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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 1,249 CAD cases and 841 controls in Population I, 1,260 cases and 833 controls in Population II and 790 cases and 1,212 controls in Population III (a total of 3,299 cases and 2,886 controls). We identified significant association between rs3807989 and CAD in three independent populations and in the combined population (P_{adj}=2.18×10^{-5}, OR=1.19 for minor allele A). We also detected significant association between rs3807989 and MI (P_{adj}=5.43×10^{-5}, OR=1.23 for allele A). Allele A of SNP rs3807989 was also

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Conflicts of interests/disclosures

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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 *CAV1* (but not *CAV2*). Thus, *CAV1* becomes a strong candidate susceptibility gene for CAD/MI in humans.

Keywords

Coronary artery disease (CAD) and myocardial infarction (MI); Atherosclerosis; Single nucleotide polymorphism (SNP); rs3807989; *CAV1 and CAV2*; Genome-Wide Association Study (GWAS)

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 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% to 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 6,185 subjects (3,299 CAD cases and 2,886 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, hypotension, 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 6,185 subjects were characterized, including 3,299 CAD patients/cases and 2,886 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 1,249 CAD cases and 841 controls in Population I, 1,260

cases and 833 controls in Population II, and 790 cases and 1,212 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 the *GAPDH* reference gene (internal standard). The ΔΔCq method ($RQ=2^{-\Delta\Delta Cq}$,$\Delta\Delta C_q$ is for the individual, ΔC_q is the calibrator) was used to determine the differences of the mean expression levels of *CAV1* and *CAV2* among different genotypes for SNP rs3807989 as described [15, 18, 47].

2.4. Measurement of expression levels of CAV1 protein in human serum samples

Serum samples were collected from whole blood samples from human study subjects. We measured the serum concentration of the CAV1 protein using an enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp. , USA) according to the instructions from the manufacturer. Absorbance was read on a spectrophotometer (VERSA max microplate reader, USA) at 450 nm.

2.5. Statistical analyses

Power analysis of each replication population was conducted using the Power and Sample Size Calculation program (PS version 3.0.43) (http://biostat.mc.vanderbilt.edu). Linkage disequilibrium (LD) analysis was performed using the physical map information of *CAV1* and *CAV2* and the MAF of the CHB population from HapMap (http://www.hapmap.org, phase1, 2 & 3) and Haploview 4.2. (http://www.broadinstitute.org). The assumptions for LD analysis were r^2 of 0.80 and MAF of 0.20. Hardy–Weinberg linkage disequilibrium analysis was performed with PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/archive.shtml) in each control population. The 2×2 Pearson χ^2 contingence tables were used for allelic association analysis. The 2×3 Pearson χ^2 contingence tables were used for genotypic association analysis. Odds ratios (ORs) and corresponding 95% confidential intervals were calculated using PLINK version 1.07 or SPSS version.17.0. For association analyses, we also performed multiple logistic regression analysis to adjust significant covariates for CAD using SPSS version.17.0.

To detect the association between SNP rs3807989 with serum lipid levels, we used SPSS version.17.0 to assess genotypic association by linear regression under an additive, dominant and recessive genetic model.

For real-time qRT-PCR data, unpaired student's *t* tests and ANOVA were used for statistical analysis with SPSS version.17.0.

For ELISA data, unpaired student's *t* tests and ANOVA were used for statistical analysis with SPSS version.17.0.

3. Results

3.1. Significant allelic association between CAV1 SNP rs3807989 and CAD

CAV1 is a small gene that spans a genomic region of 36,391 bp. Haploview 4.2 analysis showed that *CAV1* contained only one LD with four SNPs (rs3807986, rs3807989, rs11773845, and rs1997572) when r^2 was 0.80 and MAF was 0.20 (Fig. 1A). Any one of the four SNPs can sufficiently capture all genomic information of *CAV1*. Therefore, we selected rs3807989 in intron 2 as the tagSNP for *CAV1*. To determine whether *CAV1* tagSNP rs3807989 was associated with CAD, we performed genetic association analysis in three independent Chinese Han populations. Populations I, II, and III consisted of 1,249 CAD cases and 841 controls, 1,260 cases and 833 controls, and 790 cases and 1,212 controls, respectively (Table 1). The demographic and clinical characteristics of the study populations are shown in Table 1.

