Association study of *ACE2* (angiotensin I-converting enzyme 2) gene polymorphisms with coronary heart disease and myocardial infarction in a Chinese Han population

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ABSTRACT

Results are accumulating that ACE2 (angiotensin I-converting enzyme 2) might act as a protective protein for cardiovascular diseases; however, only a few studies in human populations have been carried out. This prompted us to perform a case-control study to investigate the relationship of *ACE2* polymorphisms with CHD (coronary heart disease) and MI (myocardial infarction). Three single nucleotide polymorphisms in the *ACE2* gene (1075A/G, 8790A/G and 16854G/C) were genotyped by PCR-RFLP (restriction-fragment-length polymorphism) in 811 patients with CHD (of which 508 were patients with MI) and 905 normal controls in a Chinese population. The polymorphisms were in linkage disequilibrium ($r^2 = 0.854$–0.973). Analyses were conducted by gender, because the *ACE2* gene is on the X chromosome. In females, an association was detected with MI for 1075A/G ($P = 0.026$; odds ratio = 1.98) and 16854G/C ($P = 0.028$; odds ratio = 1.97) in recessive models after adjusting for covariates. In male subjects, two haplotypes (AAG and GGC) were common in frequency. In male subjects not consuming alcohol, the haplotype GGC was associated with a 1.76-fold risk of CHD [95% CI (confidence interval), 1.15–2.69; $P = 0.007$] and a 1.77-fold risk of MI (95% CI, 1.12–2.81; $P = 0.015$) with environmental factors adjusted, when compared with the most common haplotype AAG. In conclusion, the results of the present study indicate that common genetic variants in the *ACE2* gene might impact on MI in females, and may possibly interact with alcohol consumption to affect the risk of CHD and MI in Chinese males.

Key words: association study, angiotensin I-converting enzyme 2 (ACE2), coronary heart disease, gender, myocardial infarction, single nucleotide polymorphism.

Abbreviations: Ang, angiotensin; ACE, Ang I-converting enzyme; BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CI, confidence interval; DBP, diastolic BP; HDL-C, high-density lipoprotein-cholesterol; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; LDL-C, low-density lipoprotein-cholesterol; MAF, minor allele frequency; MI, myocardial infarction; OR, odds ratio; RAS, renin–angiotensin system; SBP, systolic BP; SNP, single nucleotide polymorphism; TC, total cholesterol.

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INTRODUCTION

CHD (coronary heart disease), including its most severe complication MI (myocardial infarction), is a leading cause of death worldwide. It is believed to be caused by many genetic and environmental factors as well as their interactions. The prevailing view is that CHD/MI is a disease of inflammation and immune responses [1], and atherosclerosis underlies the major pathophysiological mechanisms [2].

Genes in the RAS (renin–angiotensin system) have been widely used for association studies of cardiovascular diseases, in particular the *ACE* [Ang (angiotensin) I-converting enzyme 1] gene [3]. ACE cleaves the inactive decameric peptide Ang I into the active octomer Ang II, which is the biological effector of the RAS system. Ang II is widely related to pro-atherosclerotic and inflammatory actions [4].

ACE2 (Ang I-converting enzyme 2), a homologue of *ACE*, has recently been identified and considered to act as an 'anti-RAS' element [5]. It is predominantly expressed in vascular endothelial cells of the kidney and heart. It cleaves a single residue from Ang I to generate Ang-(1–9), and metabolizes Ang II to generate Ang-(1–7) [6,7], which is a potent vasodilator and antitrophic peptide that has generated considerable interest during the past decade [8,9]. Thus ACE2 may counteract the function of ACE either by converting Ang II into Ang-(1–7) or by competing with ACE for the same substrate Ang I [10]. Besides the Angs, it also hydrolyses other peptide substrates. For instance, it can degrade bioactive opioid peptides and generate apelin peptides and kinin metabolites that activate APJ (Ang-receptor-like 1) and B_1 receptors respectively [11].

The *ACE2* gene is located on the X chromosome (Xp22) with 19 exons plus a newly identified 5′-untranslated exon [6,7,12]. It has been mapped to a QTL (quantitative trait locus) associated with hypertension in three rat models of high BP (blood pressure) [10]. In *ACE2*-deficient mice, there is an increase in Ang II levels, even though no apparent effect on BP was observed. A reduction in cardiac contractility and a significant decrease in aortic and ventricular pressure was also observed in these mice [10]. A potential role for ACE2 in contributing to the cardioprotective effect of Ang-(1–7) was demonstrated when long-term infusions of Ang-(1–7) reversed cardiac dysfunction and restored vascular endothelial responses in animals after MI [13]. Burrell et al. [14] found that MI increased ACE2 expression in rats and humans. These data suggested a relationship between the *ACE2* gene and CHD.

