

# Lack of association between CYP11B2 -344T/C polymorphism and transient ischemic attack in a Chinese population

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## Abstract

**Objective:** This study investigated whether the CYP11B2 -344T/C polymorphism is correlated with transient ischemic attack (TIA) susceptibility.

**Methods:** We recruited 100 TIA patients and 100 control subjects and analyzed the CYP11B2 -344T/C polymorphism using restriction fragment length polymorphism (PCR–RFLP).

**Results:** The frequency in TIA patients and controls was 42% compared with 48% for TT genotypes, 51% compared with 45% for TC genotypes, and 7% compared with 7% for CC genotype, respectively. Allele frequencies in TIA patients and controls were 67.5% compared with 70.5% for T-allele and 32.5% compared with 29.5% for C-allele, respectively. No association between the CYP11B2 -344T/C polymorphism and TIA was observed in all comparisons.

**Conclusion:** Our data suggest that there was no association between the CYP11B2 -344T/C polymorphism and TIA in a Chinese population.

## Keywords

Angiotensin-converting enzyme gene, CYP11B2, polymorphism, transient ischemic attack, allele, restriction fragment length polymorphism

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## Introduction

Transient ischemic attack (TIA) is a brief episode of neurological dysfunction that is caused by a regional reduction in blood flow (i.e., ischemia). TIA is not a rare disease, with an incidence of 0.7 to 0.8 per 1000 people evaluated.<sup>1</sup> Twenty percent of patients with ischemic stroke present with a TIA during the hours and days immediately preceding the stroke.<sup>2</sup> Recent studies have reported that about 1.4% to 3.4% of patients who present with a TIA will have a stroke within the next 90 days.<sup>3,4</sup> Both TIA and ischemic stroke arise from the same etiology and pathophysiology. Cardioembolic etiology accounts for approximately 34% of TIAs, other etiologies include small artery disease and large artery atherothrombosis.<sup>5</sup> Stroke prevention requires management of modifiable vascular risk factors, such as hypertension, hyperlipidemia, and diabetes mellitus, as well as tobacco use.

Aldosterone plays a detrimental role on the cerebrovascular and the cardiovascular system, and it increases oxidative stress, which causes endothelial dysfunction and accumulation of collagen, leading to reduced elasticity of vessel walls.<sup>6</sup> The aldosterone synthase gene (CYP11B2) locus is an important candidate region in the development of hypertension,<sup>7</sup> diabetic nephropathy,<sup>8</sup> type 2 diabetes mellitus,<sup>9</sup> and atrial fibrillation.<sup>10</sup> Because it has been suggested in a meta-analysis that the CYP11B2 -344T/C polymorphism was associated with ischemic stroke,<sup>11</sup> we suspect that this polymorphism is probably associated with the development of TIA.

## Materials and methods

### Subjects

One hundred TIA patients at Changshu No. 1 People's Hospital from February

2012 to February 2014 were selected as the TIA patient group, while 100 individuals undergoing physical examinations at the hospital during the same period were selected as the control group. Diagnosis was made using diffusion-weighted magnetic resonance imaging (MRI) for brain imaging following the transient ischemic diagnostic criteria.<sup>12</sup> Patients with trauma, any invasive procedure, or cerebral hemorrhage were excluded from the study. This study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all patients and controls.

### Gene polymorphism analysis

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The CYP11B2 genotype polymorphism was determined in genomic DNA using an allele-specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, as previously described.<sup>13</sup> The primers used for PCR were 5'-CAGGAGGAGACCCCATGTGAC-3' (sense) and 5'-CCTCACCCCTGTTCAGCC-3' (antisense). The amplified fragments were digested with Hae III restriction enzyme and were subjected to electrophoresis on 2% ethidium bromide stained agarose. The uncut -344T allele was 273 bp in size, and cut fragments (C allele) were 202 bp in size.

