



Association between G-217A polymorphism in the *AGT* gene and essential hypertension: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the angiotensinogen (*AGT*) G-217A gene polymorphism and essential hypertension risk. However, the results have been inconsistent. We examined whether the *AGT* G-217A gene polymorphism confers essential hypertension risk by conducting a meta-analysis. We conducted a literature search of the Google Scholar, PubMed, and China National Knowledge Infrastructure databases for relevant studies that examined the G-217A polymorphism and risk of essential hypertension. Statistical analyses were carried out using Stata 12.0 to combine all relevant studies. Crude odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the strength of this association. A total of 2017 patients with psoriasis and 1708 controls from 7 comparative studies were included in this meta-analysis. We found a significant association between the *AGT* G-217A gene polymorphism and the risk of essential hypertension (AA vs GG: OR = 2.52, 95%CI = 1.68-3.78; AA vs GA: OR = 2.26, 95%CI = 1.48-3.45; dominant model: OR = 0.38, 95%CI

= 0.26-0.57; recessive model: OR = 1.20, 95%CI = 1.03-1.39). Further stratified analyses were conducted by ethnicity and sample size and produced similar results. No evidence of publication bias was found. This meta-analysis confirms that the *AGT* G-217A gene polymorphism is associated with essential hypertension susceptibility.

Key words: AGT; Essential hypertension; Meta-analysis; Polymorphism

INTRODUCTION

Hypertension is thought to be a multifactorial disorder and causes severe damage to human health. It is estimated that over 95% of adult hypertension is essential hypertension (Dosh, 2002). Approximately 20-60% of blood pressure variability in the general population is heritable (Kurtz and Spence, 1993). Some environmental factors may result in essential hypertension, including physical inactivity, obesity, high sodium and low potassium diet, and alcohol consumption (Binder, 2007). In addition, genetic factors also play an important role in the development of essential hypertension, which contributes 30-50% to the pathogenesis of this disorder (Newhouse et al., 2005).

Human angiotensinogen (AGT) is produced by the liver and converted to angiotensin I (AngI) via renin. Increased plasmatic levels of AngI may induce the alternative pathway of AngI metabolism, which is associated with the neutral endopeptidase. This enzyme plays a complex role in the generation and metabolism of vasoactive peptides. Next, AngI is converted to angiotensin II, which is a physiologically important regulator of blood pressure that increases vascular tone and promotes sodium retention. The *AGT* gene is located at 1q42-43 and consists of 5 exons (Benigni et al., 2010), and DNA polymorphisms found in the human *AGT* gene have been associated with plasmatic levels of their coded proteins (Schelleman et al., 2007).

Different genetic polymorphisms in *AGT* have been reported to be associated with essential hypertension. Recent studies have shown that the promoter variants G-217A (the polymorphism at position -217 of the *AGT* promoter region) significantly influence the rate of *AGT* transcription and plasma AGT levels (Dickson et al., 2007). To date, several studies have identified an association between the *AGT* G-217A gene polymorphism and risk of essential hypertension. However, the results of previous studies have been inconsistent. Meta-analysis is useful for evaluating rare allele frequency polymorphisms (Tiret et al., 1995). In this study, we performed a meta-analysis to investigate whether the G-217A polymorphism is associated with essential hypertension risk.

MATERIAL AND METHODS

Study selection

We performed an electronic search of the PubMed, Google Scholar, and China National Knowledge Infrastructure databases to retrieve papers examining the association between the *AGT* G-217A gene polymorphism and susceptibility to essential hypertension published through June 2014 in English and Chinese using the following key words: “essential hypertension” or “blood pressure”, “angiotensinogen” or “AGT,” “polymorphism” or “allele”

or “genetic variant” or “variants.” The criteria for hypertension were defined as mean systolic blood pressure >140 mmHg and/or mean diastolic blood pressure >90 mmHg. Studies reported by the same authors were checked for possible overlapping participant groups. If the studies had partially overlapping subjects, the largest sample size was selected for analysis. If necessary, we attempted to contact the corresponding authors of retrieved articles to request additional information.