In contrast with the rapid accumulation of findings about the important role of the *ACE2* gene in cardiovascular diseases from animal-model-based experiments, only a few studies have been carried out to investigate the gene in human populations. A study in Australians of white Anglo–Celtic origin reported no association of *ACE2* gene polymorphisms with essential hypertension [15], and Chen et al. [16], in a meeting abstract, suggested associations between two polymorphisms of this gene and a number of cardiovascular end points in a Caucasian population. A recent study in a German population [17] found a haplotype consisting of four SNPs (single nucleotide polymorphisms) spanning the gene associated with higher risk of left ventricular mass index, septal wall thickness and left ventricular hypertrophy. As far as we know, whether the *ACE2* gene is associated with human CHD/MI has not been directly addressed yet. This led us to conduct an association study between *ACE2* gene polymorphisms and human CHD/MI.

MATERIALS AND METHODS

Subjects

The enrolment criteria of the CHD cases and controls for the Beijing Atherosclerosis Study have been reported in detail previously [18]. We recruited 811 patients with CHD from patients hospitalized at the Fu Wai Hospital and Cardiovascular Institute (Beijing, China) between October 1997 and December 2001. Eligible patients were those who survived an acute MI or were documented by coronary angiography to have evidence of at least a 70% stenosis in a major epicardial artery. Patients with congenital heart disease, cardiomyopathy, valvular disease and renal or hepatic disease were excluded. As a subset of the patients with CHD, the MI group consisted of 508 patients. A total of 905 control subjects were randomly selected from individuals participating in a community-based survey of cardiovascular risk factors. The controls were judged to be free of ischaemic changes by ECG, to have no symptoms of chest pain and to be free of CHD by medical history, the Rose questionnaire [19] and clinical examination. This study was approved by the local Research Ethnics Committee, and all subjects gave written informed consent.

A set of questionnaires was completed that included details of medical history, family history of CHD, drug intake, cigarette smoking and alcohol consumption. BP, weight, height, waistline and hip circumference were recorded, and BMI (body mass index) was calculated. Hypertension was defined as SBP (systolic BP) $\geqslant$ 140 mmHg and/or DBP (diastolic BP) $\geqslant$ 90 mmHg, a history of hypertension, or the patient taking anti-hypertension medication.

Venous blood was drawn from all subjects after an overnight fast. Blood, serum and plasma were separated immediately and stored at -70°C.

SNP selection and genotyping

Three SNPs, 1075A/G (rs1978124 in intron 1), 8790A/G (rs2285666 in intron 3) and 16854G/C (rs4646142 in

intron 7), were selected as proxies to study *ACE2* polymorphisms. This was justified by the strong LD (linkage disequilibrium) throughout the gene which has been suggested in various studies [15–17,20] (details in the Discussion section). All three SNPs had been involved in other studies [15,16,20].

Genomic DNA was isolated from white blood cells using the phenol/chloroform method and was stored in 400 μl of TE [10 mM Tris/HCl and 1 mM EDTA (pH 8.0)].

The three SNPs were genotyped using standard PCR-RFLP (restriction-fragment-length polymorphism) protocols. Primers and conditions for amplification are available from the authors upon request.

Quality control was performed by re-genotyping all three SNP in 98 subjects randomly selected from the control group. The replication of genotyping was performed by another team member. A comparison between the two showed that the discrepancy was 1.36 %, with 95 % CI (confidence interval) ranging from 0.7–2.04 %.

Statistical analyses

Analyses were taken separately for the three SNPs and were followed by haplotype analyses. In consideration of the different number of copies of the X chromosome in males and females, separate analyses were taken for each subgroup.

Departure from the HWE (Hardy–Weinberg equilibrium) was tested among females by the χ^2 test. LD was estimated using GOLD software [21]. For single locus analyses, genotype and allele frequency distributions across healthy individuals and patients were tested by χ^2 test, and for female genotypes, association was analysed under additive, dominant and recessive models respectively. Furthermore, covariates, including age, BMI, smoking, alcohol consumption, history of hypertension, triacylglycerols (triglycerides), TC (total cholesterol), HDL-C (high-density lipoprotein-cholesterol) and glucose were adjusted by logistic regression to investigate the independent role of each polymorphism.

