

Association between angiotensinogen T174M polymorphism and ischemic stroke: A meta-analysis

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Background: Numerous studies have evaluated the association between the angiotensinogen (AGT) T174M polymorphism and ischemic stroke (IS) risk. However, the specific association is still controversial. **Materials and Methods:** In order to explore this association more deeply, we performed a meta-analysis. All of the relevant studies were identified from PubMed, Embase, and Chinese National Knowledge Infrastructure database up to October 2014. Statistical analyses were conducted with STATA 12.0 software. Odds ratio (OR) with 95% confidence interval (CI) values were applied to evaluate the strength of the association. **Results:** Six studies with 1290 cases and 1125 controls were included. No significant variation in IS risk was detected in any of the genetic models in the overall (MM vs. TT: OR = 1.64, 95% CI = 0.51-5.28; MT vs. TT: OR = 0.93, 95% CI = 0.66-1.31; dominant model: OR = 1.08, 95% CI = 0.69-1.72; recessive model: OR = 0.61, 95% CI = 0.20-1.91). Taking into account the effect of ethnicity, further stratified analyses were performed. The results showed that AGT gene T174M polymorphism might be associated with IS risk in Asians (MM vs. TT: OR = 3.28, 95% CI = 1.79-6.02; recessive model: OR = 0.31, 95% CI = 0.17-0.57). **Conclusion:** In conclusion, the AGT T174M polymorphism may be a susceptible predictor of the risk of IS in Asians. Further, large and well-designed studies are needed to confirm this conclusion.

Key words: Angiotensinogen gene, ischemic stroke, meta-analysis, T174M polymorphism

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INTRODUCTION

Stroke is the third most common cause of death and the most common cause of disability in developed countries. Due to the aging population, this burden will rise greatly during the next 20 years. About 80% of strokes are ischemic. It has been reported by the World Health Organization (WHO) that about 15 million people suffer from ischemic stroke (IS) every year, of which 5 million were dead and 5 million became permanently disabled.^[1] Stroke consumes about 2-4% of total healthcare costs around the world. Therefore, the prevention of IS is urgent. To date, a variety of risk factors have been identified to contribute to IS, including hypertension, smoking, diabetes, obesity, advanced age,

and so on.^[2,3] However, stroke risk remains insufficiently explained by these factors. Twins, families, and animal-based studies provide substantial evidence that genetic factors are important in the pathogenesis of IS.^[4,5]

Angiotensinogen (AGT) is a component of the renin-angiotensin system, which is converted to angiotensin I by renin, and subsequently forms angiotensin II via angiotensin I-converting enzyme. Angiotensin II is responsible for the regulation of systemic blood pressure, salt and water homeostasis, and the maintenance of vascular tone, as well as increasing the levels of vasopressin and adrenocorticotrophic hormone in the central nervous system.^[6] The AGT gene is located at 1q42-43 and consists of five exons, and express pre AGT or AGT precursor protein in the liver. A threonine to methionine substitution at amino acid 174 is a common

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polymorphism called T174M (rs699), designating the T and M alleles, respectively.^[7]

Previous meta-analyses demonstrated that AGT T174M polymorphism was associated with susceptibility to coronary heart disease and hypertension.^[8,9] However, little is known regarding the association between T174M polymorphism and susceptibility to IS. Over the past decade, several case-control studies have focused on the association between T174M polymorphism and IS risk. However, the results remain controversial. In the present study, we investigated whether the AGT T174M polymorphism is associated with IS risk by performing a meta-analysis.

MATERIALS AND METHODS

Search strategy

In this study, the terms "IS" or "cerebral infarction" or "cerebrovascular disease" or "stroke" in combination with "angiotensinogen" or "AGT" or "T174M" and "polymorphism" or "variant" or "gene" were used to search the electronic databases (PubMed, Embase and China National Knowledge Infrastructure databases) for all studies on AGT T174M polymorphism and IS (last search was updated on October 2014). No publication language restrictions were imposed. All the searched studies were retrieved, and their references were checked for other relevant publications. If sequential or multiple publications from the same data occurred, the publication that reported data from the largest or most recent study was included.

Eligibility criteria

Human studies were included if they met the following criteria:

1. Case-control studies that addressed IS cases and healthy controls;
2. Studies that evaluated the association between AGT T174M polymorphism and IS risk;
3. All patients with clinically diagnosed IS;
4. Studies that included sufficient genotype data for extraction;
5. The studies contained at least two comparison groups (case group vs. control group);
6. The studies included detailed genotyping data.

In addition, the following exclusion criteria were also used:

1. Not case-control studies that evaluated the association between AGT T174M polymorphism and IS risk;
2. Animal studies;
3. Studies that were based on incomplete raw data or no usable data reported;
4. Duplicated publications.