SNP rs3807989 was genotyped in Population I. Genotypic frequencies of rs3807989 were in Hardy-Weinberg equilibrium in the control population (P_{hwe}=0.85). Significant allelic association was identified between SNP rs3807989 and CAD with an odds ratio or OR of 1.24 for minor allele A ($P_{obs}=3.23\times10^{-3}$) (Table 2). After adjusting for significant covariates of hypertension, DM, smoking, alcohol consumption, gender and age, the association remained significant ($P_{adj}=8.04\times10^{-3}$, OR=1.27 for minor allele A) (Table 2). Because rs3807989 was shown to be associated with AF, we further adjusted the association for AF and found it to remain significant ($P_{adj}=1.40\times10^{-2}$, OR=1.25 for minor allele A). Therefore, the A allele of SNP rs3807989 is the risk allele in development of CAD.

For case control association studies, initial novel, significant association needs to be replicated in at least one other independent population. Therefore, we studied SNP rs3807989 in Population II and Population III. Statistical power analysis showed that Population II and Population III had a power of >80% and >90%, respectively, to replicate the association between SNP rs3807989 and CAD based on the OR and MAF from Population I.

SNP rs3807989 was genotyped in Populations II and III. Genotypic frequencies of rs3807989 were in Hardy-Weinberg equilibrium in the two control populations (P_{hwe}=0.11 in Population II and P_{hwe}=0.34 in Population III). Significant allelic association was identified between SNP rs3807989 and CAD in both Population II and Population III ($P_{obs}=6.87\times10^{-3}$, OR=1.21 for minor allele A in Population II; $P_{obs}=5.89\times10^{-3}$, OR=1.21 for minor allele A in Population III) (Table 2). After adjusting for significant covariates of hypertension, DM, smoking, alcohol drinking, gender and age, the association remained significant in both replication populations ($P_{adj}=1.98\times10^{-3}$, OR=1.29 for minor allele A in Population II; $P_{adj}=5.67\times10^{-3}$, OR=1.21 for minor allele A in Population III) (Table 2). These data validate the finding from the discovery population (I) that the minor allele A of SNP rs3807989 in *CAV1* conferred a risk of CAD. The association remained significant after further adjustment for AF in Population II ($P_{adj}=1.24\times10^{-3}$, OR=1.31 for minor allele A). No adjustment for AF was performed in Population III due to lack of clinical data on AF.

3.2. Significant allelic association between CAV1 SNP rs3807989 and MI

We also analyzed the association between rs3807989 and MI. There are 568, 609 and 304 MI patients in Population I, II and III, respectively. Significant allelic association was identified between SNP rs3807989 and MI in all three independent populations ($P_{obs}=1.71\times10^{-2}$, OR=1.23 for minor allele A in Population I; $P_{obs}=1.51\times10^{-2}$, OR=1.23 for minor allele A in Population II; $P_{obs}=4.35\times10^{-4}$, OR=1.40 for minor allele A in Population III) (Table 2). After adjusting for significant covariates of hypertension, DM, smoking, alcohol drinking, gender and age, the association remained significant ($P_{adj}=2.87\times10^{-2}$, OR=1.27 for minor allele A in Population I; $P_{adj}=9.60\times10^{-3}$, OR=1.30 for minor allele A in Population II; $P_{adj}=4.62\times10^{-4}$, OR=1.40 for minor allele A in Population III) (Table 2). These data suggest that the minor allele A of SNP rs3807989 in *CAV1* conferred a risk of MI. The association remained significant after further adjustment for AF in Population I ($P_{adj}=3.72\times10^{-2}$, OR=1.27 for minor allele A) and II ($P_{adj}=8.13\times10^{-3}$,

OR=1.32 for minor allele A). No adjustment for AF was performed in Population III due to lack of clinical data on AF.

3.3. Significant allelic association of SNP rs3807989 with CAD and MI in the combined population

To further assess the association between SNP rs3807989 and CAD or MI, we combined the three populations together. This generated a large case control association study population for CAD (3,299 cases and 2,886 controls) and for MI (1,481 cases and 2,886 controls). The association between SNP rs3807989 and CAD or MI became much more significant before and after adjustment of covariates in the combined CAD population ($P_{obs}=1.84\times10^{-4}$, OR=1.16; $P_{adj}=2.18\times10^{-5}$, OR=1.19) and in the combined MI population ($P_{obs}=2.25\times10^{-4}$, OR=1.20; $P_{adj}=5.43\times10^{-5}$, OR=1.23) (Tables 2). When the study populations were divided into male groups and female groups, the association between rs3807989 and CAD and MI remained significant in both male populations and female populations (Table 2). The data from three independent populations and the combined population provided strong genetic evidence that the minor allele A of SNP rs3807989 conferred a risk of both CAD and MI.