Male haplotypes were readily available, and we used the EM algorithm to account for missing genotypes (13 missed for 16854G/C). Both χ^2 test and backward stepwise logistic regression were used to assess the association between the male haplotypes and CHD/MI. In females, the association of haplotypes with diseases was analysed using the Haplo.score approach, as outlined by Schaid et al. [22]. This approach was implemented in the haplo.stats software developed using the R language (http://www.R-project.org). The method helps with the inference of haplotypes and accounts for haplotype ambiguity. It can be used to adjust for covariates. Estimated haplotype pairs were also taken for diplotype analysis.

We analysed further the interaction between the genetic effects (genotypes and haplotypes) and effects of alcohol consumption, smoking and BMI by introducing the corresponding product terms into the logistic regression models.

General calculations, including χ^2 tests and logistic regression, were performed using the Stata software (Stata/SE 8.0 for windows). We used $P < 0.05$ to define statistical significance. In backward stepwise logistic regression, we used significant levels of 0.2 for removal from the model and of 0.05 for addition into the model.

RESULTS

The demographic details of the patients and controls are given in Table 1. Compared with the control group, the CHD group had more patients with hypertension, higher mean BMI and SBP, higher levels of serum triacylglycerols, LDL-C (low-density lipoprotein-cholesterol) and fasting glucose, and lower HDL-C levels. However, DBP was significantly lower in patients with CHD, which could be a result of medication taken after they were diagnosed, as 11.7 % of the controls and 44.5 % of the patients were taking antihypertension medication. Characteristics of the MI group of patients were similar with those of the CHD group, except that TC and LDL-C levels were not significantly different compared with the controls, and that the MI group had larger proportions of smokers and alcohol consumers.

Genotype and allele frequencies for each polymorphism in male and female subjects are shown separately in Tables 2 and 3 respectively. MAF (minor allele frequency) was higher in the female controls compared with the male controls for each of the SNPs, but this difference was not statistically significant. HWE could be tested in only the female population and showed no significant deviation for all of the three sites in the control group and in patients with CHD. However, in female patients in the MI group, HWE was not in accordance with expectation for any of the SNPs. Pairwise LD tests indicated that all of the three SNPs investigated were in strong LD with each other ($D' = 0.929$–0.995, $P < 0.0001$; $r^2 = 0.854$–0.973).

None of the polymorphisms had any association with CHD or MI in male patients (Table 2), and neither did they have any association with female CHD in additive, dominant or recessive models (Table 3). Calculating the ORs (odds ratios) for female patients with CHD and MI suggested a recessive model effect, and association tests showed that carriers of the 1075AA and 16854GG genotypes were at higher risks of MI ($P = 0.028$ and 0.044 respectively; OR = 1.74 and 1.67 respectively; Table 3). Subsequent stepwise logistic regression to adjust for covariates revealed that the 1075A/G and 16854G/C polymorphisms were associated with MI in female patients ($P = 0.026$ and 0.028; OR, 1.98 and 1.97 respectively) in a recessive mode of inheritance. The same trend was found with 8790A/G, but this was only marginally significant.

Table 1 Characteristics of the patients with CHD and MI compared with controls

For continuous characteristics, value are means ± S.D.; for discrete characteristics, values are the number of observations. The MI group are a subset of the CHD group. Not all measurements were available for all participants, but a maximum of 11 values were absent at the most for each characteristic. *$P < 0.05$ compared with controls.

		Patients with	
Characteristic	Controls	CHD	MI
Sex (male/female)	665/240	636/175*	416/92*
Age (years)	52.45 ± 10.34	54.46 ± 8.90*	54.22 ± 9.46*
BMI (kg/m^2)	24.79 ± 3.30	26.52 ± 3.33*	26.56 ± 3.21*
TC (mg/dl)	198.31 ± 37.72	202.36 ± 43.74*	200.06 ± 41.09
HDL-C (mg/dl)	49.39 ± 11.80	42.12 ± 9.65*	41.47 ± 9.53*
Triacylglycerols (mg/dl)	128.69 ± 85.84	161.12 ± 108.00*	165.81 ± 121.71*
LDL-C (mg/dl)	122.66 ± 32.94	127.74 ± 39.75*	125.49 ± 39.17
Glucose (mg/dl)	99.80 ± 28.96	108.31 ± 37.34*	109.37 ± 40.43*
SBP (mmHg)	127.26 ± 18.04	131.36 ± 20.69*	129.82 ± 20.28*
DBP (mmHg)	80.11 ± 9.90	76.3 ± 11.12*	76.07 ± 10.87*
Hypertension (yes/no)	299/606	514/297*	297/211*
Smokers (yes/no)	522/383	505/306	341/167*
Alcohol consumers (yes/no)	385/510	381/430	248/260*

Table 2 Genotype and allele frequencies of *ACE2* polymorphism in male subjects

P values are compared with controls. 16854G/C genotype was missing for 13 male subjects.