Data extraction

The extraction of data from all eligible publications was performed by two investigators independently, according to the inclusion and exclusion criteria listed above. If there was a discrepancy between them, it was settled by discussion until a consensus was reached. The following data were extracted: The first author's name, year of publication, country of the study, ethnicity, numbers of genotyped cases and controls and deviation from Hardy-Weinberg Equilibrium (HWE) of the control group.

Statistical analysis

We assessed HWE in the controls for each study using χ^2 test and a $P < 0.05$ was considered as significant disequilibrium.^[10] The odd ratios (ORs) together with the 95% confidence intervals (CIs) were used to assess the strength of association. The combined ORs and 95% CIs were calculated respectively for a homozygote comparison (MM vs. TT), a heterozygote comparison (MT vs. TT), a dominant model (MM + MT vs. TT), and a recessive model (TT + MT vs. MM) between groups. Between-study heterogeneities were estimated using I^2 test. I^2 values of 25, 50 and 75% were defined as low, moderate and high estimates, respectively.^[11] When $I^2 > 50\%$ indicated heterogeneity across studies, the random effects model was used for meta-analysis, or else the fixed effects model was used. Subgroup analyses were based on ethnicity to identify possible causes of heterogeneity and assess the robustness of the relationships. Different ethnicities were categorized as Asians and Africans. Only one study was performed in African patients; therefore, the result of subgroup analysis by ethnicity could not be reliable for Africans. Sensitivity analysis was performed to assess the stability of the results by excluding one study with genotype distributions not in HWE. And publication bias was examined by plotting a Begg's funnel plot ($P < 0.05$ was considered representative of statistically significant publication bias). All analyses were performed by STATA 12.0 (STATA College Station, TX, USA).

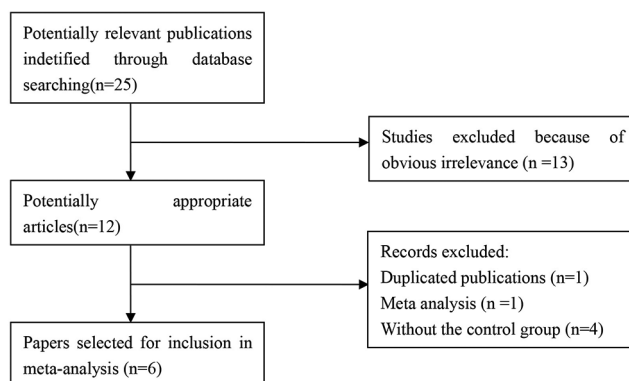


Figure 1: The flow chart of the included studies in the meta-analysis

RESULTS

Study characteristics

With our search criterion, 25 individual records were found, and 12 full-text publications were preliminarily identified for further detailed evaluation. According to the exclusion criteria, six publications were excluded, including one duplicate study, one meta-analysis, and four without sufficient data for extraction. Finally, as shown in Figure 1, six studies with 1290 cases and 1125 healthy controls were included into this meta-analysis.^[12-17] The ethnicity of eligible subjects involved populations ranging from African ($n = 1$) and Asian ($n = 5$). Only one^[15] of the six studies was deviated from HWE. In addition, the controls in two^[13,14] of these eligible studies were based on common populations, while the others were based on hospital populations. Furthermore, all of the genotyping methods applied in the included studies are PCR-based. The publication year of the included studies ranged from 2004 to 2014. The main characteristics of eligible studies were summarized in Table 1.

Main results, subgroup analyses

The main results of meta-analysis and heterogeneity are listed in Table 2. Four models suggested remarkable between-study heterogeneity, thus both fixed effects model and random effects model were performed to calculate the pooled estimates. Overall, no significant association between AGT T174M polymorphism and IS was observed under all genetic models. (MM vs. TT: OR = 1.64, 95% CI = 0.51-5.28, $P = 0.01$; MT vs. TT: OR = 0.93, 95% CI = 0.66-1.31, $P = 0.07$; dominant model: OR = 1.08, 95% CI = 0.69-1.72, $P = 0.00$; recessive model: OR = 0.61, 95% CI = 0.20-1.91, $P = 0.02$).

In the subgroup analysis based on ethnicity, results of subgroup analysis confirmed that there was significant associations between AGT T174M polymorphism and IS risk in Asian populations (Figure 2, MM vs. TT: OR = 3.28, 95% CI = 1.79-6.02, $P = 0.32$; MT vs. TT: OR = 0.96, 95% CI = 0.61-1.53, $P = 0.04$; dominant model: OR = 1.17, 95% CI = 0.65-2.09, $P = 0.00$; recessive model: OR = 0.31, 95% CI = 0.17-0.57, $P = 0.35$).