### Statistical analysis

The TIA patient and control groups were compared using a two-tailed unpaired Student's *t*-test for continuous variables and the Pearson's Chi-square test for categorical variables. The Hardy-Weinberg equilibrium (HWE) was tested for the controls. The association was assessed by calculating the pooled odds ratio (OR)

with the 95% confidence interval (95%CI) from multivariate logistic regression adjusted for age and gender. SPSS 21.0 (IBM Corp., Armonk, NY, USA) was used for data processing. P-values below 0.05 were considered to be statistically significant.

## Results

### Characteristics

The statistical analysis of the basic characteristics in the 100 TIA patient and 100 controls is presented in Table 1. The patients' age (mean  $\pm$  standard deviation [SD]) was  $63.5 \pm 6.8$  years in the TIA patient group and  $62.6 \pm 8.6$  years in the control group. There were 61 men and 39 women in the TIA patient group, and 64 men and 36 women in the control group. No difference between age or sex was observed between the two groups. TIA patients had significantly higher values for TIA risk parameters such as glucose, blood pressure, and serum lipid compared with controls ( $P < 0.05$ ).

### HWE test

The genotype distributions of the CYP11B2 -344T/C polymorphism in the control group met the criteria for the HWE. Therefore, this

sample of subjects was considered to be representative of the population.

### Association of the -344C/T polymorphism in patients with CYP11B2 with TIA

A comparison was made between the CYP11B2 -344T>C genotype and allele frequency in the TIA and control groups (Table 2). There were no significant differences in genotype and allele frequencies between the two groups. There were no association between the CYP11B2 -344T/C polymorphism and TIA in all comparisons.

## Discussion

As previously indicated, the CYP11B2 TT genotype was associated with higher plasma aldosterone levels.<sup>14-16</sup> However, the CYP11B2 -344T/C polymorphism was not associated with TIA occurrence in our study. In this study, we only investigated the association between one single polymorphism and TIA, and the influence of gene-gene or gene-environment interaction was not considered. Because of the complex effect of genetic polymorphisms on TIA progression, the lack of a relationship between the CYP11B2 -344T/C polymorphism and

**Table 1.** Basic characteristics of transient ischemic attack patients and controls.

Clinical characteristics	TIA patients (n = 100)	Controls (n = 100)	P value
Age	63.5 $\pm$ 6.8	62.6 $\pm$ 8.6	N.S.
Male	61 (61%)	64 (64%)	N.S.
Female	39 (39%)	36 (36%)	N.S.
Body weight (kg)	67.5 $\pm$ 10.7	68.3 $\pm$ 13.6	N.S.
Hypertension, yes	70 (70%)	54 (54%)	0.006
Diabetes, yes	35 (35%)	18 (18%)	0.007
Hyperlipidemia, yes	55 (55%)	35 (35%)	0.005
TC (mmol/L)	5.2 $\pm$ 1.0	4.6 $\pm$ 1.1	<0.0001
TG (mmol/L)	1.4 $\pm$ 0.4	1.1 $\pm$ 0.3	<0.0001
HDL-C (mmol/L)	1.4 $\pm$ 0.4	1.3 $\pm$ 0.4	0.08
LDL-C (mmol/L)	3.2 $\pm$ 0.7	2.9 $\pm$ 0.8	0.005

TIA, transient ischemic attack; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N.S., not significant.

**Table 2.** The genotype and allele frequencies of the CYP11B2 -344C/T polymorphism between TIA patients and controls.

CYP11B2 -344T>C	TIA patients (n = 100)	Controls (n = 100)	OR (95% CI)*	P value
TT	42 (42%)	48 (48%)	0.86 (0.29–2.66)	N.S.
TC	51 (51%)	45 (45%)	1.12 (0.35–3.51)	N.S.
CC	7 (7%)	7 (7%)	1.000 (Reference)	
Recessive model (TT vs. TC+CC)			0.77 (0.46–1.39)	N.S.
Dominant model (TT+TC vs. CC)			1.01 (0.35–2.99)	N.S.
Overdominant model (TC vs. TT+CC)			1.25 (0.71–2.23)	N.S.
T allele	135 (67.5%)	141 (70.5%)	0.88 (0.54–1.31)	N.S.
C allele	65 (32.5%)	59 (29.5%)	1.000 (Reference)	

\*Adjusted by age and gender.