			Patients with			
SNPs	Alleles	Controls	CHD	*P* value	MI	*P* value
1075A/G	A/G					
	Count (*n*)	381/284	350/286		221/195	
	MAF (%)	42.7	45.0	0.41	46.9	0.18
8790A/G	A/G					
	Count (*n*)	381/284	348/288		220/196	
	MAF (%)	42.7	45.3	0.35	47.1	0.16
16854G/C	C/G					
	Count (*n*)	378/275	350/285		221/194	
	MAF (%)	42.1	44.9	0.32	46.7	0.15
	Total (*n*)	665	636		416	

In male subjects, two haplotypes, HAP1 (AAG) and HAP2 (GGC), were common in frequency. The other five haplotypes in all constituted < 5 % of the total number. Frequencies of haplotypes in males were not significantly different between controls and patients with CHD or MI whether environmental covariates were adjusted for or not. In females, haplotype was associated with neither CHD nor MI, but diplotype analysis revealed the carriers of two copies of HAP1 were at higher risk of MI (OR = 1.81, $P = 0.022$) compared with other diplotypes, as expected. When environmental factors were adjusted for, this association was more significant ($P = 0.016$ and OR = 2.1 for diplotype HAP1/HAP1 compared with the others).

Alcohol consumption might interact with the male SNPs and haplotypes, as the corresponding product terms in logistic regression analysis had marginal significant contributions ($P = 0.032$–0.082) for CHD. This association was investigated further in subgroups of male subjects. In males not consuming alcohol, HAP2 carriers had a significantly increased risk of CHD [OR = 1.76 (95 % CI, 1.15–2.69); $P = 0.007$] and MI [OR = 1.77 (95 % CI, 1.12–2.81); $P = 0.015$] after adjusting for environmental factors when compared

Table 3 Genotype and allele frequencies of the *ACE2* polymorphisms in female subjects

P values are compared with controls. 16854G/C genotype was missing for three female subjects.

			Patients with			
SNPs	Alleles	Controls	CHD	*P* value (r/d*)	MI	*P* value (r/d*)
1075A/G	AA/AG/GG					
	Count (*n*)	69/112/59	59/73/43		38/30/24	
	Frequency (%)	28.8/46.7/24.6	33.7/41.7/24.6	0.51 (0.31/0.98)	41.3/32.6/26.1	0.04 (0.028/0.78)
	MAF (%)	47.9	45.4	0.52	42.4	0.43
8790A/G	AA/AG/GG					
	n	68/116/56	57/75/43		36/33/23	
	Frequency (%)	28.3/48.3/23.3	32.6/42.9/24.6	0.52(0.39/0.75)	39.1/35.9/25.0	0.09(0.058/0.79)
	MAF (%)	47.5	46	0.72	42.9	0.33
16854G/C	GG/GC/CC					
	n	68/116/53	58/76/41		37/33/22	
	Frequency (%)	28.7/48.9/22.4	33.1/43.4/23.4	0.51(0.37/0.78)	40.2/35.9/23.9	0.07(0.044/0.76)
	MAF (%)	46.8	45.1	0.68	41.8	0.29
	Total (*n*)	240	175		92	

* r/d, *P* values under recessive/dominant models.

with HAP1. However, in males consuming alcohol, the distribution of haplotypes and genotypes between cases and controls was not significantly different ($P = 0.22$–0.84).