The control study subjects are mostly individuals undergoing physical examinations offered free for the active working groups by the government agencies or individual's working units/ institutions. The free physical examinations are not readily available for retired individuals (men, 60 years old or above; women, 50-55 years old or above). Therefore, the average age of the controls was younger than that of CAD/MI patients/cases (Table 1). To further minimize the confounding of age and sex, we generated case control populations for CAD and MI by matching each individual case to a control (Table 3). There are 2,187 cases and 2,187 controls matched for age and sex in the CAD population. Among 2,187 CAD cases, 958 study subjects were affected with MI, too (Table 3). The association between rs3807989 and CAD was highly significant ($P_{adj}=6.48\times10^{-6}$, OR=1.24) (Table 3). When divided into a male group and a female group, the association remained significant in both male populations and female populations (Table 3). Similar findings were made for MI (Table 3).

3.4. Genotypic association between rs3807989 and serum lipids levels

Atherosclerosis is usually associated with lipid levels, thus, we analyzed potential association between SNP rs3807989 and lipid levels, including the plasma concentrations of total cholesterol (TC), LDL-C, HDL-C and triglyceride (TG). For association analysis with lipid levels, we excluded any study subject with potential use of satins and other lipid lowering medications both in cases and controls to rule out the interference of pharmacological interventions. The means ± SE serum LDL-C levels were 2.63 ± 0.06 mmol/L for the AA genotype, 2.68 ± 0.05 mmol/L with for the AG genotype, and 2.68 ± 0.08 mmol/L for the GG genotype (Table 3). We found that SNP rs3807989 was significantly associated with LDL-C levels under an additive model and a dominant model (P_{adj} =0.03 and 0.02, respectively) (Table 4). Thus, the minor allele A of SNP rs1122608 was associated with a decreased level of LDL-C. No significant association was identified between SNP rs3807989 and TG, TC, or HDL-C.

3.5. Real-Time RT-PCR and ELISA analyses identified significant association between SNP rs3807989 and the expression level of CAV1, but not that of CAV2

SNP rs3807989 is located in the 2nd intron of the *CAV1* gene, therefore it may be associated with the expression level of *CAV1*. We performed real-time qRT-PCR analysis with two sets of total RNA samples isolated from leukocytes: one with 83 study subjects and the other with 88 subjects. Real-time qRT-PCR analysis showed that the expression levels of the *CAV1* mRNA were significantly different among carriers with the AA, AG, and GG genotypes in the first 83 subjects (P=0.003) (Fig. 1B). The expression levels of the *CAV1* mRNA were significantly higher in carriers with AA genotype than those with AG or GG genotypes (Fig. 1B). In order to validate the finding, we performed real-time qRT-PCR analysis in another 88 subjects (Fig. 1C). The expression levels of the *CAV1* mRNA were still significantly higher in carriers with the AA genotype than those with AG or GG genotypes ($P=1.76\times10^{-9}$) (Fig. 1C). When the two groups of study subjects were combined, significant association between SNP rs3807989 and the expression levels of *CAV1* remained highly significant ($P=8.00\times10^{-13}$) (Fig. 1D).

Further LD analysis revealed that *CAV1* and *CAV2* are located in the same LD block and rs3807989 can capture genomic information for both *CAV1* and *CAV2* (Fig. 2A). Therefore, it is important to determine whether tagSNP rs3807989 is also associated with the expression level of *CAV2*. We studied another independent group of study subjects with real-time qRT-PCR analysis. As shown in Fig. 2B, the expression levels of the *CAV1* mRNA were, as expected, significantly different among carriers with the AA, AG, and GG genotypes (P=0.02). On the other hand, rs3807989 was not associated with the expression level of the *CAV2* mRNA (P=0.38) (Fig. 2C).

To further confirm the association between SNP rs3807989 and the expression level of *CAV1*, we also performed ELISA analysis to measure the expression level of the CAV1 protein with 65 human serum samples, including 14 carriers with the AA genotype, 31 carriers with the AG genotype and 20 carriers with the GG genotype. As shown in Fig. 3, the expression levels of the CAV1 protein were significantly different among carriers with the AA, AG, and GG genotypes (P=0.018).

Together, these data suggests that SNP rs3807989 is significantly associated with the expression level of *CAV1* but not that of *CAV2*.

