Clinical study

Evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischaemic stroke

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Abstract

Angiotensin-converting enzyme (ACE) gene polymorphism has been associated with increased incidence of stroke in some populations, although contradictory results have been reported. The aim of this study was to determine the allelic frequency and the genotypic distribution for ACE gene polymorphism in Turkish patients with ischemic stroke compared to appropriate healthy controls and to correlate the genetic findings with stroke type. One hundred and eight patients with ischemic stroke versus 79 healthy controls were studied for the presence of ACE gene polymorphism detected by PCR. Genotypes were defined as DD, II and ID according to the presence of the D (deletion) and I (insertion) alleles. There was no statistically significant difference in either the genotypic distribution or allelic frequency between the patients versus healthy controls ($\chi^2 = 0.105; df = 1; p = 0.430$). There was also no significant difference for ACE genotype distribution and allelic frequency within the stroke group classified according to Bamford criteria ($\chi^2 = 4.827; df = 3; p = 0.185$). Our data supports lack of association between DD genotype and/or D allele and ischemic stroke or subtypes of ischaemic stroke in the Turkish population.

Keywords: Angiotensin-converting enzyme; Gene polymorphism; Ischaemic stroke; Allelic frequency; Genotype

1. Introduction

Stroke is the third most common cause of death following cardiac disease and cancer worldwide. Ischemic stroke accounts for 75–85% of all strokes and 15–33% of these result in severe morbidity and/or mortality.1 The pathogenesis of stroke is multifactorial and the major risk factors defined are hypertension, hyperlipidemia and diabetes mellitus. It has also been suggested that genetic background may predispose individual patients to increased risk either as an independent risk factor for cerebrovascular disease or by modulating the effects of classical risk factors.2,3

There is substantial evidence suggesting a role of the renin angiotensin system (RAS) in the development of hypertension and cardiovascular disease.4 Cerebrovascular endothelium has been shown to be rich in angiotensin-converting enzyme (ACE) by histochemical studies.5,6 Furthermore, in experimental stroke models in spontaneously hypertensive rats, ACE has been an important mediator of vascular changes.7 ACE has also been demonstrated in human studies to have an important role in the pathogenesis of white material lesions and lacunar infaracts.8

The human ACE gene is located on the short arm of chromosome 17 at position 23 (17q23).9 A deletion polymorphism of a 287-bp fragment of intron 16 of the ACE gene was detected, resulting in allele D if deletion is present and in allele I if absent.10 Homozygous presence of the deletion polymorphism has been associated with higher plasma ACE activity.10 The ACE gene polymorphism has
been investigated for its possible association with essential hypertension, coronary artery disease, atherosclerosis of the carotid artery and cerebral white matter lesions in patients with essential hypertension, and the findings seem to vary between populations of different genetic and environmental backgrounds.\textsuperscript{11–19} ACE gene polymorphism has also been associated with increased incidence of stroke in some populations, although contradictory results have been reported.\textsuperscript{8,17,20–25}

The aim of the present study was to determine the allelic frequency and the genotypic distribution for ACE gene polymorphism in Turkish patients who presented with ischemic stroke compared to appropriate healthy controls and to correlate the genetic findings with the type and incidence of stroke.

2. Materials and methods

One hundred and eight patients (M:F, 45:63) who presented with acute stroke to Marmara University Hospital Neurology Department from March 2001–March 2002 and 79 (M:F, 36:43) healthy individuals matched for age and gender formed the control group. Informed consent was taken from all subjects included in the study.

Detailed medical histories and risk factors for stroke including hypertension, hyperlipidemia, diabetes mellitus, coronary heart disease, dysrhythmias and smoking status were ascertained for each subject. Hypertension was defined as blood pressure of 160/95 mmHg or greater at repeated measurements and hyperlipidemia was defined as plasma total cholesterol >200 mg/dL and/or plasma triglycerides >200 mg/dL. Those subjects whose fasting glucose levels were >110 mg/dL or who were taking antidiabetic medication were defined as diabetic. Patients with cerebral hemorrhage, transient ischemic attack, valvar heart disease, recent myocardial infarction and known cardiac rhythm disturbances were excluded from the study.