DISCUSSION

In contrast with the rapid accumulation of findings on the important role of the *ACE2* gene in cardiovascular diseases from animal-model-based experiments, only a few studies have been carried out to investigate the gene in human populations. Benjafield et al. [15] found no association between *ACE2* polymorphisms and hypertension, and stated that the role of *ACE2* in CHD remained to be examined. Chen et al. [16] reported in a meeting abstract that two SNPs in the *ACE2* locus were associated with a number of cardiovascular end points, although they did not provide any specific detailed information on the SNPs or the disease of interest. A recent study in a German population [17] found a haplotype consisting of four SNPs spanning the *ACE2* gene that was associated with a higher risk of left ventricular mass index, septal wall thickness and left ventricular hypertrophy in males and a similar, but less, pronounced trend in females. In the present study, two sites of all three of the SNPs studied and the HAP1/HAP1 diplotype were associated with MI in females in recessive models, and the haplotypes were associated with both CHD and MI in males not consuming alcohol. Thus the findings of the present study do not contradict these previous observations in humans.

Table 4 Comparison of MAFs in different studies

	Mainland Chinese*		Hong Kong Chinese†		Australian Caucasians‡	
	Male	Female	Male	Female	Male	Female
n	665	240	174	154	104	89
MAF (%)						
1075A/G	42.7	47.9	—	—	29.0	17.0
8790A/G	42.7	47.5	49.0	45.0	20.0	18.0
16854G/C	42.1	46.8	48.0	45.0	—	—

* Data from the present study

† Data from a study of severe acute respiratory syndrome in Hong Kong Chinese subjects [20].

‡ Data from Benjafield et al. [15]

The three SNPs have also been genotyped in previous studies [15,20] and thus the MAFs were able to be compared with those from the present study. As shown in Table 4, MAFs for 8790A/G and 16854G/C in our present study were approximately equal to that in a Hong Kong Chinese population [20], but these were much lower for 1075A/G and 8790A/G in an Australian Caucasian population of Anglo–Celtic origin in both males and females [15]. This suggests a consistency within Chinese populations, but a large race-wise difference between Chinese and Caucasian populations.

The HapMap website (http://www.hapmap.org/) provides information on LD across the whole genome in four different populations, including a Chinese Han population. However, the data quality for the *ACE2* gene are questionable. Only 11 out of 71 genotyped SNPs in this gene were considered valid by Haploview

software when analysed using default configurations. Two of the SNPs investigated in the present study, 1075A/G and 16854G/C, were contained within the HapMap listing, but both had MAFs of 0 in a Chinese population. However, these SNPs are obviously common polymorphisms in both Chinese and Caucasian populations, as they have been reported in the previous studies mentioned above. In the HapMap project, more than 1 million SNPs were genotyped from only 45 Chinese, 45 Japanese, 90 African and 90 European subjects, and this might partly explain the situation.

The *ACE2* gene appears to be in a single haplotype block, even though no information about this is available from the HapMap database. In our present study, the three SNPs, 1075A/G (intron 1), 8790A/G (intron 3) and 16854G/C (intron 7), were in strong LD ($D' = 0.929$–0.995; $r^2 = 0.854$–0.973). Benjafield et al. [15] showed that 1075A/G (intron 1), 8790A/G (intron 3), rs879922 (intron 11) and rs714205 (intron 16) were also in LD ($D' = 0.54$–1; $P = 0.05$–0.001). Chen et al. [16] reported that an SNP in intron 3 and an inserting/deletion SNP in intron 9 were in strong LD ($D' = 0.93$; $P < 0.001$). In a recent study in a German population [17], four SNPs in introns 3, 11, 14 and 16 respectively, also had high pairwise LD. Even though these studies were not in the same populations, strong LD across the gene was supported. This implies that studying a few SNPs in this LD block will provide enough information for all other SNPs. As an example of this, in the present study, any of the three SNPs provides almost the same information.

In females, carriers of the 1075AA and 16854GG genotypes were at higher risk of MI in recessive models, which was confirmed by subsequent logistic regression analyses. Co-incidentally, only the genotypes among female patients with MI were in discordance with HWE for each of the SNPs. Lee [23] has proposed a method for searching disease-susceptibility loci by testing for Hardy–Weinberg disequilibrium in a gene bank of affected individuals. Deviation from HWE among cases, while HWE does not deviate in controls, could reflect an underlying association. Thus the co-incidence might be explained by a real association. Diplotype analysis provided further support for this suggestion, but we cannot exclude the possibility of false positives, due to the low sample size in our female population (240 controls and 92 patients with MI).