TIA, transient ischemic attack; CI, confidence interval; N.S., not significant.

the risk of TIA might result from the renin–angiotensin–aldosterone system (RAAS) polymorphisms, such as renin (REN), angiotensinogen (ATG), angiotensin converting enzyme I (ACE), angiotensin II type 1 receptor (AT1), and aldosterone synthase (CYP11B2) genes from the RAAS pathway, which could influence aldosterone expression.<sup>17–19</sup> Aldosterone-induced reactive oxygen species (ROS) play an important role in endothelial dysfunction.<sup>20,21</sup> Aldosterone causes increased oxidative stress, decreased nitric oxide bioavailability, and has a direct effect on NADPH activity, which increases glucose-6-phosphate dehydrogenase enzyme levels and epidermal growth factor receptor expression, further reducing vascular elasticity.<sup>22</sup> Aldosterone also potentiates the stimulatory effect of angiotensin II on activation of extracellular signal-regulated kinase (ERK1/2) and the c-Jun N-terminal kinase (JNK) signaling pathway to induce vascular fibrosis.<sup>23</sup> Evidence from experimental and clinical studies suggests that excessive circulating aldosterone levels can cause adverse vascular sequelae that are independent of hypertension.<sup>24</sup> The association of angiotensinogen,

CYP11B2, and angiotensin-converting enzyme polymorphisms showed a possible positive interaction in the development of renal insufficiency among hypertensive subjects.<sup>25</sup> The interaction between the CYP11B2 -344T/C polymorphism, age, and smoking status is also associated with an increased risk of coronary artery disease.<sup>26</sup> Thus, interactions between genetic and environmental factors may play an important role in TIA pathogenesis.

This study has some limitations. Because the age range of controls was consistent with TIA patients, there were more cases of hypertension, diabetes, and hyperlipidemia among controls. Another weakness of the study is that changes in aldosterone levels in the TIA patients and controls were not determined. This will be analyzed in the TIA patients in future research. The third weakness of the study is that there was no evaluation the patients' condition 6 months after their treatments.

In conclusion, the present study provides evidence that it is unlikely that the CYP11B2 -344T/C polymorphism plays a role in the genetic susceptibility to TIA in the Chinese population.


## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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## References

