Meta Gene 2 (2014) 661–669

Contents lists available at ScienceDirect

Meta Gene

ACE-II genotype and I allele predicts ischemic stroke among males in south India

Murali Vijayan [a], Rathika Chinniah [b], Padma Malini Ravi [b], Arun Kumar Mosses Joseph [c], Neethi Arasu Vellaiappan [d], Jeyaram Illiayaraja Krishnan [e], Balakrishnan Karuppiah [b,*]

[a] Department of Biotechnology & Genetic Engineering, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India
[b] Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai-625 021, Tamil Nadu, India
[c] Hannah Joseph Hospital, Madurai-625 020, Tamil Nadu, India
[d] Neethi Arasu Neuro Hospital, Madurai-625 020, Tamil Nadu, India
[e] Narayana Institute Health City, Bangalore-560 099, Karnataka, India

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 22 August 2014
Accepted 2 September 2014
Available online 28 September 2014

Keywords:
Ischemic stroke
Large vessel disease
ACE (I/D)
Genotype
Polymorphism
Hypertension

ABSTRACT

Two hundred ischemic stroke patients and 193 age and sex matched healthy controls were studied for the presence of Angiotensin Converting Enzyme Insertion/Deletion (ACE I/D) gene polymorphism. The PCR studies revealed that ACE 'II' (OR = 2.055; $p = 0.004$) genotype and 'I' (OR = 1.411; $p = 0.018$) alleles were significantly associated with IS patients. Gender specific analysis revealed a strong association of 'II' (OR = 2.044; $p = 0.014$) genotype and 'I' (OR = 1.531; $p = 0.011$) allele with male sex. Classification of patients based on TOAST criteria, revealed a significant association for 'II' genotype (OR = 1.713; $p = 0.043$) and 'I' (OR = 1.382; $p = 0.039$) allele in LVD patients only. When the data was stratified based on age and sex, a statistically significant association was observed for ACE 'II' genotype (OR = 2.288; $p = 0.006$) and 'I' allele (OR = 1.395; $p = 0.054$) in IS male patients of >50 years of age. The ACE 'D' allele was found to be increased in controls (OR = 0.709; p = 0.018) than IS patients. Multivariate logistic regression analysis showed that smoking and diabetes were the most powerful independent risk factor in LVD type of stroke. Thus, we presented here an evidence for a strong association of ACE 'II' genotype and 'I' allele compounded by factors such as smoking and diabetes among south Indian IS patients.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

* Corresponding author. Tel.: +91 452 2458418.
E-mail address: immunobala@mkuniversity.org (B. Karuppiah).

http://dx.doi.org/10.1016/j.mgene.2014.09.003
2214-5400/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Introduction

Stroke is the second leading cause of death and the third leading cause of disability-adjusted life-years (DALYs) worldwide (Lozano et al., 2012). The prevalence rate of stroke in India varies across regions (90–222/lakh) (Dalal et al., 2007). Stroke can be broadly classified in two major clinical types: ischemic stroke and hemorrhagic stroke (Rothwell et al., 2004). For the ischemic stroke (IS), the classification into etiological subtypes is usually done according to the validated TOAST diagnostic criteria (Adams et al., 1993). This classification denotes five subtypes of ischemic stroke: Large Vessel Disease (LVD), Small Vessel Disease (SVD) and Cardio Embolic (CE) stroke, stroke of other determined etiology and stroke of undetermined etiology. IS is the most predominant type of stroke accounting for approximately 87% of stroke cases (Rosamond et al., 2008). It usually results from atherosclerosis, heart disease, vasculitis, and hypertension, and compounded by smoking and alcoholism. Atherosclerosis is an inflammatory disease which induces arterial stenosis and occlusion leading to ischemic stroke. Age, ethnicity, race, gender and genetic factors have also been identified as risk factors for stroke (Brass et al., 1992; Elbaz and Amarenco, 1999; Goldstein et al., 2001).