4. Discussion

In this study, we identified significant association between tagSNP rs3807989 in intron 2 of the *CAV1* gene and CAD as well as MI. The genetic evidence was particularly strong. The association between SNP rs3807989 and CAD was significant in all three independent populations and in the large combined population (3,299 cases and 2,886 controls). The association was significant before and after adjustment of significant covariates of CAD ($P_{obs}=1.84\times10^{-4}$, OR=1.16 for minor allele A; $P_{adj}=2.18\times10^{-5}$, OR=1.19) (Table 2). Similar findings were made for MI (1,481 cases and 2,886 controls; $P_{obs}=2.25\times10^{-4}$, OR=1.20; $P_{adj}=5.43\times10^{-5}$, OR=1.23) (Table 2). Minor allele A conferred a risk in development of CAD and MI, whereas the major allele G played a protective role. This is the first time that

rs3807989 in the *CAV1* gene is linked to CAD and MI in humans. To the best of our knowledge, no GWAS to date reported significant association between rs3807989 or other SNPs in *CAV1* and CAD/MI. We also mined two GWAS databases for CAD and found that one SNP, rs193567, showed a nominal *P* value of 0.04 for association with CAD in a Wuhan population, but the association was not significant in a Beijing population (*P*=0.59).

We then tried to identify the underlying mechanism by which SNP rs3807989 increases risk of CAD. Because SNP rs3807989 is located in the 2nd intron of the *CAV1* gene, we hypothesized that it affected the expression level of *CAV1*. We performed real-time qRT-PCR analysis in two groups of study subjects and found that the expression levels of the *CAV1* mRNA were significantly higher in carriers with the AA genotype than those with AG or GG genotypes in two independent populations as well as in the combined population (Fig. 1B-1D). Further eQTL analysis showed that although *CAV1* and *CAV2* are located in the same LD block, rs3807989 was associated with the expression level of *CAV1*, but not *CAV2* (Fig. 2B-2C). ELISA analysis also revealed significant association between rs3807989 and the expression level of the CAV1 protein (Fig. 3). These data indicate that the minor allele A of SNP rs3807989 was associated significantly with an increased expression level of *CAV1*. Therefore, it is likely that SNP rs3807989 increased expression of *CAV1*, which then leads to development of CAD and MI. The human findings were consistent with the observations made in *Cav1*-null mice. Frank et al [49] reported that $Cav1^{-/-}$ knockout mice showed a 70% decrease in atherosclerotic lesion area under $ApoE^{-/-}$ background. Engel et al [50] reported that $Cav1^{-/-}ApoE^{-/-}$ mice showed a 15-fold reduction in plaque size and infiltration of fewer inflammation cells such as macrophages, T cells and neutrophils. Fernandez-Hernando et al [32] reported that $Cav1^{-/-}$ $ApoE^{-/-}$ mice showed inhibition of atherosclerosis, but re-expression of *Cav1* in endothelial cells expanded atherosclerotic lesions. The same group later created transgenic mice that overexpress *Cav1* in endothelial cells under $ApoE^{-/-}$ background and these mice showed increased atherosclerosis [34]. Together, all these human and mouse studies suggest that increased *CAV1* expression increases risk of CAD, whereas decreased *CAV1* expression or *Cav1* deficiency inhibits development of CAD.

We also found that minor allele A of SNP rs3807989 was associated with a decrease of the LDL-C levels although the effect was very small. This result is consistent with the studies from $Cav1^{-/-}$ knockout mice with increased large, apoB-containing and remnant lipoproteins (VLDL-sized and IDL/LDL-sized fractions) when fed with Western diet [49]. This may be related to the role of caveolin-1 in transcytosis of LDL [50].

GWAS in populations of European ancestry found that SNP rs3807989 was significantly associated with atrial fibrillation (AF), however, controversial results were reported in Chinese replication studies [51, 52]. We have recently carried out additional replication studies and follow-up meta-analysis with 4 Asian populations and found a significant association between rs3807989 and AF ($P=3.40\times10^{-4}$, OR=0.81 for minor allele A or 1.24 for major allele G) [48]. However, in contrast to CAD and MI, the major allele G of rs3807989 is the risk allele for AF, whereas the minor allele A is a protective allele for AF. We speculate that the opposite effects of SNP rs3807989 on CAD/MI and AF may be related to the distinct roles of *CAV1* in cardiomyocytes (AF) and endothelial cells (CAD and

MI). In cardiomyocytes, caveolin-1 plays an important role in regulation of ionic currents by interacting with ion channels such as Kir2.1, KCNH2, HCN4, Nav1.8 and Nav1.5 [53-57]. Major allele G of rs3807989, which decreases expression of *CAV1*, may alters cardiac potassium or sodium currents, leading to development of AF. In contrast, Fernandez-Hernando et al [32, 34] showed that the role of *Cav1* in atherosclerosis involves endothelial cells. Minor allele A of rs3807989, which increases expression of *CAV1*, may decrease endothelial cell proliferation and migration and nitric oxide production, leading to increased risk of CAD.