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation* 2015; 131: e29–e322.
2. Rothwell PM and Warlow CP. Timing of TIAs preceding stroke: time window for prevention is very short. *Neurology* 2005; 64: 817–820.
3. Wasserman JK, Perry JJ, Sivilotti ML, et al. Computed tomography identifies patients at high risk for stroke after transient ischemic attack/nondisabling stroke: prospective, multicenter cohort study. *Stroke* 2015; 46: 114–119.
4. Anticoli S, Pezzella FR, Pozzessere C, et al. Transient ischemic attack fast-track and long-term stroke risk: role of diffusion-weighted magnetic resonance imaging. *J Stroke Cerebrovasc Dis* 2015; 24: 2110–2116.
5. Marnane M, Duggan CA, Sheehan OC, et al. Stroke subtype classification to mechanism-specific and undetermined categories by TOAST, A-S-C-O, and causative classification system: direct comparison in the North Dublin population stroke study. *Stroke* 2010; 41: 1579–1586.
6. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab* 2013; 98(12): 4826–4833.
7. Niu S, Zhang B, Zhang K, et al. Synergistic effects of gene polymorphisms of the renin-angiotensin-aldosterone system on essential hypertension in Kazakhs in Xinjiang. *Clin Exp Hypertens* 2015: 1–8.
8. Xu H, Wang X, Liu M, et al. Association of aldosterone synthase (CYP11B2) -344 T/C polymorphism with diabetic nephropathy: a meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2016; 17: 1470320316633896.
9. Ichikawa M, Konoshita T, Nakaya T, et al. Genetic variant of the renin-angiotensin system and prevalence of type 2 diabetes mellitus: a modest but significant effect of aldosterone synthase. *Acta Diabetol* 2014; 51: 595–599.
10. Fu X, Ma X, Zhong L, et al. Relationship between CYP11B2-344T>C polymorphism and atrial fibrillation: a meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2015; 16: 185–188.
11. Yu Y. The CYP11B2 -344C/T variant is associated with ischemic stroke risk: an updated meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2015; 16: 382–388.
12. Easton JD, Saver JL, Albers GW, et al. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009; 40: 2276–2293.
13. Pawlik M, Mostowska A, Lianeri M, et al. Association of aldosterone synthase (CYP11B2) gene -344T/C polymorphism with the risk of primary chronic glomerulonephritis in the Polish population. *J Renin Angiotensin Aldosterone Syst* 2014; 15: 553–558.
14. Nicod J, Bruhin D, Auer L, et al. A biallelic gene polymorphism of CYP11B2 predicts increased aldosterone to renin ratio in selected hypertensive patients. *J Clin Endocrinol Metab* 2003; 88: 2495–2500.
15. Russo P, Siani A, Venezia A, et al. Interaction between the C(-344)T

- polymorphism of CYP11B2 and age in the regulation of blood pressure and plasma aldosterone levels: cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. *J Hypertens* 2002; 20: 1785–1792.
16. Abdel Ghafar MT. Association of aldosterone synthase CYP11B2 (-344C/T) gene polymorphism with essential hypertension and left ventricular hypertrophy in the Egyptian population. *Clin Exp Hypertens* 2019; 41: 779–786.
  17. Brenner D, Labreuche J, Poirier O, et al. Renin-angiotensin-aldosterone system in brain infarction and vascular death. *Ann Neurol* 2005; 58: 131–138.
  18. Kolder IC, Michels M, Christiaans I, et al. The role of renin-angiotensin-aldosterone system polymorphisms in phenotypic expression of MYBPC3-related hypertrophic cardiomyopathy. *Eur J Hum Genet* 2012; 20: 1071–1077.
  19. Ellis KL, Palmer BR, Frampton CM, et al. Genetic variation in the renin-angiotensin-aldosterone system is associated with cardiovascular risk factors and early mortality in established coronary heart disease. *J Hum Hypertens* 2013; 27: 237–244.
  20. Nishizaka MK, Zaman MA, Green SA, et al. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. *Circulation* 2004; 109: 2857–2861.
  21. Lai S, Petramala L, Mastroluca D, et al. Hyperaldosteronism and cardiovascular risk in patients with autosomal dominant polycystic kidney disease. *Medicine (Baltimore)* 2016; 95: e4175.
  22. Briet M and Schiffrin EL. Vascular actions of aldosterone. *J Vasc Res* 2013; 50: 89–99.
  23. Mazak I, Fiebeler A, Muller DN, et al. Aldosterone potentiates angiotensin II-induced signaling in vascular smooth muscle cells. *Circulation* 2004; 109: 2792–2800.
  24. Rocha R and Stier CT Jr. Pathophysiological effects of aldosterone in cardiovascular tissues. *Trends Endocrinol Metab* 2001; 12: 308–314.
  25. Fabris B, Bortoletto M, Candido R, et al. Genetic polymorphisms of the renin-angiotensin-aldosterone system and renal insufficiency in essential hypertension. *J Hypertens* 2005; 23: 309–316.
  26. Jia EZ, Xu ZX, Guo CY, et al. Renin-angiotensin-aldosterone system gene polymorphisms and coronary artery disease: detection of gene-gene and gene-environment interactions. *Cell Physiol Biochem* 2012; 29: 443–452.