancestry populations, but recently GWAS in the Chinese population have been reported, too. Our group reported the first GWAS for CAD in the Chinese population and identified the *C6orf105* gene (now referred to as *ADTRP*) as a susceptibility gene for CAD in the Chinese population only [15]. Later, another GWAS reported 4 SNPs associated with CAD in the Chinese population [16]. Recently, we developed a candidate pathway GWAS that combines eQTL analysis and mining of GWAS data and identified two SNPs in the complement system associated with CAD [18]. Some CAD variants identified by GWAS showed susceptibility of CAD across multiple ethnic populations, for example, SNPs on 9p21 [19–22]. However, some CAD risk variants showed ethnic specificity, for example, rs3184504 on 12q24 and rs17228212 on 15q22 with a 0 or very low frequency of the minor allele (MAF) in the Chinese population, rs12619285 on 2q13, rs4857855 on 3q21 and rs4143832 on 5q31 not replicated in the Chinese population [23,24], and rs6903956 on 6p24 increasing risk of CAD only in the Chinese population to date [15,25,26]. Nevertheless, all genomic variants identified to date in aggregate accounted for <20% of heritability of CAD [17]. Considering the estimated heritability of 40%–60% of CAD, a majority of CAD heritability remains missing, an observation referred to as missing heritability [27,28]. Therefore, a major challenge for the field of genetics of CAD is to identify the rest of genomic variants that account for missing heritability.

The candidate gene approach is potentially one of the effective strategies to identify missing heritability of CAD. Almost all candidate gene studies failed to identify true CAD/MI risk variants before GWAS mostly due to the small sample sizes used in those studies and lack of rigorous independent replication. The small sample size generated false positive signals that are rarely replicated. With large sample cohorts available now for CAD/MI, we speculate that the candidate gene approach may become one of the prevailing strategies to identify missing heritability in the post-GWAS era. In this study, we employed the candidate gene approach to identify significant association between *CAV1* and CAD.

The *CAV1* gene is a small gene with 3 exons that encodes caveolin-1, one of the three members of the caveolin family, that assembles caveolae as a coat and scaffolding protein [29]. The caveolae plays an important role in many signaling pathways, ionic conductance and lipid regulation [29,30]. Interesting studies on *Cav1*-null mice indicated that *Cav1* was involved in insulin resistance, hypertension, atherosclerosis and lipoprotein metabolism [31–35]. Because insulin resistance, hypertension, and lipoprotein metabolism are all associated with risk of atherosclerosis, we hypothesized that genomic variant in *CAV1* was associated with susceptibility to CAD and/or MI in humans. Thus, we selected a tagSNP in *CAV1*, rs3807989, and tested its association with CAD and MI using a case–control study design. Previous GWAS in populations of European ancestry found that SNP rs3807989 was associated with the electrocardiographic PR interval and QRS duration [36–38] as well as AF [39,40], but not with CAD and/or MI. We studied three independent Chinese populations with a total of 6185 subjects (3299 CAD cases and 2886 controls) from GeneID [15,18,41–48]. Significant association was found between SNP rs3807989 and CAD/MI in all three populations and the large combined population. Moreover, eQTL analysis and ELISA protein expression analysis found that allele A of SNP rs3807989 was associated with an increased expression level of *CAV1* mRNA or protein.

2. Materials and methods

2.1. Study subjects

The subjects in this study were all from the Chinese GeneID database, which is one of the largest GeneBank databases for cardiovascular diseases and contains more than 80,000 study subjects with several different types of diseases, including CAD/MI, atrial fibrillation, ventricular arrhythmias, hypertension, stroke, congenital heart disease and controls in China. All study subjects were all of Han ethnic origin by self-description. This study was approved by appropriate local institutional review boards on human subject research and conformed to the guidelines set forth by the Declaration of Helsinki. All participants have provided written informed consent.

A total of 6185 subjects were characterized, including 3299 CAD patients/cases and 2886 non-CAD controls. The subjects were from three independent populations: Population I as the discovery population and Population II and Population III as independent replication populations (Table 1). There were 1249 CAD cases and 841 controls in Population I, 1260 cases and 833 controls in Population II, and 790 cases and 1212 controls in Population III (Table 1). The numbers of MI patients in Population I, II and III were 568, 609 and 304, respectively (Table 1).

The diagnosis of CAD was made by at least two independent cardiologists according to the standard guidelines established by the ACC/AHA. A patient with >70% of luminal stenosis in one or more main vessels detected by coronary angiography, coronary artery bypass graft (CABG), percutaneous coronary intervention (PCA), and/or MI was diagnosed as a CAD case. The diagnosis of MI was based on typical chest pain sustained for at least 30 min, characteristic electrocardiographic patterns of acute MI, and elevation of troponin I or T and cardiac enzymes such as creatine kinase-MB and lactate dehydrogenase. Patients with congenital heart disease, childhood hypertension, type I diabetes mellitus, myocardial spasm, and myocardial bridge identified by angiography were excluded [15,18,46]. Subjects without history of MI or detectable stenosis evaluated by coronary angiography were defined as controls. The demographic and other relevant clinical information, if present, were all obtained from the medical records.

2.2. Isolation of genomic DNA and genotyping of SNP rs3807989

Human genomic DNA was extracted from peripheral blood samples using the Wizard Genomic DNA Purification Kit (Promega Corporation).

SNP rs3807989 was genotyped using a Rotor-Gene™ 6000 High Resolution Melt system (Corbett Life Science, Concorde, NSW, Australia). The procedures of PCR and the high-resolution melting analysis (HRM) were described by us previously [15,41,43,45–48]. The sequences for primers for HRM genotyping and sequencing are: HRM forward primer, 5′-CGC GAC CCT AAA CAC CTC AA-3′ and reverse primer, 5′-TGA TTC TTT TTT GTC CTC TGG TGT C-3'; Sequencing forward primer, 5′- ATC CCT CCT CTC TGT TCA AGT TC-3′ and sequencing reverse primer, 5′- TGG CCT CAC GTG TTC ATT ATC-3′.

2.3. Real -time quantitative RT-PCR

Total RNA was isolated from human peripheral blood leukocytes using the Trizol reagent (Life Technologies, Gaithersburg, MD). The RNA samples were quantified, reverse-transcribed and used for real-time qRT-PCR analysis with the Faststart Universal SYBR Green Master Kit (Roche Applied Science, Indianapolis, IN) as described by us previously [15,18,47]. The primers used in the study were 5′-CGC GAC CCT AAA CAC CTC AA-3' (forward) and 5′-TGC CGT CAA AAC TGT GTG TCC-3' (reverse) for *CAV1*, 5′-GCC ATG CCC TCT TTG AAA TCA-3' (forward) and 5′-AAG GCA GAA CCA TTA GGC AGG-3' (reverse) for *CAV2*, and 5′-AAG GTG AAG GTC GGA GTC AAC-3' (forward) and 5′-GGG GTC ATT GAT GGC AAC AAT A-3' (reverse) for