In conclusion, for the first time we identified the significant association between SNP rs3807989 in intron 2 of *CAV1* and CAD and MI. We also demonstrated that minor allele A of rs3807989 was associated with an increased expression level of *CAV1* (but not *CAV2*), thereby leading to development of CAD and MI. These data identifies a new candidate susceptibility gene for CAD and MI and further our understanding of genetic bases of CAD.

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Highlight

- Identification of a new susceptibility gene (*CAV1*) for CAD and MI;
- First demonstration of association between a genomic variant in *CAV1* and CAD/MI;
- The minor allele A of *CAV1* tagSNP rs3807989 increases risk of CAD and MI;
- Allele A of rs3807989 is associated with increased *CAV1* expression, but not *CAV2* expression.

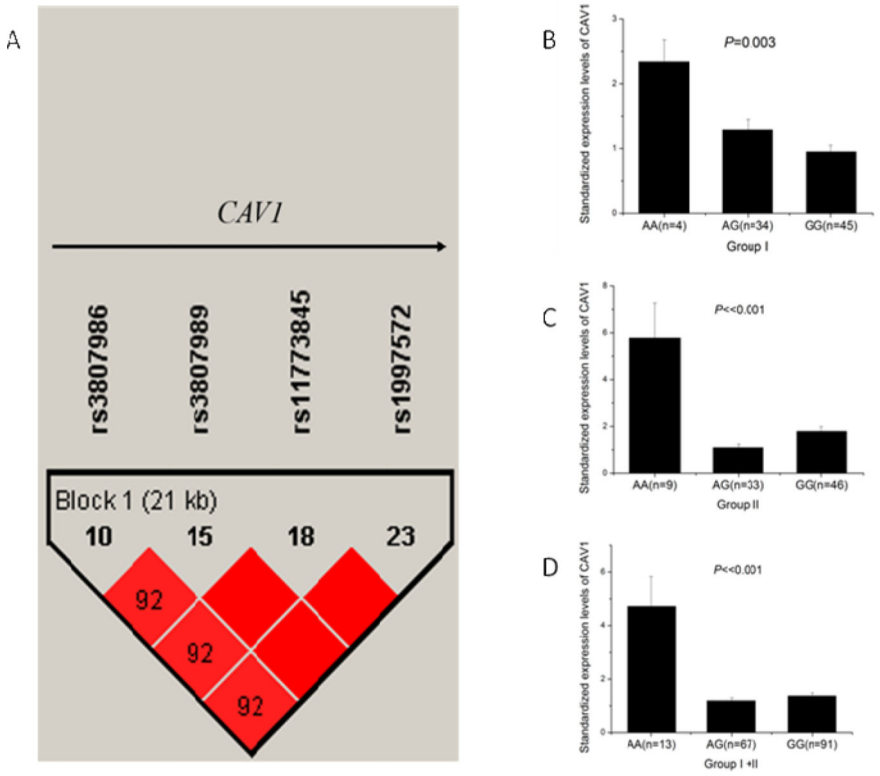

Fig. 1.

Identification of a tagSNP for *CAV1* by linkage disequilibrium (LD) analysis and significant association of SNP rs3807989 with expression of *CAV1* mRNA. (A) Overview of LD of the 36.4 kb genomic region spanning *CAV1*. The LD structure was derived from MAF of SNPs for the Chinese population from the HapMap database (http://www.hapmap.org, phase1, 2&3) using Haploview 4.2. *CAV1* has only one LD and rs3807989 captures all genomic information of the LD. (B-D) eQTL analysis of rs3807989 for *CAV1* in two independent groups of study subjects. Major allele G of rs3807989 was associated with decreased expression of *CAV1* mRNA , whereas minor allele A was associated with increased expression of *CAV1* mRNA under an additive model in two independent groups of samples (B, C) and in the combined group (D).