Sensitivity analysis

In the subgroup analysis based on HWE, we detected no significant association between AGT T174M polymorphism and IS risk (MM vs. TT: OR = 1.08, 95% CI = 0.53-2.21, $P = 0.11$; MT vs. TT: OR = 0.86, 95% CI = 0.68-1.09, $P = 0.09$; dominant model: OR = 0.90, 95% CI = 0.62-1.32, $P = 0.05$; recessive model: OR = 0.91, 95% CI = 0.44-1.85, $P = 0.12$). No material alteration was detected, indicating that our results were statistically robust.

Publication bias

The funnel plot and Begg's test was used to assess the publication bias. There was no evidence of publication bias in our study [Figure 3]. The results implied that the publication bias was low in the present meta-analysis.^[18]

DISCUSSION

Ischemic stroke is a multifactorial disease and its pathogenesis is not yet fully understood.^[17] Accumulated evidence indicate incontestably that IS is determined by a complex interaction of environmental and genetic factors. AGT is the component of RAS that is released from the liver and is cleaved by rennin, which has been shown to play a

Table 1: Distribution of AGT T174M polymorphism genotype between cases and controls and genotyping methods

Study included	Year	Area	Race	Source of controls	Cases/controls	Genotypes for cases			Genotypes for controls			HWE test
						MM	MT	TT	MM	MT	TT	
Liu et al.	2004	China	Asian	HB	90/90	5	12	73	0	11	79	0.54
Yue et al.	2008	China	Asian	PB	82/82	1	12	69	2	20	60	0.83
Saidi et al.	2009	Tunisia	African	PB	444/329	4	73	367	8	60	261	0.05
Sun et al.	2010	China	Asian	HB	180/130	39	22	119	9	13	108	0.00
Zhang et al.	2010	China	Asian	HB	374/193	4	47	283	0	41	152	0.10
Park et al.	2013	Korea	Asian	HB	120/301	2	27	91	4	50	247	0.42

PB = Population-based; HB = Hospital-based; HWE = Hardy-Weinberg equilibrium

Table 2: Summary of different comparative results

Variables	Number	Cases/controls	MM versus TT			MT versus TT			Dominant model			Recessive model		
			OR (95% CI)	P	I ² (%)	OR (95% CI)	P	I ²	OR (95% CI)	P	I ²	OR (95% CI)	P	I ²
Total	6	1290/1125	1.64 (0.51-5.28)	0.01	65.4	0.93 (0.66-1.31)	0.07	50.7	1.08 (0.69-1.72)	0.00	76.8	0.61 (0.20-1.91)	0.02	63.2
Ethnicity														
Asian	5	846/796	3.28 (1.79-6.02)	0.32	15	0.96 (0.61-1.53)	0.04	60.1	1.17 (0.65-2.09)	0.00	79.0	0.31 (0.17-0.57)	0.35	10.0
African	1	444/329	3.36 (0.11-1.19)			0.87 (0.59-1.26)			0.81 (0.56-1.16)			2.74 (0.82-9.18)		
HWE														
Yes	5	1110/995	1.08 (0.53-2.21)	0.11	46.4	0.86 (0.68-1.09)	0.09	49.8	0.90 (0.62-1.32)	0.05	58.3	0.91 (0.44-1.85)	0.12	45.0
No	1	180/130	3.93 (1.82-8.50)			1.54 (0.74-3.20)			2.52 (1.45-4.37)			0.27 (0.13-0.58)		

I² = Inconsistency index; CI = Confidence interval; OR = Odds ratio

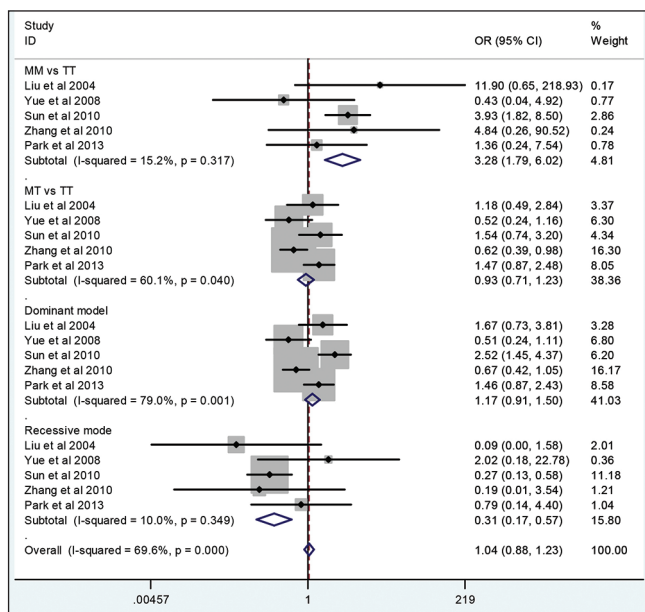


Figure 2: Forest plots for the association of angiotensinogen T174M polymorphism with risk of ischemic stroke in Asians. For each study, the estimates of odds ratio (OR) and its 95% confidence interval (CI) were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI

