



Association between the *CYP11B2* gene -344T>C polymorphism and coronary artery disease: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the *CYP11B2* gene -344T>C polymorphism and coronary artery disease (CAD) risk. However, the specific association is still controversial. To address this issue, PubMed, EMBASE, and China National Knowledge Infrastructure databases were searched for eligible articles that reported on the relationship between the *CYP11B2* gene -344T>C polymorphism and CAD, and were published before April 2014. Data from five separate studies with 3687 subjects were analyzed by meta-analysis. No significant variation in CAD risk was detected by any of the genetic models in the overall study population. Taking into account the effect of ethnicity, further stratified analyses demonstrated significant association in both Caucasian (TT vs TC: OR = 0.80, 95%CI = 0.64-1.00) and Asian populations (TT vs TC: OR = 1.25, 95%CI = 1.01-1.54; dominant model: OR = 0.80, 95%CI = 0.66-0.98). The pooled ORs were not substantially altered after the exclusion of one study in the control group that deviated from Hardy-Weinberg equilibrium, highlighting the reliability of our meta-

analysis results. In conclusion, this meta-analysis suggested that the -344T>C polymorphism in the *CYP11B2* gene might be associated with susceptibility to CAD in Caucasians and Asians.

Keywords: -344T>C polymorphism; Coronary artery disease; *CYP11B2*

INTRODUCTION

Global trends predict a steady rise in cardiovascular disease in the next decade, and by 2020 coronary artery disease (CAD) is predicted to be the leading cause of death worldwide (Khor, 2001). CAD presents as a complex traits disease that includes myocardial infarction and coronary atherosclerosis. Despite much investigation, the underlying causes of CAD are not yet fully understood. The classic risk factors include family history, body mass index, smoking habits, hypertension, diabetes mellitus, and serum lipid levels. In addition, genetic factors also play important roles in the pathogenesis of CAD. It has been estimated that approximately 50% of the variability of the major risk factors for CAD is determined by genetics (Sekuri et al., 2005).

The endocrine renin-angiotensin system (RAS) plays an important role in circulatory homeostasis. Angiotensin-converting enzyme acts upon angiotensin I (Ang I) to yield the vasoconstrictor Ang II, whose action at the type 1 receptor drives synthesis and release of the adrenal pressor hormone aldosterone. Aldosterone is a mineralocorticoid hormone, which, via renal actions, has been shown to increase sodium resorption and intravascular volume and thus helps to regulate blood pressure (White, 1994). In addition, aldosterone may have several direct actions on the heart including promoting the development of cardiac hypertrophy and fibrosis.

The regulation of aldosterone secretion occurs largely at the level of expression of the final enzyme required for its biosynthesis, aldosterone synthase (*CYP11B2*). The activity of the *CYP11B2* gene is primarily regulated by the RAS via the actions of Ang II. Additionally, variations in the *CYP11B2* gene have been described that may influence its activity as well. One potentially interesting polymorphism is located in the 5'-flanking region of the *CYP11B2* gene, 344 nucleotides upstream from the start of translation within a binding site for the transcription factor steroidogenic factor-1; this position may contain either a C or T nucleotide (-344T>C) (White and Slutsker, 1995).

To date, several studies have shown that the *CYP11B2* gene -344T>C polymorphism is associated with CAD (Jia et al., 2012; Mishra et al., 2012). However, the specific association remains controversial. The aim of this meta-analysis was to investigate the association between the *CYP11B2* -344T>C polymorphism and CAD risk by conducting a meta-analysis from all eligible published case-control studies.

MATERIAL AND METHODS

Literature search

PubMed and EMBASE database searches were performed to retrieve papers linking the -344T>C polymorphism in the *CYP11B2* gene and CAD risk. Papers available online by April 2014 were searched without language restrictions, using the following key words:

“CYP11B2”, “-344T>C”, “gene polymorphism”, “coronary artery disease/CAD”, and “single nucleotide polymorphism”. In addition, we performed a manual search of reference lists for original articles. The reference lists of major textbooks, review articles, and of all the included articles identified by the search were then individually searched to identify other potentially eligible studies.

Inclusion and exclusion criteria

To be eligible for inclusion in this meta-analysis, the following criteria were established: 1) case-control studies that addressed patients with CAD versus healthy controls; 2) studies on the association of the -344T>C polymorphism and susceptibility to CAD; and 3) studies that included sufficient genotype data for extraction. The exclusion criteria were as follows: 1) studies that evaluated the association between the -344T>C polymorphism and CAD risk that were not based on a case-control design; 2) case reports, letters, reviews, meta-analyses, and editorial articles; 3) studies that were based on incomplete raw data and those with no usable data reported; and 4) inclusion of data duplicated in other studies.

