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Research paper

# Genetic insight into the role of *MRAS* in coronary artery disease risk

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ABSTRACT

The muscle Ras (*MRAS*) gene polymorphisms have been reported to be associated with coronary artery disease (CAD) in white Europeans. The aim of this study was to ascertain the role of *MRAS* gene polymorphisms in conferring susceptibility to CAD, and to explore the effect on severity of CAD in Chinese population. We genotyped 5009 Chinese individuals (2466 CAD cases and 2543 controls) for eight single nucleotide polymorphisms (SNPs) around *MRAS* and used logistic regression analysis to determine whether they were associated with CAD. The association of the SNP loci on the severity of CAD was analyzed using a logistic and linear regression analysis, respectively. Our results revealed that an intron SNP, rs1199337, tends to be marginally associated with CAD as previously reported in Caucasians (nominal $P = 0.01$, OR 1.10, 95% CI 1.01–1.20). However, this association did not retain statistically significant levels after applying Bonferroni's correction for multiple testing (corrected $P = 0.08$). There was no significant association between other loci and CAD (nominal $P > 0.05$). We did not observe any significant association between the SNPs and severity of CAD (all $P$ values $> 0.05$). From the above results, the *MRAS* gene loci might have a minor effect in conferring susceptibility to CAD in Chinese population.


## 1. Introduction

Coronary artery disease (CAD), which manifests in many forms from chronic stable angina to that of sudden death in asymptomatic persons, is the leading cause of mortality and disability worldwide (He et al., 2005; Mathers and Loncar, 2006; Go et al., 2013). It is a disease that both genetic and environmental determinants (Gibbons et al., 2004). Over the past 5 years, researchers have completed many genome-wide association (GWA) studies to map underlying common susceptibility variants for CAD (Zeller et al., 2012). However, most GWA studies were performed in populations of European ancestry, and the identified loci altogether explain only a small fraction of the risk for CAD (Maouche and Schunkert, 2012). As linkage disequilibrium (LD) patterns of the human genome and allele frequency vary across populations (Lonjou et al., 2003; International HapMap Consortium, 2005), it is necessary to establish whether previously reported loci have a consistent effect across ethnic groups if they are to be used in cardiovascular risk assessment.

To date, GWA studies have successfully identified ~46 loci with common genetic variants associated with CAD risk in individuals of European and south Asian ancestry (Deloukas et al., 2013). Among these, the muscle Ras (*MRAS*) gene resides on chromosome 3q22.3 and encodes a member of the membrane-associated Ras small GTPase proteins, which function as signal transducers in multiple processes including cell growth and differentiation (Kimmelman et al., 1997; Yoshikawa et al., 2007; Watanabe-Takano et al., 2010). There have been reported that four single nucleotide polymorphisms (SNPs) (rs9818870, rs2306374, rs1720819 and rs1199337) within the *MRAS* gene are nominally associated with genetic susceptibility for CAD in European populations (Erdmann et al., 2009; Lluis-Ganella et al., 2010; O'Donnell et al., 2011; Reilly et al., 2011; Schunkert et al., 2011), and part of results have been replicated in Saudi populations (Alshahid et al., 2013). However, unlike in populations of European origin, the frequency of the rs9818870 T-allele and rs2306374 C-allele is relatively low (<1%) in the HapMap Han Chinese Beijing (CHB) databank (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp), raising the question of whether these variants are major contributors of CAD in the Chinese population. Furthermore, the association study of the genetic polymorphism around *MRAS* with CAD in the Chinese population has been lacking.

Detailed characterization of *MRAS* genetic variability could help to elucidate the role of *MRAS* in CAD and to identify potential CAD pathways. In contrast to previous studies focusing on the top hit, we assessed the impact of the whole genetic variability of *MRAS* on CAD in the Chinese population. Therefore, we conducted an association study in 5009 unrelated subjects (including 2466 CAD cases and 2543 controls) from a Han Chinese population. SNPs tagging common variations across

*Abbreviations:* CAD, coronary artery disease; GWA, genome-wide association; LD, linkage disequilibrium; MRAS, muscle Ras; CHB, Han Chinese Beijing; HWE, Hardy–Weinberg Equilibrium; VD, vessel disease; MAF, minor allele frequency.

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the *MRAS* gene were selected according the HapMap (CHB + JPT), and additional SNPs with previously reported to be associated with CAD in populations of European ancestry and potentially functional variants by resequencing were genotyped. We further assessed the effects of the *MRAS* genetic variants on severity of CAD.

## 2. Methods

### 2.1. Study subjects

We carried out a case–control association study involving 2466 unrelated cases that were consecutively selected from patients admitted to the Tongji Hospital in Wuhan (Hubei, China) with a diagnosis of CAD. The diagnostic criteria for CAD are as our described previously (Liu et al., 2013) and include >50% luminal narrowing in at least one vessel by coronary angiography, percutaneous coronary angioplasty, coronary artery bypass graft, and myocardial infarction. Patients with congenital heart disease, cardiomyopathy, valvular heart disease, and renal or hepatic disease were excluded from the study.