The diagnosis of stroke was made by clinical findings of neurological deficits persisting longer than 24 h. Ischemic strokes were differentiated by computed tomography scans and magnetic resonance imaging and classified according to Bamford criteria as defined in the literature.\textsuperscript{26}

Genomic DNA was isolated from peripheral lymphocytes and the 16th intron of the polymorphic ACE gene was amplified by polymerase chain reaction (PCR) as previously described.\textsuperscript{27,25} In the first PCR, a 190 bp fragment representing the D allele and a 490 bp fragment representing the I allele were obtained using 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' as a forward primer; and 5'-GAT GTG GCC ATC ACA TTC TGC GTC AGA T-3' as a reverse primer, and the ACE DD genotype was confirmed by an insertion-specific PCR amplification as previously described using 5'-TGG GAC CAC AGC GCC CGC CAC TAC T-3' as a forward primer and 5'-TGG CCA GCC CTC CCA TGC CCA TAA-3' as a reverse primer.\textsuperscript{16} The resultant PCR products were subjected to electrophoresis on an 8% polyacrylamide gel, followed by silver staining.

The association between ACE polymorphism and ischemic stroke, as well as ischemic stroke subclasses, was studied using a chi-squared test as 3 × 2 factorial of ACE genotype versus the presence or absence of ischemic stroke. The odds ratio for the DD genotype and D allele carrier state versus other genotypes and I allele carrier state respectively was calculated with an approximately 95% confidence interval. Significance was defined as $p<0.05$.

3. Results

A total of 187 subjects (108 with ischemic stroke; 79 healthy controls) were included in the study. Demographic data of the population studied are given in Table 1. Analysis of the baseline data of the stroke patients demonstrated that 84% had hypertension, 58% had hyperlipidemia, 30% had coronary heart disease, 27% had diabetes and 41% were either current or ex-smokers. In the control group, 38% were current or ex-smokers and 14% had hyperlipidemia. None of the subjects in the control group had hypertension or diabetes mellitus. Genotypic distribution of ACE polymorphism and allelic frequency for D and I alleles in patients and control subjects are presented in Table 2. There was no statistically significant difference in either the genotypic distribution or allelic frequency between the patients and healthy controls. ($\chi^2 = 0.105; df = 1; p = 0.430$). The difference within the ischemic stroke group was also not significant for ACE genotype distribution and allelic frequency when classified according to Bamford criteria ($\chi^2 = 4.827; df = 3; p = 0.185$) (Table 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic characteristics of the study population</th>
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<tbody>
<tr>
<td></td>
<td>Demographic data</td>
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<tr>
<td>Age</td>
<td>70.9 ± 8.4</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>45:63</td>
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<tr>
<td>Hypertension n (%)</td>
<td>92 (84.4)</td>
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<tr>
<td>Hyperlipidemia n (%)</td>
<td>64 (58)</td>
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<tr>
<td>Diabetes mellitus n (%)</td>
<td>29 (27)</td>
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<tr>
<td>Coronary artery disease</td>
<td>33 (30)</td>
</tr>
<tr>
<td>Current/ex-smokers n (%)</td>
<td>45 (41)</td>
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</tbody>
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<tr>
<th>Table 2</th>
<th>ACE genotypes and allelic frequencies in the study population</th>
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<tr>
<td>Study groups</td>
<td>ACE Genotypes</td>
</tr>
<tr>
<td>II</td>
<td>DD</td>
</tr>
<tr>
<td>Patients n (%)</td>
<td>17 (16)</td>
</tr>
<tr>
<td>Controls n (%)</td>
<td>12 (15)</td>
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$p = NS$ between groups. Odds ratio for DD genotype and D allele as an independent risk factor in ischemic stroke was $0.949$ (95% CI: 0.692–1.301) and $0.994$ (95% CI: 0.878–1.125) respectively.
ACE genotypes and allelic frequencies in stroke patients classified according to Bamford criteria