Data extraction

Two reviewers (Y. Liu and H.L. Liu) independently performed data extraction and then together compared the results. The following information was extracted from the studies included: first author, year of publication: region; number of patient and control individuals; genotype frequencies in patients and controls; and evidence of Hardy-Weinberg equilibrium (HWE) in controls. For conflicting evaluations, agreement was reached following discussion.

Statistical analysis

We assessed HWE in the controls for each study using the chi-square test; $P < 0.05$ was considered to be significant disequilibrium. The strength of the associations between -344T>C and susceptibility to CAD was estimated by odds ratio (OR) and 95% confidence interval (95%CI) under a homozygote (TT vs CC) or heterozygote comparison (TT vs TC), or a dominant (CC + TC vs TT) or recessive model (TT + TC vs CC) between groups. We quantified the effect of heterogeneity by the I^2 test. I^2 ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than chance. I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When $I^2 > 50\%$ indicated heterogeneity across studies, the random-effect model was used for meta-analysis; else the fixed-effect model was used. Sensitivity analysis was performed through comparison of random-effect model values compared to fixed-effect values. Publication bias was investigated by Begg's funnel plot, and $P < 0.05$ was considered as statistically significant publication bias. Meta-analysis was performed using STATA package version 12.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Study characteristics

A total of 33 potentially relevant publications on the association between the *CYP11B2* gene -344T>C polymorphism and CAD risk published prior to April 2014 were systematically identified through PubMed, EMBASE and China National Knowledge Infrastructure databases. Based on our preliminary search criteria, 28 were excluded because they did not satisfy the inclusion criteria. In total, 2122 cases and 1565 controls were included in the meta-analysis (Hautanen et al., 1999; Patel et al., 2000; Franco et al., 2007; Jia et al., 2012; Mishra et al., 2012). The characteristics of the selected studies are summarized in Figure 1. Among the five case-control studies, there were three studies in Caucasians (Hautanen et al., 1999; Patel et al., 2000; Franco et al., 2007) and two studies in Asians (Jia et al., 2012; Mishra et al., 2012). The distribution of genotypes in the controls was consistent with HWE in all studies except for one (Franco et al., 2007). The baseline characteristics of all studies included are summarized in Table 1 and Figure 1.

Table 1. Characteristics of the studies included for meta-analysis.

Study included	Year	Area	Race	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test
					TT	TC	CC	TT	TC	CC	
Hautanen et al.	1999	Finland	Caucasians	141/270	31	74	36	78	132	60	0.77
Patel et al.	2000	England	Caucasians	542/500	168	281	93	154	237	109	0.32
Franco et al.	2007	Italy	Caucasians	201/201	59	55	87	69	35	97	0.00
Mishra et al.	2012	India	Asians	518/234	190	262	66	77	124	33	0.13
Jia et al.	2012	China	Asians	720/360	394	268	58	174	154	32	0.80

HWE, Hardy-Weinberg equilibrium.

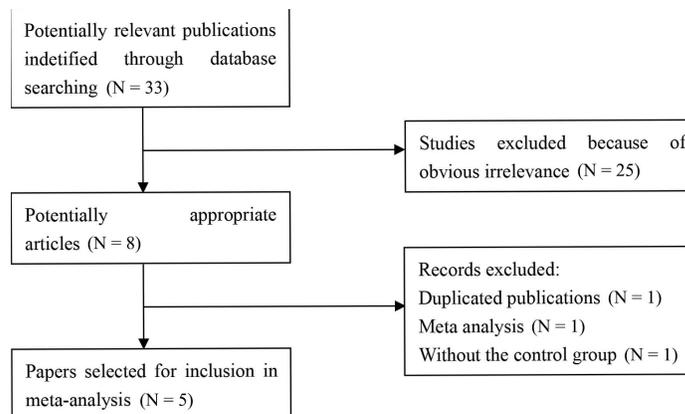


Figure 1. Flow diagram of study searching and selection process.