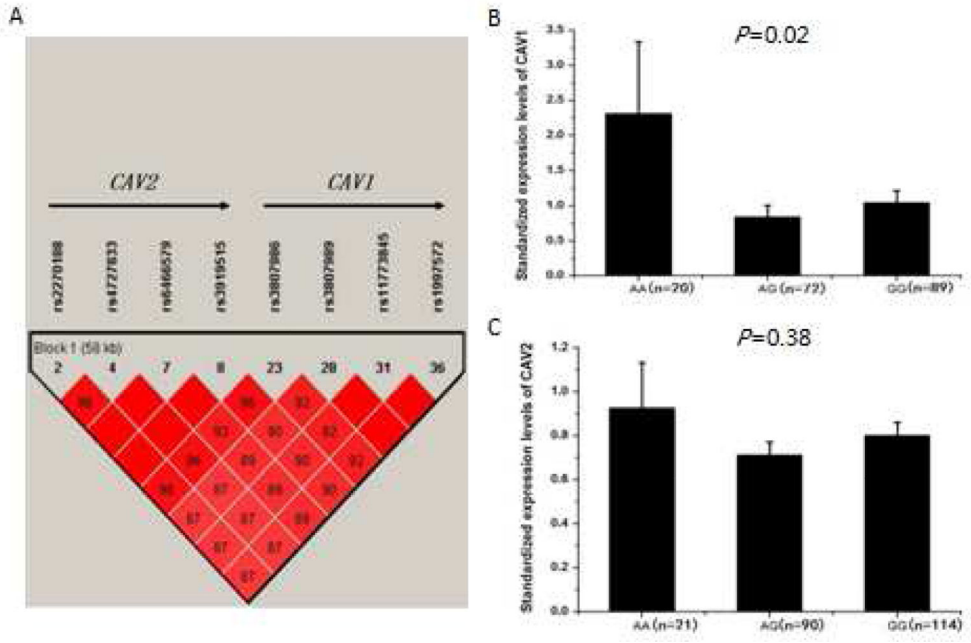

Fig. 2.

Significant association of SNP rs3807989 with expression of *CAV1* mRNA, but not with expression of *CAV2*. **(**A**)** Overview of LD of the 61.8 kb genomic region spanning both *CAV1* and *CAV2*. The LD structure was computed as in Fig. 1A. *CAV1* and *CAV2* are in the same LD and rs3807989 captures all genomic information of the LD. (B, C) eQTL analysis of rs3807989 for both *CAV1* and *CAV2* located at the same LD block. Major allele G of rs3807989 was associated with decreased expression of *CAV1* mRNA (B), but not with expression of *CAV2* (C) under an additive model in a group of study subjects.

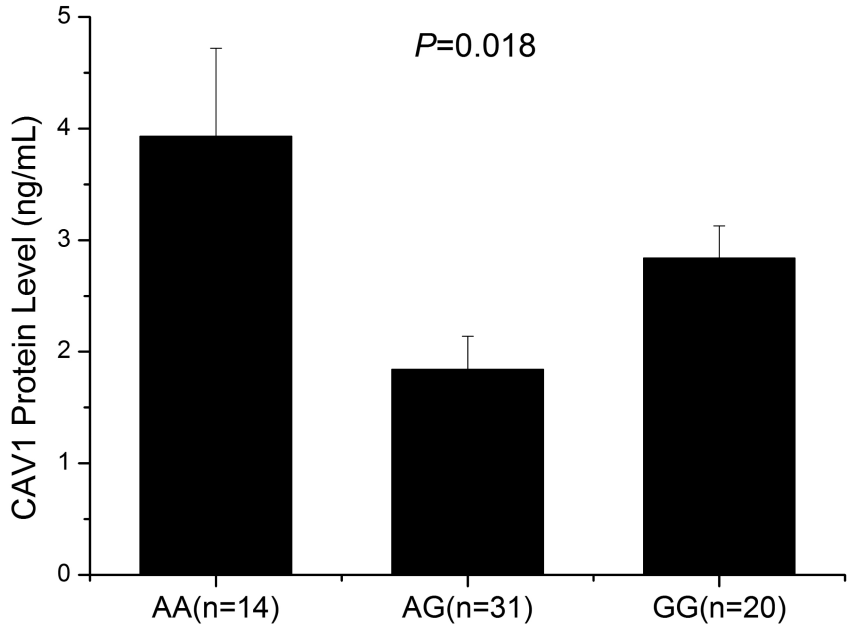

Fig. 3.

Significant association of SNP rs3807989 with expression of the CAV1 protein in serum samples. ELISA analysis showed that major allele G of rs3807989 was associated with decreased expression of the CAV1 protein.

Table 1

Clinical and Demographic Characteristics of Study Subjects.