<table>
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<tr>
<th>Classification</th>
<th>ACE genotypes</th>
<th>Alles</th>
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<tbody>
<tr>
<td></td>
<td>II</td>
<td>DD</td>
</tr>
<tr>
<td>TACI n (%)</td>
<td>4 (27)</td>
<td>7 (48)</td>
</tr>
<tr>
<td>PACI n (%)</td>
<td>4 (17)</td>
<td>6 (25)</td>
</tr>
<tr>
<td>POCI n (%)</td>
<td>7 (18)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>LACI n (%)</td>
<td>2 (7)</td>
<td>15 (52)</td>
</tr>
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</table>

\( p = NS. \)

\( ^a \) TACI = total anterior circulation syndrome, PACI = partial anterior circulation syndrome, POCI = posterior circulation syndrome, LACI = lacunar syndrome.

4. Discussion

This study is the first to investigate the possible association of ACE gene polymorphism and ischaemic stroke in Turkish patients. The results of our study suggest no association between ischaemic stroke and the presence of DD genotype or D allele in our patient population. The results of prior studies of ACE polymorphism in stroke patients have been inconsistent with some groups reporting a positive association between the DD genotype and/or D allele and stroke, while others reported to the contrary. Doi et al. reported a significant association between ACE gene polymorphism and thrombotic brain infarction in patients age 60 years or younger in a Japanese population. Furthermore, they found a significant association between the presence of D allele and mortality following ischaemic stroke. Pföhl et al. also reported a lack of significant relationship between the history of stroke and the deletion polymorphism. Zee et al. has addressed this issue in a large prospective trial including 348 patients and reported a lack of association between ACE polymorphism and ischaemic stroke in an American population. On the other hand, Kostulas et al. reported a positive correlation between ACE gene polymorphism and both carotid artery stenosis and ischaemic cerebrovascular disease. Recently, Sharma reported a meta-analysis analyzing all known publications involving the ACE polymorphism in ischaemic stroke. In this meta-analysis 1918 white subjects were studied and no difference in ACE genotype or I/D allele frequency was found between cases and controls. However, the overall OR for the D allele as an independent risk factor in ischaemic stroke was 1.31 under a recessive model and 1.14 in a dominant model and the author concluded that the D allele may be a modest but independent risk factor for ischaemic stroke onset. Furthermore, recently the DD genotype has been associated with silent brain ischaemia and/or lacunar syndromes which mainly result from essential hypertension. Our results support the data in the literature suggesting a lack of association between ACE polymorphism and ischaemic stroke.

The fact that ischemic stroke may be considered the cerebral counterpart of generalized arteriosclerosis leads to the speculation that ACE activity may have an impact in cerebrovascular disease. However, this tempting speculation has been questioned by Catto et al. who reported significantly decreased ACE activity in stroke patients at presentation and lower ACE activity seemed to correlate with mortality. They have also reported an association between increased mortality and the D allele. These findings and lack of association between ACE polymorphism and stroke in the literature suggest that ACE activity may be determined by other factors than ACE gene polymorphism in stroke patients. ACE could be involved in the pathogenesis of cerebrovascular disease by several biological mechanisms, including activation of angiotensin I and inactivation of bradykinin, resulting in decreased tissue perfusion, vascular smooth muscle cell growth, and stimulation of plasminogen activator inhibitor type 1. However, the role of ACE either as a direct mediator of or secondary following an acute cerebrovascular event is not clear, although ACE has been demonstrated in the nigrostriatal pathway and basal ganglia. There are very few studies in the literature addressing ACE activity in stroke patients. Furthermore, in these studies plasma ACE activity has been measured with a vertically cross-sectional time relationship to stroke, therefore ACE activity might actually fluctuate before, during or after the stroke has occurred. The contribution of ACE activity to the development of stroke therefore needs to be further investigated.

The limitation of our study, as of the other studies in the literature, may be the absence of ACE activity measurement and its correlation to genetic data.

In conclusion, our data supports lack of association between DD genotype and/or D allele and ischemic stroke or subtypes of ischaemic stroke in the Turkish population. Further studies are needed to investigate the role and determinants of ACE activity in patients with cerebrovascular events.

References


34. Erdős EG, Skidgel RA. The angiotensin I-converting enzyme. Lab Invest 1987;56:345–8.