Quantitative synthesis

Summaries of the meta-analysis findings are shown in Table 2 and Figure 2. We found no significant association between the *CYP11B2* gene -344T>C polymorphism and CAD risk in any of the genetic models tested (TT vs CC: OR = 1.10, 95%CI = 0.90-1.35; TT vs TC:

OR = 0.94, 95%CI = 0.72-1.24; dominant model: OR = 0.95, 95%CI = 0.82-1.09; recessive model: OR = 1.16, 95%CI = 0.97-1.39). In subgroup analysis by ethnicity, the studies included were divided into Asian and Caucasian populations, and significant association was found between the -344T>C polymorphism and CAD risk in Asians (TT vs CC: OR = 1.24, 95%CI = 0.88-1.74; TT vs TC: OR = 1.25, 95%CI = 1.01-1.54; dominant model: OR = 0.80, 95%CI = 0.66-0.98; recessive model: OR = 1.12, 95%CI = 0.81-1.54); and Caucasians (TT vs CC: OR = 1.03, 95%CI = 0.81-1.33; TT vs TC: OR = 0.80, 95%CI = 0.64-1.00; dominant model: OR = 1.12, 95%CI = 0.92-1.37; recessive model: OR = 1.18, 95%CI = 0.95-1.47). Sensitivity analysis was performed by omission of one non-HWE study (Franco et al., 2007) and the result was not notably altered, indicating that the meta-analysis results were statistically significant (Table 2).

Table 2. Summary ORs and 95%CI of the CYP11B2 gene -344T>C polymorphism and CAD risk.

Subgroup	Genetic model	Sample size	Type of model	Test of heterogeneity		Test of association		Test of publication bias	
				I ²	P	OR	95%CI	z	P
		Case control							
Overall	TT vs CC	2122/1565	Fixed	10.1%	0.35	1.10	0.90-1.35	0.24	0.81
	TT vs TC		Random	64.8%	0.02	0.94	0.72-1.24	0.24	0.81
	Dominant model		Fixed	48.2%	0.10	0.95	0.82-1.09	0.24	0.81
	Recessive model		Fixed	0.0%	0.58	1.16	0.97-1.39	0.24	0.81
Caucasians	TT vs CC	884/971	Fixed	46.4%	0.16	1.03	0.81-1.33	0.00	1.00
	TT vs TC		Fixed	35.2%	0.21	0.80	0.64-1.00	0.00	1.00
	Dominant model		Fixed	9.1%	0.33	1.12	0.92-1.37	0.00	1.00
	Recessive model		Fixed	28.3%	0.25	1.18	0.95-1.47	0.00	1.00
Asians	TT vs CC	1238/594	Fixed	0.0%	0.97	1.24	0.88-1.74	0.00	1.00
	TT vs TC		Fixed	0.0%	0.62	1.25	1.01-1.54	0.00	1.00
	Dominant model		Fixed	0.0%	0.67	0.80	0.66-0.98	0.00	1.00
	Recessive model		Fixed	0.0%	0.98	1.12	0.81-1.54	0.00	1.00
Consistent with HWE	TT vs CC	1921/1364	Fixed	23.9%	0.27	1.14	0.91-1.43	0.34	0.73
	TT vs TC		Random	50.1%	0.11	1.04	0.83-1.32	0.34	0.73
	Dominant model		Fixed	47.6%	0.13	0.91	0.79-1.06	0.34	0.73
	Recessive model		Fixed	0.0%	0.42	1.15	0.94-1.40	0.34	0.73

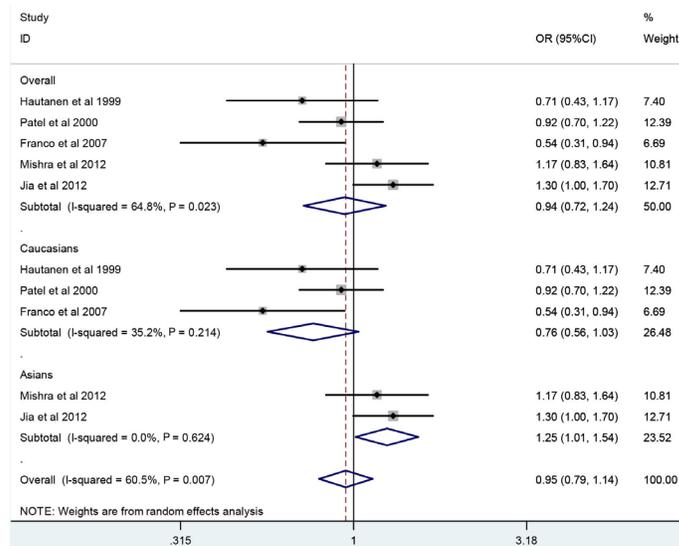


Figure 2. Meta-analysis of the relationship between the CYP11B2 gene -344T>C polymorphism and CAD risk.

Publication bias

A Begg's funnel plot was used to assess the publication bias. There was no evidence of publication bias visually from the funnel plot (Table 2 and Figure 3), which implied that the publication bias was low in the present meta-analysis (all values $P > 0.05$).