Characteristics	Population I		Population II		Population III		Population I+II+III (Combined)	
	CAD (n=1,249)	Control (n=841)	CAD (n=1,260)	Control (n=833)	CAD (n=790)	Control (n=1,212)	CAD (n=3,299)	Control (n=2,886)
Male, n (%)	816 (65.33)	509 (60.52)	819 (65.00)	495 (59.42)	510 (64.56)	818 (67.49)	2,145 (65.02)	1,822 (65.13)
Age, years (mean±sd)*	65.08±12.57	58.80±10.68	66.18±12.26	59.52±10.85	58.30±10.26	57.97±11.80	63.87±12.34	58.66±11.23
Hypertension, n (%)	922 (73.82)	178 (21.17)	798 (63.33)	149 (17.89)	528 (66.84)	N/A	2248 (68.14)	N/A
DM, n (%)	265 (21.22)	39 (4.64)	261 (20.71)	41 (4.92)	136 (17.22)	N/A	662 (20.06)	N/A
Smoker, n (%)	330 (26.42)	56 (6.66)	303 (24.04)	63 (7.56)	294 (37.22)	N/A	927 (28.10)	N/A
Drinker, n (%)	195 (15.61)	27 (3.21)	203 (16.11)	46 (5.52)	14 (1.77)	N/A	412 (12.49)	N/A
Lipid lowering medication, n (%)	125 (10.00)	7 (0.83)	111 (8.80)	7 (0.84)	N/A	N/A	N/A	N/A
AF, n (%)	89 (7.12)	10 (1.19)	94 (7.46)	15 (1.80)	35 (4.43)	N/A	243 (7.37)	N/A
MI, n (%)	568 (45.48)	N/A	609 (48.33)	N/A	304 (38.48)	N/A	1,481(44.89)	N/A

*For quantitative traits, data were presented as means±SD.

Table 2

Allelic association of SNP rs3807989 with CAD and MI in Chinese Han populations and the combined population.

Study population		Sample size	Major/Risk Allele	MAF (case/control)	Before adjustment		After adjustment	
		Case/Control			P_{obs}	OR (95%CI)	P_{adj}	OR (95%CI)
CAD	Population I	1,249/841	G/A	0.277/0.237	3.23E-03	1.24 (1.07-1.43)	8.04E-03	1.27 (1.06-1.51)
	Population II	1,260/833	G/A	0.300/0.261	6.87E-03	1.21 (1.05-1.39)	1.98E-03	1.29 (1.10-1.52)
	Population III	790/1,212	G/A	0.344/0.302	5.89E-03	1.21 (1.06-1.38)	5.67E-03	1.21 (1.06-1.39)
	Combined	3,299/2,886	G/A	0.302/0.269	1.84E-04	1.16 (1.07-1.26)	2.18E-05	1.19 (1.10-1.29)
Subgroups	Male	2,145/1,822	G/A	0.306/0.273	1.43E-03	1.17 (1.06-1.29)	4.71E-04	1.19 (1.08-1.32)
	Female	1,154/1,064	G/A	0.295/0.268	0.052	1.14 (0.99-1.30)	1.76E-02	1.19 (1.03-1.36)
MI	Population I	568/841	G/A	0.276/0.237	1.71E-02	1.23 (1.04-1.46)	2.87E-02	1.27 (1.03-1.58)
	Population II	609/833	G/A	0.305/0.261	1.51E-02	1.23 (1.04-1.44)	9.60E-03	1.30 (1.07-1.58)
	Population III	304/1,212	G/A	0.370/0.302	4.35E-04	1.40 (1.16-1.68)	4.62E-04	1.40 (1.16-1.69)
	Combined	1,481/2,886	G/A	0.306/0.269	2.25E-04	1.20 (1.09-1.32)	5.43E-05	1.23 (1.11-1.36)
Subgroups	Male	1,036/1,822	G/A	0.295/0.273	8.12E-03	1.17 (1.04-1.32)	4.60E-03	1.19 (1.06-1.34)
	Female	445/1,064	G/A	0.313/0.268	8.04E-03	1.26 (1.06-1.50)	3.25E-03	1.33 (1.10-1.62)

P_{obs}: *P* value for association before adjusting for covariates by 2×2 contingence tables using PLINK version 1.07; P_{adj}: *P* value for association after adjusting for covariates of hypertension, DM, smoking, alcohol intake, gender and age by multiple logistic regression analysis using SPSS version17.0; OR: odds ratio; 95% CI: 95% confidential interval.

Table 3

Allelic association of SNP rs3807989 with CAD and MI in age- and sex-matched case-control populations.