vital role in affecting the cardiovascular system. Recently, a variety of studies have focused on the association between the AGT T174M polymorphism and IS. However, the observed associations of these studies were inconclusive. The most likely reason for the inconsistencies among these studies is that they are single case-control studies with small sample sizes.^[18] The aim of meta-analysis is to combine the same kind of studies to increase the sample size and statistical power, and thereby get a more authentic result.

To our knowledge, this is the first meta-analysis examining the association between T174M polymorphism and IS risk. And a total of six studies with 1290 cases and 1125 controls were included to systematically explore the association between the AGT T174M polymorphism and the risk of IS in this meta-analysis. From the combined statistical results, the meta-analysis did not show any significant association between T174M polymorphism and IS risk in the overall populations.

Because of the difference in genetic backgrounds and the environment in which they lived, we perform an ethnicity-specific subgroup analysis, and we found a significant association between AGT T174M polymorphism and IS risk in Asians. For only one study was performed in Africans and no study aiming at Caucasians, these results still need further investigation in Africans and Caucasians.

Moreover, if the distribution of genotypes in the control groups were not in HWE, the results of genetic association studies might be spurious.^[19] When limiting the analysis to the studies within HWE, no significant relationship was

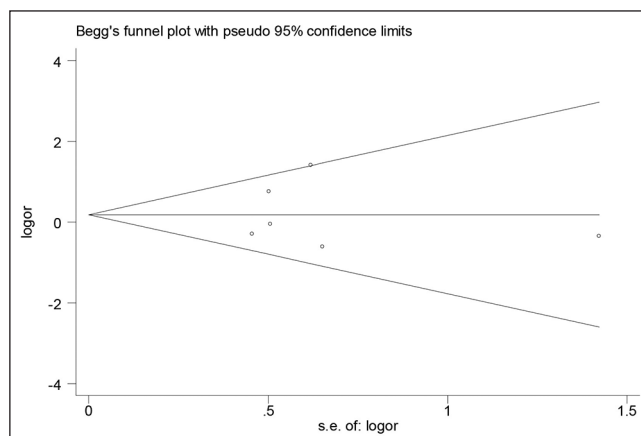


Figure 3: Funnel plot of angiotensinogen gene T174M polymorphism and susceptibility of ischemic stroke (MM vs. TT)

detected, suggesting that this factor probably had little effect in the present meta-analysis. No evidence showed publication bias in this meta-analysis.

The mechanism of the AGT gene T174M relates to IS risk is still unclear. The serum AGT level was shown to be higher in subjects carrying the T allele.^[20] And AGT interacts with renin to produce angiotensin II, which activates vascular cell apoptosis, contributing to vascular remodeling, and cardiomyocyte loss in ischemia-reperfusion.^[21] In addition, the M235T polymorphism was in strong linkage disequilibrium with the T174M polymorphism.^[22] A recent meta-analysis showed that the AGT M235T polymorphism was a risk factor for IS among Asians,^[23] the linkage disequilibrium of M235T and T174M in exon 2 of the AGT gene may synergistically increase the risk of IS.

There are still some limitations in this meta-analysis. First, heterogeneity was observed in some models, so all cases and controls should be matched for age, gender, ethnicity, and type of IS, but these issues could not be analyzed precisely because of inadequate clinical information for individual person. Second, the number of published studies was not sufficiently large for a comprehensive analysis, and some included studies of small size might not have had enough statistical power to explore the real association between the T174M polymorphism and susceptibility to IS. Finally, the inclusion or exclusion of confounding variables (hypertension, hyperlipidemia, coronary artery disease, diabetes) in the original studies is inconsistent.

CONCLUSION

This meta-analysis suggests that the AGT gene T174M polymorphism might be associated with IS risk in Asians. Large-scale case-control and population-based association studies are warranted to validate the risk identified in the current meta-analysis and investigate the potential gene-gene and gene-environment interactions on IS risk.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

ZLO contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. HBC contributed in the conception of the work, approval of the final version of the manuscript, and agreed for all aspects of the work. GL contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. CL contributed in revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. SYL contributed in revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. JW contributed in the conception and design of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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