Inclusion and exclusion criteria

Studies were included in the analysis if: 1) they were case-control studies; 2) contained original data; 3) contained sufficient data to calculate odds ratios (ORs), and 4) were studies in which the genotype distribution of controls were in Hardy-Weinberg equilibrium (HWE). Major reasons for the exclusion of studies were: 1) studies containing overlapping data, incomplete data, and those with no usable data reported; 2) studies in which the number of null and wild-type genotypes or alleles could not be ascertained; 3) studies in which family members had been studied because their analysis was based on linkage considerations; and 4) studies in which the genotype distribution of controls were not in HWE.

Data extraction

Using a standardized form, data from published studies were extracted independently by 2 reviewers. From each of the articles included, the following information was retrieved: first author’s last name, year of publication, ethnicity of the population studied, source of cases and controls, number of cases and controls, sample, polymorphisms, genotypes frequency, and evidence of HWE in controls. For conflicting evaluations, an agreement was reached following discussion.

Statistical analysis

HWE was assessed by the Fisher exact test and $P < 0.05$ was considered to indicate significant disequilibrium. The strength of the associations between the G-217A polymorphism and susceptibility to essential hypertension was estimated by the OR and 95% confidence interval (CI) under the co-dominant model (AA vs GG, AA vs GA), dominant model (GG+GA vs AA), and recessive model (AA+GA vs GG), which were calculated using the fixed-effect model or the random-effect model. Pooled ORs were obtained using either the fixed- or random-effect models. The fixed-effect model was used in the absence of heterogeneity, while the random-effect model was used for heterogeneous samples. Between-study heterogeneities were estimated using the I^2 test with a range from 0-100%, representing the proportion of between-study variability attributable to heterogeneity rather than to chance (Higgins and Thompson, 2002). I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. Subgroup analysis by ethnicity and sample size were performed to examine the source of heterogeneity. To test for robustness in the summary effects, sensitivity analysis was performed by comparing the random-effect model values with the fixed-effect model values to ensure the stability of the findings. Publication bias was assessed by visual inspection of funnel plots and the Begg’s rank correlation method ($P < 0.05$ was considered to be statistically significant). All analyses were conducted using Stata 12.0 (Stata-Corp LP, College Station, TX, USA). The power of each study was computed as the probabil-

ity of detecting an association between the G-217A polymorphism and essential hypertension using a significance level of 0.05, assuming an OR of 1.5 (small effect size). Power analysis was performed using the statistical program PS: Power and Sample Size Calculation (<http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize>).

RESULTS

Study characteristics

A total of 79 potentially relevant papers were identified in the literature search. Of these, 64 papers were excluded because of obvious irrelevance by reading the titles and abstracts, with 15 articles remaining for full publication review. Of these, 8 were excluded because they did not meet the inclusion criteria. Finally, a total of 7 studies were included in our meta-analysis (Jain et al., 2002; Liu et al., 2004; Wu et al., 2003, 2004; Kong et al., 2004; Zhan et al., 2007; Jiang et al., 2009), which included 2017 essential hypertension cases and 1708 controls (Figure 1). Study characteristics are summarized in Table 1. All studies were published between 2002 and 2009. Studies were conducted in the USA and China. The genotype distributions among the controls of all studies were consistent with HWE. Of the 7 retrospective studies, 5 used population-based controls and 2 studies used hospital-based controls (Zhan et al., 2006; Jiang et al., 2009). The study by Wu et al. (2004) used the quick change site-directed mutagenesis method, while the other studies used polymerase chain reaction/restriction fragment length polymorphism for genotyping. The statistical powers of these 7 studies ranged from 9.3 to 28%. None of the studies had a statistical power that exceeded 80%. The mean frequency of the A allele of the *AGT* G-217A gene polymorphism was 14.9% among all normal controls, and Asians (China) had lower A allele prevalence than the other ethnic groups (14.7%). Among normal controls, the frequencies of the A allele in Asian, African, and Caucasian populations were 20, 19, and 14.7%, respectively (Table 1).

