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.1 Twenty percent of patients with ischemic stroke present with a TIA during the hours and days immediately preceding the stroke.2 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.3,4 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.5 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.6 The aldosterone synthase gene (CYP11B2) locus is an important candidate region in the development of hypertension,7 diabetic nephropathy,8 type 2 diabetes mellitus,9 and atrial fibrillation.10 Because it has been suggested in a meta-analysis that the CYP11B2 -344T/C polymorphism was associated with ischemic stroke,11 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.12 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.13 The primers used for PCR were 5‘-CAGGAGGAGA CCCCCATGTGAC-3’ (sense) and 5‘-CCTC CACCCTGTTCAGCC-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 ± standard deviation [SD]) was 63.5 ± 6.8 years in the TIA patient group and 62.6 ± 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.14–16 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 (years) | 63.5 ± 6.8 | 62.6 ± 8.6 | N.S. |
| Sex | | | |
| Male | 61 (61%) | 64 (64%) | N.S. |
| Female | 39 (39%) | 36 (36%) | N.S. |
| Body weight (kg) | 67.5 ± 10.7 | 68.3 ± 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 ± 1.0 | 4.6 ± 1.1 | <0.0001 |
| TG (mmol/L) | 1.4 ± 0.4 | 1.1 ± 0.3 | <0.0001 |
| HDL-C (mmol/L) | 1.4 ± 0.4 | 1.3 ± 0.4 | 0.08 |
| LDL-C (mmol/L) | 3.2 ± 0.7 | 2.9 ± 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.
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. Aldosterone-induced reactive oxygen species (ROS) play an important role in endothelial dysfunction. 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. 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. Evidence from experimental and clinical studies suggests that excessive circulating aldosterone levels can cause adverse vascular sequelae that are independent of hypertension. The association of angiotensinogen, CYP11B2, and angiotensin-converting enzyme polymorphisms showed a possible positive interaction in the development of renal insufficiency among hypertensive subjects. The interaction between the CYP11B2 -344T/C polymorphism, age, and smoking status is also associated with an increased risk of coronary artery disease. 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 of 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.

### 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.
Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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