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Association between rs10118757(A/G) in *methylthioadenosine phosphorylase* gene and coronary artery disease in Chinese Hans

Xiaofei Lv [a,b,1], Yuan Zhang [c,1], Shaoqi Rao [d,1], Dongfang Su [a,1], Dan Feng [e], Min Wang [a], Xinrui Li [a], Dan Li [a], Honghui Guo [f], Xiaoyu Zuo [e], Min Xia [a], Haimei Ouyang [b], Wenhua Ling [a], Jian Qiu [c,*]

[a] *Guangdong Provincial Key Laboratory of Food, Nutrition and Health, Department of Nutrition, School of Public Health, Sun Yat-Sen University (Northern Campus), 74 Zhongshan Road 2, Guangzhou, 510080, China*
[b] *Department of Obesity and Metabolism, Guangdong Women and Children Hospital, 521 Xingnan Road, Guangzhou, 511400, China*
[c] *Department of Cardiology, Guangzhou Military Region General Hospital, 111 Liuhua Road, Guangzhou, 510070, China*
[d] *Department of Medical Statistics and Epidemiology, School of Public Health, Guangdong Medical College, 1 New City Avenue, Songshan Lake Science and Technology Industry Park, Dongguan 523808, Guangdong, China*
[e] *Department of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University (Northern Campus), Guangzhou, China*
[f] *Department of Food Science, Yingdong College of Bioengineering, Shaoguan University, Daxue Road, Shaoguan, 512005, Guangdong Province, China*

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ABSTRACT

Studies focusing on the association of gene *methylthioadenosine phosphorylase* (*MTAP*) with the risk of coronary artery disease (CAD) and myocardial infarction (MI) are limited.
In this study, we explored the effects of rs10118757 in *MTAP* gene on CAD and MI by performing association analysis in a Chinese Han population. rs10118757 was genotyped in 1007 CAD patients (including 338 MI patients) and 885 healthy controls. Allelic analysis showed that allele A of rs10118757 was associated with increased risk of CAD, with OR (95%CI) = 1.193 (1.035–1.376), and $P = 0.015$. After adjusted for age, BMI, gender, hypertension and smoking, rs10118757 was still significantly associated with CAD under additive and dominant models, with OR (95%CI) = 1.252 (1.070–1.465), $P = 0.005$, and OR (95%CI) = 1.698 (1.168–2.467), $P = 0.006$, respectively. Compared to additive model, dominant model may be the best-fitting model ($P = 6.63E-10$ vs $P = 6.70E-10$). As reported previously, rs10118757 was not associated with MI in the current study.
Our study firstly reported that SNP rs10118757 was associated with CAD risk in a Chinese Han population, indicating that *MTAP* gene may play a potential role in the pathophysiological process of CAD.

1. Introduction

Coronary artery disease (CAD) is a worldwide health concern in both developing and developed countries. The global endemic of CAD calls for improved risk assessment. Identification of the underlying genetic architecture of CAD may provide improved risk assessment and better measures for prevention and treatment. Up to date, GWAS have identified thousands of risk genes for CAD, but studies on the relationship between *methylthioadenosine phosphorylase* (*MTAP*) gene and CAD or myocardial infarction (MI) are few.

Gene *MTAP* encodes methylthioadenosine phosphorylase (MTAP), which is the first enzyme in a salvage pathway that allows reuse of sulfur from 5-methythioadenosine for methionine biosynthesis. In the major pathway of methionine metabolism, methionine is converted to S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), and then homocysteine. Gene *MTAP* spans 63 kb, and SNP rs10118757 is located at the fifth intron. Li et al. reported that rs10118757 was associated with ischemic stroke in a Chinese population (Li et al., 2009). CAD and ischemic stroke may share common pathogenic mechanisms. Thus, here we tested the association between SNP rs10118757 in gene *MTAP* with CAD and MI, in a case–control study conducted in a Chinese Han population.

2. Methods

2.1. Subjects

In this study, the recruit criteria of CAD patients and healthy controls were described previously (Lv et al., 2012). CAD was diagnosed

Abbreviations: BMI, body mass index; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; CI, confidence interval; DNA, deoxyribonucleic acid; IFN-γ, interferon-γ; MAF, minor allele frequency; MI, myocardial infarction; MTAP, methylthioadenosine phosphorylase; OR, odds ratio; PTCA, percutaneous transluminal coronary angioplasty; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SNP, single nucleotide polymorphism.