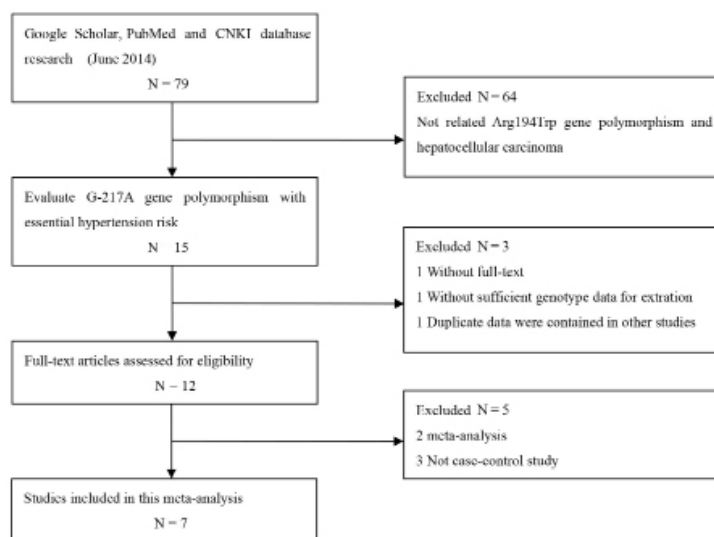


Figure 1. Flow chart showing study selection procedure.

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

Study included	Year	Area	Race	Cases/controls	Genotypes for cases			Genotypes for controls			HWE test	Power (%)
					GG	GA	AA	GG	GA	AA		
Jain et al.	2002	USA	African	186/156	94	76	16	102	48	6	0.91	28
Jain et al.	2002	USA	Caucasian	127/135	91	33	3	89	45	1	0.06	9.5
Wu et al.	2003	China	Asian	390/388	267	96	25	275	95	7	0.71	12
Liu et al.	2004	China	Asian	185/185	136	42	7	123	53	9	0.30	10
Wu et al.	2004	China	Asian	456/325	317	112	27	237	82	6	0.72	9.5
Kong et al.	2004	China	Asian	298/198	222	68	8	153	43	2	0.59	9.3
Zhan et al.	2007	China	Asian	177/86	128	43	6	66	18	2	0.57	10
Jiang et al.	2009	China	Asian	198/235	126	63	9	167	63	5	0.80	13.1

Quantitative synthesis

A meta-analysis of all essential hypertension patients and of each ethnic group was performed. A summary of the meta-analysis findings regarding the relationship between the G-217A polymorphism and essential hypertension is shown in Figure 2 and Table 2. The meta-analysis indicated that the G-217A polymorphism was significantly associated with essential hypertension under AA vs GG (OR = 2.52, 95%CI = 1.68-3.78), AA vs GA (OR = 2.26, 95%CI = 1.48-3.45), dominant model (OR = 0.38, 95%CI = 0.26-0.57), and recessive model (OR = 1.20, 95%CI = 1.03-1.39). In the subgroup analysis by race, the G-217A variant was significantly associated with essential hypertension in the Asian population (Figure 3, Table 2). Further subgroup analysis using sample size ($N > 500$) and stratification produced similar results (Table 2). The cumulative meta-analyses showed a clear association between the *AGT* G-217A gene polymorphism and risk of essential hypertension as information accumulated in the analyses (Figure 3).