Study population		Sample size	Major/Risk Allele	MAF (case/control)	Before adjustment		After adjustment	
		Case/Control			P_{obs}	OR (95%CI)	P_{adj}	OR (95%CI)
CAD		2,187/2,187	G/A	0.309/0.265	6.39E-06	1.24 (1.13-1.36)	6.48E-06	1.24 (1.13-1.36)
Subgroups	Male	1,505/1,505	G/A	0.311/0.268	2.21E-04	1.23 (1.10-1.38)	2.23E-04	1.23 (1.10-1.38)
	Female	682/682	G/A	0.304/0.260	9.43E-03	1.25 (1.06-1.48)	9.49E-03	1.25 (1.06-1.48)
MI		958/2,187	G/A	0.313/0.265	1.24E-04	1.26 (1.12-1.42)	1.20E-04	1.26 (1.12-1.42)
Subgroups	Male	718/1,505	G/A	0.306/0.268	9.04E-03	1.20 (1.05-1.38)	9.16E-03	1.20 (1.05-1.38)
	Female	240/682	G/A	0.333/0.260	1.93E-03	1.43 (1.14-1.79)	1.67E-03	1.44 (1.15-1.80)

P_{obs}: *P* value for association before adjusting for covariates by 2×2 contingence tables using PLINK version 1.07; P_{adj}: *P* value for association after adjusting for covariates of gender and age by multiple logistic regression analysis using SPSS version17.0; OR: odds ratio; 95% CI: 95% confidential interval.

Table 4

Clinical and demographic characteristics of serum lipids levels

Lipid	Case (2,592)	Control (707)	*P* value
Total cholesterol (TC, mmol/L) *	4.32±0.02	4.56±0.07	<0.001
LDL-C (mmol/L) *	2.65±0.04	3.25±0.14	<0.001
HDL (mmol/L) *	1.16±0.03	1.49±0.23	0.02
Triglyceride (TG, mmol/L) *	1.69±0.03	1.56±0.05	0.02

* For quantitative traits, data were shown as means±SE

Table 5

Genotypic association between rs3807989 and serum lipids levels under three different genetic models.

Lipid	Subgroups	P_{adj}			Effect		
		Additive*	Recessive*	Dominant*	Additive*	Recessive*	Dominant*
Total cholesterol (TC, mmol/L)	Combined	0.158	0.747	0.100	−0.024 (−0.119 to 0.019)	−0.006 (−0.184 to 0.132)	−0.028 (−0.164 to 0.014)
	Male	0.597	0.625	0.338	−0.012 (−0.119 to 0.068)	0.011 (−0.161 to 0.267)	−0.021 (−0.179 to 0.062)
	Female	0.067	0.146	0.118	−0.053 (−0.188 to 0.006)	−0.042 (−0.388 to 0.058)	−0.045 (−0.228 to 0.026)
LDL-C (mmol/L)	Combined	0.030	0.289	0.027	−0.038 (−0.280 to −0.014)	−0.018 (−0.469 to 0.140)	−0.039 (−0.366 to −0.022)
	Male	0.252	0.476	0.283	−0.025 (−0.231 to 0.061)	−0.016 (−0.455 to 0.212)	−0.024 (−0.291 to 0.085)
	Female	0.058	0.431	0.043	−0.055 (−0.514 to 0.009)	−0.023 (− 0.841 to 0.359)	−0.059 (−0.693 to −0.011)
HDL (mmol/L)	Combined	0.804	0.318	0.376	−0.004 (−0.192 to 0.149)	0.017 (−0.192 to 0.590)	−0.016 (−0.321 to 0.121)
	Male	0.490	0.082	0.930	0.015 (−0.126 to 0.264)	0.038 (−0.051 to 0.842)	−0.002 (−0.263 to 0.241)
	Female	0.270	0.700	0.222	−0.032 (−0.503 to 0.141)	−0.011 (−0.883 to 0.594)	−0.035 (−0.682 to 0.158)
Triglyceride (TG, mmol/L)	Combined	0.696	0.505	0.898	−0.007 (−0.082 to 0.055)	−0.011 (−0.210 to 0.103)	−0.002 (−0.094 to 0.083)
	Male	0.672	0.653	0.770	0.009 (−0.072 to 0.112)	0.010 (−0.162 to 0.259)	0.006 (−0.101 to 0.136)
	Female	0.138	0.041	0.439	−0.042 (−0.171 to 0.024)	−0.058 (−0.455 to −0.010)	−0.022 (−0.177 to 0.077)

P_{adj}, *P* value obtained from multiple linear modeling after adjustment for age, gender, hypertension, DM, smoking, and alcohol intake using SPSS v17.0; β, effect size related to the minor allele, using SPSS v17.0

*Additive model = AA/AG/GG; recessive model = AA/AG+GG; dominant model = AA+AG/GG.