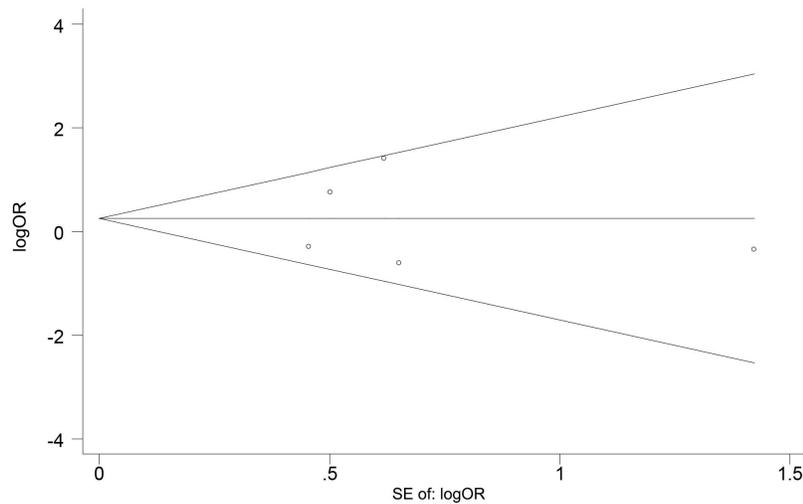


Figure 3. Begg's funnel plot with pseudo 95% confidence limits.

DISCUSSION

CAD is a multifactorial disease and its pathogenesis is not yet fully understood. Accumulated evidence indicates incontestably that CAD is determined by a complex interaction of environmental and genetic factors. The RAS system is an important regulator of blood pressure, and polymorphisms in genes that encode components of this system have been associated with physiological risk factors for CAD. The most consistent of these is the angiotensinogen (*AGT*) gene M235T polymorphism (Wang and Pan, 2012). Aldosterone is one of the main effectors of the RAS system. Aldosterone secretion is regulated by the aldosterone synthase enzyme, which is encoded by the *CYP11B2* gene. Recently, a variety of studies have focused on the association between the *CYP11B2* gene -344T>C polymorphism and CAD (Jia et al., 2012; Mishra et al., 2012). However, the results from these studies are inconsistent. A likely reason for the inconsistencies among these studies is that they are single case-control studies with small sample sizes. To help resolve these conflicting results, we conducted a meta-analysis to combine similar studies to increase the sample size and statistical power, and thereby yield a more reliable result.

This represents the first systematic study of the association between the *CYP11B2* gene -344T>C polymorphism and CAD risk using the strategy of meta-analysis. In all, five case-control studies were included and assessed, with a total of 2122 patients with CAD and 1565 healthy control individuals. The results suggested that no significant association existed

between the *CYP11B2* gene -344T>C polymorphism and CAD risk in the overall study population. Because of the difference in genetic backgrounds and the environments in which the subjects lived, we performed an ethnicity-specific subgroup analysis and found a significant association between the *CYP11B2* gene -344T>C polymorphism and CAD risk in both Caucasians and Asians. Although deviation of allelic distributions from HWE may contribute to between-study heterogeneity, sensitivity analysis performed by limiting this meta-analysis to those studies that were consistent with HWE revealed that this meta-analysis was realistic and reliable. Furthermore, there was no evidence to suggest publication bias for the *CYP11B2* -344T>C polymorphism in this meta-analysis. However, as the number of eligible studies was limited in the meta-analysis, caution should be exercised when considering the study conclusions.

The underlying mechanism of how the *CYP11B2* -344T>C polymorphism relates to CAD risk is still unclear. Aldosterone is known to have effects on the cardiovascular system that are independent of blood pressure. It is possible that presence of the -344T>C polymorphism in conjunction with cardiovascular disease would have the effect of increasing expression of CYP11B2, thereby increasing aldosterone secretion. It is also possible that potential functional effects of the -344T>C polymorphism might be influenced through gene-environment interaction. For example, evidence has suggested that the -344T>C polymorphism and smoking status synergistically increased CAD risk (Jia et al., 2012). However, as the only study that examined smoking status could not be included in our subgroup meta-analysis, studies of gene-environment interaction should be taken into consideration in future analyses.

There were some limitations to our meta-analysis. The sample size was still relatively small and might not have provided sufficient power to estimate the association between the *CYP11B2* gene -344T>C polymorphism and CAD. Second, because of incomplete raw data or publication limitations, some relevant studies could not be included in our analysis. Finally, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs, or, when they did, the ORs were not adjusted by the same potential confounders, such as age, gender, ethnicity, and exposures. Lacking full information for data analysis might have caused a serious confounding bias.

In conclusion, our meta-analysis indicated that the *CYP11B2* gene -344T>C polymorphism might be associated with an increased risk of CAD. Considering the limitations of the present meta-analysis, it is necessary to conduct further research with standardized unbiased methods, studies based on larger sample sizes, and well-matched controls.

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