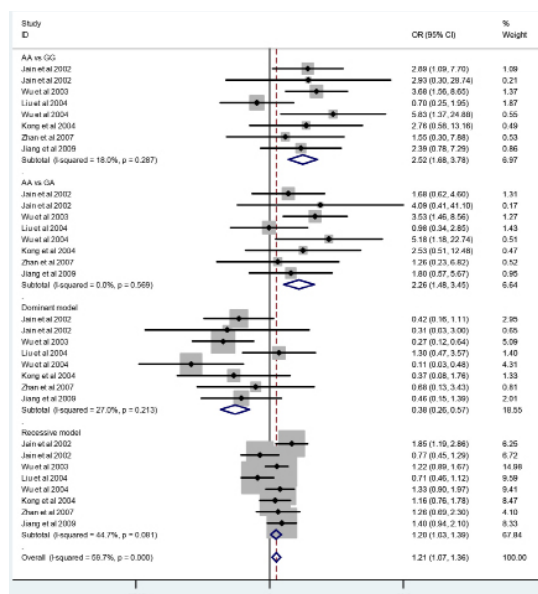
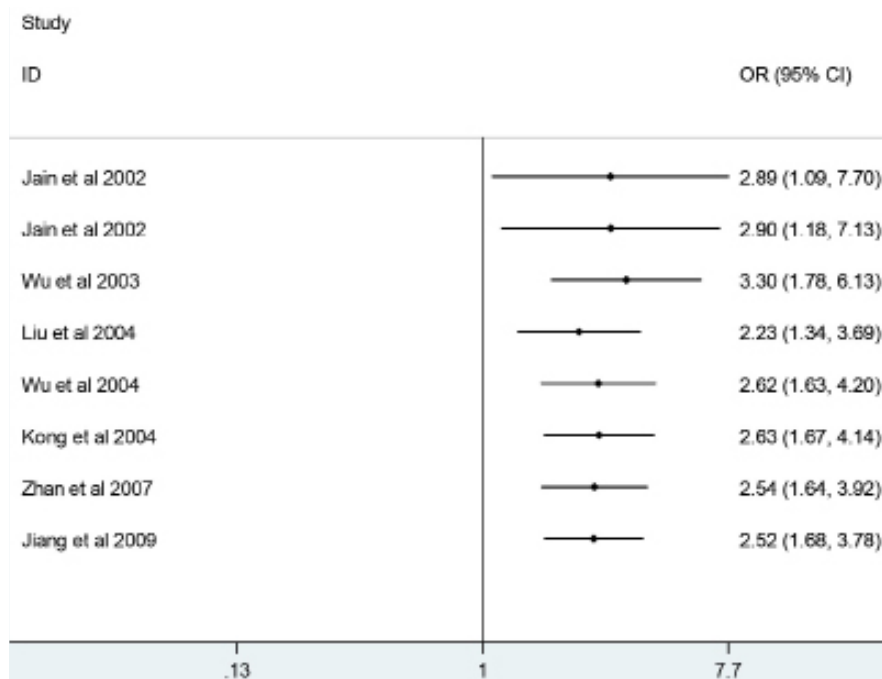


Figure 2. Forest plot of essential hypertension risk associated with *AGT* G-217A gene polymorphism in the overall population. The squares and horizontal lines correspond to the study-specific odds ratios (OR) and 95% confidence intervals (CI), respectively.

Table 2 Summary ORs and 95%CI of AGT G-217A gene polymorphism and essential hypertension 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	AA vs GG	2017	1708	Fixed	18.0%	0.29	2.52	1.68-3.78	0.00	1.00
	AA vs GA			Fixed	0.0%	0.57	2.26	1.48-3.45	0.00	1.00
	Dominant model			Fixed	27.0%	0.21	0.38	0.26-0.57	0.00	1.00
	Recessive model			Fixed	44.7%	0.08	1.20	1.03-1.39	0.00	1.00
Asians	AA vs GG	1704	1417	Fixed	40.0%	0.14	2.43	1.54-3.83	0.34	0.73
	AA vs GA			Fixed	4.3%	0.39	2.35	1.46-3.78	0.34	0.73
	Dominant model			Fixed	47.7%	0.09	0.38	0.24-0.59	0.34	0.73
	Recessive model			Fixed	16.9%	0.30	1.17	1.00-1.39	0.34	0.73
Sample size >500	AA vs GG	846	713	Fixed	0.0%	0.59	4.30	2.06-8.98	0.00	1.00
	AA vs GA			Fixed	0.0%	0.66	4.01	1.88-8.57	0.00	1.00
	Dominant model			Fixed	10.8%	0.29	0.20	0.10-0.42	0.00	1.00
	Recessive model			Fixed	0.0%	0.73	1.27	0.99-1.62	0.00	1.00

**Figure 3.** Cumulative meta-analysis for AGT G-217A gene polymorphism in the fixed-effect pooled odds ratios (OR) with the corresponding confidence interval at 95% (95%CI).

Sensitivity analysis

Sensitivity analysis was performed to evaluate the stability of the results using random-effect model values compared to the fixed-effect values. By using the random-effect model, the significant association did not materially alter the final decision (AA vs GG: OR = 2.37, 95%CI 1.47-3.82; AA vs GA: OR = 2.14, 95%CI 1.38-3.30; dominant model: OR = 0.42, 95%CI 0.25-0.70; recessive model: OR = 1.18, 95%CI 0.96-1.45), suggesting that the outcomes were robust.