* Corresponding author. Tel./fax: +86 20 36653568.
E-mail addresses: lvxfei@mail3.sysu.edu.cn (X. Lv), fly19803133@yahoo.com.cn (Y. Zhang), raoshaoq@gdmc.edu.cn (S. Rao), sdfbest@126.com (D. Su), fengdan3@mail.sysu.edu.cn (D. Feng), wangmin1605@tom.com (M. Wang), xinrui_603@163.com (X. Li), dolphin_danli@yahoo.com.cn (D. Li), guohh1999@hotmail.com (H. Guo), zuoxiaoyu1984@126.com (X. Zuo), xiamin@mail.sysu.edu.cn (M. Xia), 13729882809@163.com (H. Ouyang), lingwh@mail.sysu.edu.cn (W. Ling), grace198033@126.com (J. Qiu).
[1] Contributed equally to this work.

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following the definition described by Amouyel et al. (2009). Patients with diabetes, valvular heart disease or myocardiopathy were excluded. All subjects were of the ethnic Han origin and are not related to each other. Full hospital records were reviewed to confirm the diagnosis. At the enrollment, anthropometric measures for both cases and controls were collected by well-trained interns and physicians. Body mass index (BMI) was calculated using the formula: BMI = weight in kilograms/(height in meters × height in meters). The study was approved by the Ethics Committees of the participating hospital and institutes. All subjects signed the written informed consent forms. The investigation conformed to the principles outlined in the Declaration of Helsinki.

2.2. Biological samples collection

Fasting blood was drawn from the cubital vein and immediately stored at 0 °C. Within 4 h, the samples were centrifuged at 1734 *g* for 15 min at 4 °C, then isolated and separated into plasma and white blood cells. Finally, they were sealed and stored at −80 °C until analysis.

2.3. Genotyping

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood lymphocytes using the TIANamp Blood DNA Kit (Tiangen Inc.; Beijing; China). rs10118757 was genotyped using Illumina Golden Gate Genotyping Bead Chips (Illumina Inc.; San Diego; USA).

2.4. Quality control

The quality for SNP genotyping was assured by independently replicating the genotyping and allelic calls of 30 randomly selected samples. All the DNA samples for cases and controls were run in the same batches.

2.5. Statistical analysis

Continuous variables were expressed as the mean ± SD, and the difference between two groups was analyzed by independent *t*-test or Mann–Whitney *U* test. Categorical variables were summarized as frequency (percentage) and analyzed by Chi-square test. Statistical analyses above were performed with SPSS 13.0 (SPSS Inc., Chicago, USA).

Hardy–Weinberg equilibrium (HWE) test, minor allele frequency (MAF) calculation, allelic and genotype association tests were conducted by using the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink). The deviation from HWE was tested with a chi-square statistic. Logistic regression was used to determine the effect of rs10118757 on risk for CAD and MI. Multivariate analyses were used to adjust for several well established risk factors, age, BMI, gender, hypertension and smoking. Odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated. As only single SNP was analyzed, $P<0.05$ was considered to be statistically significant. Comparisons of goodness-of-fit between different models were tested by using likelihood ratio test.

3. Results

3.1. Study population

Blood sample from 1915 participants were genotyped, of which 23 samples were not successfully called. Consequently, 1892 participants, composed of 1007 CAD patients (388 MI patients) and 885 healthy controls, were included in the following analyses. Characteristics of the participants and genotype distribution are shown in Table 1. Compared to the controls, patients with CAD or MI were older, more likely to be current smokers and in hypertension status, and patients with CAD are more likely to have larger BMI.

Table 1
Characteristics of the study population, and genotype distribution of rs10118757.

Characteristics	CAD cases	MI cases	Healthy controls	*P* for CAD	*P* for MI
Sample size	1007	338	885	–	
Male (%)	683 (67.8%)	286 (84.6%)	563 (63.6%)	0.054	<0.001
Age	63.8 ± 5.72	61.9 ± 12.44	59.90 ± 5.63	<0.001	0.005
BMI	23.78 ± 3.45	23.7 ± 3.08	23.23 ± 3.07	<0.001	0.417
Smokers (%)	430 (42.7%)	192 (56.8%)	294 (33.2%)	<0.001	<0.001
Hypertension (%)	604 (60%)	170 (50.3%)	215 (24.3%)	<0.001	<0.001
Genotype				0.024	0.336
GG	63	23	83		
GA	403	146	362		
AA	541	169	440		

3.2. Characteristics of SNP

The genotyping success rate of rs10118757 was 99.0%. No significant deviation from the HWE was observed in controls ($P=0.52$). The frequency of the minor allele G was 0.279. The result from quality control was in perfect agreement with the initial genotyping result.