The coronary angiograms were available for 2205 CAD subjects and reviewed by two independent angiographers who were both blinded to the results of the genotype. CAD cases who were previously preformed coronary artery bypassed graft surgery and undergone percutaneous intervention for a lesion for which the degree of stenosis were excluded. The severity of CAD was assessed both by the number of diseased vessels and Gensini scores. The corresponding coronary angiograms and Modified Gensini scores were derived by a method previously described (Liu et al., 2013).

For the control group, a total of 2543 subjects, residing in the same communities as the cases, were determined to be free of CAD and peripheral atherosclerotic arterial disease by medical history, clinical examinations, and electrocardiography. All participants were of Han Chinese ancestry residing Wuhan area and underwent standard medical history and physical evaluations.

All the study protocols were approved by the institutional review board of Tongji hospital, and written informed consent was obtained from all participants. The investigation conformed to the principles outlined in the Declaration of Helsinki.

### 2.2. SNPs selection and genotyping

The TagSNPs for *MRAS* were selected by using Tagger program implemented in Haploview V4.2 from the HapMap genotype data for the Asians combined (JPT + CHB populations). For the common SNPs (minor allele frequency > 0.05), tagSNPs were selected with Tagger using a pairwise approach with an $r^2$ threshold > 0.8. This yielded 4 tagSNPs (rs1199333, rs1209710, rs1720819, rs40593). These SNPs flank a 40-kb genomic region on chromosome 3q22 (National Center for Biotechnology Information build 36.1 from 139570000 to 139610000). The initial set of 36 common variants (minor allele frequency >0.05) in this region can be well captured by this set of 4 haplotype-tagging SNPs, which were selected from the genotyped SNPs in the HapMap project (the Phase II database) using the pairwise tagging method in Haploview 4.2 (Supplementary Fig. 1). Additionally, two common SNPs (rs1199337, rs1720819) that were previously reported to be associated with CAD in Caucasians were also selected.

To identify the potentially functional variants within *MRAS*, we sequenced all the exons, exon–intron boundaries as well as 1-kb 5′ upstream from transcription initiation sites (containing putative regulatory elements) in 48 healthy controls and 48 CAD patients. Variation screening was performed according to standard protocol as previously described (Liu et al., 2013). We identified 3 common polymorphisms (rs1199332, rs40593, rs2293252) in the 3′ untranslated region and 1 polymorphism (rs3755751) in the promoter region of *MRAS*. As some SNPs are mentioned several times across the different selection approaches, 8 SNPs were selected for genotyping in the present study (Supplementary Table 1).

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (DB-S; Fujifilm Corporation, Life Science Products Division, Tokyo, Japan). Each SNP genotyping was performed according to standard TaqMan allelic discrimination assay as our previously described (Liu et al., 2013). The quality for SNP genotyping was assured by independently replicating the genotyping and allelic calls of 96 randomly selected samples. The results of quality control were 100% in agreement with the initial genotyping results.

### 2.3. Statistical analysis

Continuous variables were tested for normal distribution by using Kolmogorov–Smirnov test. Variables deviating from normal distribution were transformed by taking the natural logarithm of their value. For comparison of the baseline characteristics between groups of participants, quantifiable variables were compared with one-way ANOVA. Categorical values were compared by the Chi-square test or Fisher's test when appropriate. The distributions of genotype for variants were analyzed for deviation from Hardy–Weinberg Equilibrium (HWE) using Chi-square analysis.

The general association of genotypes with CAD was assessed by multiple unconditional logistic regression analysis and was adjusted for age, sex, body mass index (BMI), hypertension, diabetes, hyperlipidemia, smoking status. The model was fit with a generalized estimating equation technique to account for correlation among participants. Genotype was analyzed as a numeric variable representing the number (0, 1, 2) of copies of a given allele. *P* values were corrected for multiple comparisons using the Bonferroni method. Haplotype frequencies for various SNP combinations were estimated by haplo.stats version 1.2.1 for the R programming language and double-checked using Haploview 4.2.

Additional quantifying scores for severity of CAD, such as the Modified Gensini score and number of diseased vessels, were assessed by linear regression and logistic regression with adjustment for covariates, respectively. Severity of CAD in the case group was dichotomized in two vessel disease groups: (a) 1 vessel disease (VD) versus 2 and 3VD, and (b) 1 and 2 VD versus 3 VD.

The power of sample size to identify the association of investigated SNPs with CAD was calculated using Quanto version 1.2.3 (University of Southern California, Los Angeles, CA, USA). Assuming a minor allele frequency of 0.05 and disease prevalence of 0.5–1%, we had 80% power to detect genetic effects at an OR of 1.29, 3.24, and 1.27 under a dominant, recessive, and additive model in our samples, respectively.

Statistical and association analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA). All tests were two-sided and *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Characteristics of study population

The participants were all of self-reported Chinese Han population. The general characteristics of the study subjects, containing 2466 CAD cases and 2543 ethnically and geographically matched controls, are summarized in Table 1. As expected, the traditional CHD risk factors such as hypertension, hyperlipidemia, diabetes and smoking were significantly different between the cases and controls.

### 3.2. Effects of MRAS SNPs on CAD risk

The minor allele frequency (MAF) of each SNP selected for the present study (see Methods section) in the case and control groups was shown in Table 2. Genotype distributions of all the SNP loci did not deviate from HWE (all *P* value > 0.05) between the control population and case population. The *MRAS* SNPs showed no association with