Heterogeneity analysis and publication bias

Between-study heterogeneity was not observed during the meta-analyses of all types of essential hypertension. Begg's funnel plot was performed to evaluate publication bias of the literature on essential hypertension. Figure 3 displayed a funnel plot that examined the G-217A polymorphism and essential hypertension risk included in the meta-analysis for the dominant model. The shape of funnel plots did not reveal evidence of funnel plot asymmetry. The statistical results revealed no publication bias (AA vs GG: Begg's test $P = 1.00$; AA vs GA: Begg's test $P = 1.00$; dominant model: Begg's test $P = 1.00$; recessive model: Begg's test $P = 1.00$).

DISCUSSION

Although several previous studies have evaluated the association between the *AGT* G-217A gene polymorphism and essential hypertension, the association remains poorly understood. Our meta-analysis quantitatively assessed the association between the G-217A polymorphism and essential hypertension risk. A total of 7 case-control studies were included and assessed, which included a total of 2017 essential hypertension patients and 1708 healthy controls. The meta-analysis results revealed significant associations between the G-217A polymorphism and essential hypertension risk (AA vs GG: OR = 2.52, 95%CI = 1.68-3.78; AA vs GA: OR = 2.26, 95%CI = 1.48-3.45; dominant model: OR = 0.38, 95%CI = 0.26-0.57; recessive model: OR = 1.20, 95%CI = 1.03-1.39). Furthermore, we performed subgroup analysis based on ethnicity. Interestingly, the subgroup analysis results showed the same association in Asians. Because there was only 1 study of Africans and Caucasians (Jain et al., 2002), further studies examining Africans and Caucasians should be taken into consideration in future analyses. There may be selective bias regarding the relationship between the G-217A polymorphism and essential hypertension in several studies with small samples, and this association should be reevaluated in studies with large sample sizes. After stratification by sample size ($N > 500$), the subgroup analysis showed similar results, indicating no small-study bias in our meta-analysis. Further cumulative meta-analyses showed an obvious association between the *AGT* G-217A gene polymorphism and risk of essential hypertension. There was no evidence of publication bias in this meta-analysis (all $P > 0.05$).

The mechanism by which the *AGT* G-217A gene polymorphism affects essential hypertension risk is currently unclear. A previous study showed that the frequency of the -217A allele was significantly higher in hypertensive patients than in normotensive subjects (Jain et al., 2002). In addition, hypertensive patients with the -217A allele may show altered cell function, specifically regulation of the *AGT* transcription level in AGT-producing tissues (Dickson et al., 2007). Furthermore, *AGT* gene transcription may lead to increased plasma AGT levels. As a result, the RAS system is further activated to exert a potent regulatory effect on blood pressure. In addition, essential hypertension is a disease involving multiple genes and genetic polymorphisms. A previous study demonstrated high linkage disequilibrium for *AGT* G-217A with A-6G, A-20C, and M235T polymorphisms synergistically increased essential hypertension risk (Mondry et al., 2005). A previous study indicated that the risk of hypertension increased with noise exposure among TT homozygotes of *AGT* M235T gene polymorphisms (Hwang et al., 2012). Further studies examining the G-217A polymorphism should be taken into consideration to investigate potential relationships.

There were some limitations to our meta-analysis. First, a relatively small sample

size was included and significant heterogeneity among studies in overall comparisons was observed in the meta-analysis of the *AGT* G-217A gene polymorphism and essential hypertension. Second, there were varying levels of disease severity, and the activity level of essential hypertension was unclear. Further studies are required to examine whether an association exists between the G-217A polymorphism and the activity or clinical features of the disease. In addition, the effect of potential gene-gene and gene-environment interactions could not be addressed in this meta-analysis.

In conclusion, our study indicated that the *AGT* G-217A gene polymorphism is associated with essential hypertension susceptibility. Further studies examining gene-gene and gene-environment interactions should be performed to further evaluate this association.

Conflicts of interest

The authors declare no conflict of interest.

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