3.3. Allelic and genotype association analyses

The frequency of allele A was significantly higher in CAD cases than in controls (0.737 vs 0.702, $P=0.015$), with an allelic risk estimate of OR (95% CI) = 1.193 (1.035–1.376). The genotypic distribution between CAD cases and health controls were also significantly different ($P=0.024$), as shown in Table 1. Further univariate (with only SNP modeled) and multivariate logistic regression (aiming to adjust for age, BMI, gender, hypertension and smoking) revealed that rs10118757 had highly significant effects on CAD. The adjusted genotypic risk estimates under additive model and dominant model were 1.252 ($P=0.005$) and 1.698 ($P=0.006$), respectively (Table 2). According to the results, the SNP either directly exerts an effect or links to functional gene impacts CAD in additive or dominant inheritance manner, and dominant model may be the best-fitting model ($P=6.63E-10$ vs $P=6.70E-10$).

Allele frequency showed no difference between MI patients and healthy controls ($P=0.76$). Genotype association analyses showed that there was no association between rs10118757 and MI, under additive, recessive or dominant models, neither in univariate nor multivariate logistic regression models ($P\geq0.15$, $P\geq0.18$). The results were not shown here.

4. Discussion

Gene *MTAP* is often reported as a tumor suppressor gene deleted in various tumors, such as leukemia (Della Ragione et al., 1995), hepatocellular carcinoma and gastric carcinoma (Kim et al., 2011; Kirovski et al., 2011). As for its relationship to cardiovascular diseases, studies are limited. As far as we know, Li et al. reported that rs10118757 was associated with ischemic stroke in Chinese (Li et al., 2009) and Yang et al. (2009) reported that rs10118757 was not associated with MI in Chinese Han population, which was in accord with our result.

Based on Li's research on rs10118757 with ischemic stroke, and the conception that ischemic stroke and CAD may share common pathogenic mechanisms, we carried out a case–control study to assess the potential effect of rs10118757 in *MTAP* gene on CAD and MI in a Chinese Han population. We carefully designed and implemented this study. First, we recruited a large sample adequate to detect a moderate-size SNP. Second, we carefully selected the CAD cases by using a widely accepted criterion. And moreover, to avoid any racial mixture, our participants were limited in Guangdong region of confirmed Han origin. Finally, to well control confounding factors, we performed multivariate logistic regression with covariates age, BMI, gender, hypertension and smoking simultaneously modeled. We found that rs10118757 was

Table 2
Logistic regression analysis of genotypic effects of rs10118757 on CAD.

SNP	Risk allele	Genetic model	Univariate[a]		Multivariate[b]	
			OR	*P*	OR	*P*
rs10118757	A	Additive	1.195 (1.036–1.378)	0.015	1.252 (1.070–1.465)	0.005
rs10118757	A	Dominant	1.551 (1.103–2.180)	0.012	1.698 (1.168–2.467)	0.006

[a] With only SNP modeled.
[b] In addition to the SNP, age, BMI, gender, hypertension and smoking were also included.

associated with CAD but not MI. Allele A is the risk allele, and dominant model may be the most likely inheritance model.

Our results inferred that SNP rs10118757 may be in strong linkage disequilibrium with a true causal variant, or may be a causal variant. Although rs10118757 is located in the intronic region of *MTAP* gene, there is still a possibility that it may be a causal variant, by regulating the gene-expression of *MTAP* gene (Tokuhiro et al., 2003). A recent animal model provided clues, the research from Kim et al. found that compared with wild-type mice, the mice heterozygous for *MTAP* developed larger atherosclerotic lesions, and their further research suggested that the effect on lesion size was due solely to the difference in *MTAP* expression levels (Kim et al., 2012). Their results also showed that the mice heterozygous for *MTAP* had trends toward lower levels of SAM and SAH, indicating that *MTAP* may have an effect on CAD risk by regulating the methionine processing pathway. While, another study demonstrated that several variants in 9p21 region (including *CDKN2A/B*, *MTAP* and *IFNA21*) imposed an effect on CAD by impairing IFN signaling response (Harismendy et al., 2011). Further research is needed to reveal the molecular mechanisms underlying the association.

We are conscious that because of the limitations in this study, such as only including one SNP, overlooking the interaction between different genes, and not controlling other potential confounders, such as lipid and blood pressure level, the genetic parameter estimates (odds ratios, risk allelic or genotype frequencies) under a simplified model may be over-biased. We also realized that it could be better if we explore the influence of rs10118757 on methionine metabolites, such as SAM and SAH. That is what we plan to do next. To explore the full scape of pathogenic nature of *MTAP* gene, multiple repetitive and independent studies across different populations as well as molecular functional studies from the wet laboratories are essential.

5. Conclusion

SNP rs10118757 in *MTAP* gene is an independent risk factor for CAD, with allele A as the risk allele, and confers risk most likely in dominant model.

Competing interests

All of the authors declare no conflict of interest.

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Disclosure statement

None.

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