Existence of gene–environment interactions has been implicated as a complicating factor in discovering genetic models of complex human diseases. In the present study, we found a possible interaction between alcohol consumption and *ACE2* gene polymorphisms in male subjects. The effect of alcohol consumption on CHD has been studied and, in a recent review, Hill [24] argued that mild-to-moderate consumption of alcohol tends to play a protective role in the pathogenesis of CHD, but risk is promoted for heavy consumers. The effect is also gender-specific. In the present study, alcohol consumption was defined as using alcohol at least once a week for at least 6 months. Thus quantitative analysis of its effect is not feasible. It should also be noted that the data are self-reported and have to be interpreted with some caution. Additional data will be required to confirm this potential interaction. One possible approach is to include both *ADH1C* (formerly called *ADH3*) and *ACE2* genes in a similar study. *ADH1C* encodes an alcohol dehydrogenase enzyme that mediates the initial breakdown of alcohol, and there are two common genetic variants of the gene that breakdown ethanol at different speeds. Thus existence of an interaction between *ADH1C* and *ACE2* polymorphisms may confirm the interaction reported in the present study.

In our present study, one of these SNPs would have been sufficient to study when the strong LD among each pair of the SNPs is considered. Even though predetermined at the stage of study design, we proceeded with the analysis of three SNPs and haplotype analysis in the CHD and MI groups, and also performed analysis of interactions. It is encouraging that every single SNP and haplotype had an almost identical result, which reduces the possibility of making false judgments; however, it should be noted that the *P* values were not corrected for multiple testing and further work is therefore necessary.

HAP1 was associated with an increased risk of MI in females, whereas HAP2 was associated with increased risk of CHD and MI in male subjects consuming alcohol. Gender-specific effects of the RAS on cardiovascular disease like this have been observed and studied widely. Both androgens and oestrogens play a role in modulating the effects of members of the RAS, and numerous studies have demonstrated that genetic polymorphisms in *ACE*, *AGT* (angiotensinogen) or *AGTR1* (Ang II type 1 receptor) genes display more profound effects in men than in women [25,26]. As a newer member of the RAS, ACE2 might also be modulated by sex hormones to exert its effect. In the presence of interactions, the observed effect of ACE2 might be distorted when ignoring the context of other factors where it takes effect, even though it still suggests an association between disease and genotypes. This highlights the need for further gene–environment interaction studies of the *ACE2* gene in CHD using a systematic approach.

How *ACE2* polymorphisms affect the pathogenesis of CHD and MI remains largely unclear. ACE2 is obviously multifunctional given the observation that it has been widely used to study severe acute respiratory syndrome [20]. It might impact on CHD by altering the levels of Ang II and Ang-(1–7). Ang II mediates endothelial dysfunction via increased oxidative stress in cell culture, animal models and human subjects. Alternatively, Ang-(1–7), besides causing vasodilation, counteracts the growth-promoting and fibrotic effects of Ang II. However, novel roles for ACE2 in hydrolysing several

other peptides, such as the apelins, opioids and kinin metabolites raises the possibility that peptide systems other than Ang and its derivatives may also have an important role in regulating cardiovascular function [5,11].

Several limitations of our present study should be considered. First, we selected only three intronic SNPs in the 39.98 kb *ACE2* gene. This is partly because SNPs in exons are both scarce in terms of numbers and are also at low frequencies based on information from dbSNP (Single Nucleotide Polymorphism database; http://www.ncbi.nlm.nih.gov/projects/SNP) and other sources. Systematic selection of SNPs should be made considering LD throughout the gene, and variants in conservative regions should also be accounted for. However, since the whole gene is likely to be located in one LD block, the problem might not be critical. Secondly, the problem of a deficiency in sample size should be addressed when analysing gene–environment interactions. In our present study, the sample size of the female subjects was too small to afford stratification, and the male sample was not sufficient to ensure adequate power after stratification. In fact, there are 282 controls, 274 patients with CHD (including 178 patients with MI) in males not consuming alcohol. When comparison of the frequencies of HAP2 were made between the 282 male controls and 178 male patients with MI without adjustment, power to detect a true association was as low as 0.15. The problem of subgrouping for interaction analyses has been discussed elsewhere [27]. The demand for sample size grows dramatically when a confounding interactive factor is taken into account. Larger samples should be considered in subsequent studies. Finally, if alcohol consumption is quantitatively assessed, results could be more exact, as both excessive consumption and abstinence is deleterious in this scenario.

In conclusion, the present study indicates (i) the association of common genetic variants in the *ACE2* gene with MI in females, and (ii) a potential interaction between these variants and alcohol consumption to affect the risk of CHD and MI in Chinese males. Further studies with large sample sizes and based on different population backgrounds are needed to elucidate this relationship.

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