Angiotensin Converting Enzyme (ACE) gene is located on chromosome 17 at position 23 (17q23) in humans. The insertion or deletion of 287-base pair sequence of DNA in intron 16 of the gene coding for ACE mediates the circulating ACE levels. The renin–angiotensin system (RAS) has a central role in hypertension and atherosclerosis (Jiang et al., 2009). Angiotensinogen is converted to angiotensin I by the stimulation of renin. ACE converts angiotensin I to angiotensin II, the active product of the RAS system. The ACE I/D polymorphism is reported to determine circulating and tissue ACE levels such that individuals homozygous for the D allele have higher tissue and plasma ACE concentrations than heterozygous ID and II homozygous (Costerousse et al., 1993; Rigat et al., 1990). Stroke patients with hypertension were treated with ACE inhibitors as the primary and secondary prevention strategies. ACE is widely distributed on the surfaces of endothelial and epithelial cells. Cerebrovascular endothelium was reported to be rich in ACE (Ehlers and Riordan, 1989; McGeer and Singh, 1992). The ACE (I/D) gene polymorphism was shown to be associated in several diseases including diabetes mellitus (Inanir et al., 2013), nephrotic syndrome (Shahid et al., 2012), Alzheimer's disease (Narain et al., 2000), hypertension, and coronary artery disease (Dhar et al., 2012; Zhou et al., 2013). In the present study, we carried out a retrospective analysis of ACE (I/D) genotype and allele polymorphism among IS patients from south India.

Materials and methods

Subjects

For the present study 200 IS patients (154 men, 46 women: mean age of 57.5 ± 13.8 years) presenting with new or recurrent stroke as evaluated in the stroke clinics (Madurai, Tamil Nadu) were recruited over a period of one year (between July 2012 to March 2013). Each patient with acute stroke was examined and confirmed by CT scans and MRI. The stroke subtype assignment was as per the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification (Adams et al., 1993). Patients with hemorrhagic and transient ischemic attack were excluded from the study.

Totally 193 healthy individuals matched for age and sex formed the control group (144 men, 49 women: mean age: 55.9 ± 13.2 years). Demographic information, medical history, biochemical profile and established risk factors were recorded in a precoded questionnaire. Hypertension was diagnosed according to the JNC VI–VII criteria (Britov and Bystroya, 2003). Data on smoking was recorded based on self reporting by the patients and diabetes by laboratory diagnosis. Ex-smokers and occasional smokers were classified as non-smokers (Glader et al., 2000). Informed written consents were obtained from all the subjects and the study was approved by the Institutional Ethical Committee. The concentrations of total lipid profile and blood glucose were measured using the standard methods and estimated by end point assays using commercial kits (Liquizone, India) using an auto analyzer (ERBA Mannheim, Germany; model no: Erba Chem 5 v2).

Genomic DNA extraction

Two milliliters of blood was collected for the extraction of genomic DNA by modified salting out method (Miller et al., 1988) and the concentration was estimated by UV-Spectrophotometer (Milton Roy, USA; model no: 336001). DNA samples were stored at −80 °C until further use.

ACE (I/D) genotyping

The 16th intron of the polymorphic ACE gene was detected using following primers: forward 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′; and reverse 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ and the PCR was carried as described previously (Rigat et al., 1992). PCR was carried out in a total volume of 12 μl containing 7.00 μl of Milli-Q water, 1.2 μl of 10× PCR buffer, 3 μl of genomic DNA (200 ng/ml), 0.24 μl of 10 mM dNTP (CinnaGen, Iran), 0.2 μl of 5U *Taq* polymerase (GeNet Bio, Korea), 0.36 μl each of forward and reverse primers (10 mM). PCR amplification was carried with an initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 2 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min and a final extension at 72 °C for 5 (Agilent, USA; model no: G8800A). Homozygosity was identified by observing 190-bp PCR product for D allele (DD), 490-bp PCR product for I allele (II) and 190-bp and 490-bp PCR products for ID (Fig. 1). To avoid mistyping of heterozygotes (ID) DNA samples identified as a DD genotype were subsequently amplified with second set of primers designed for the insertion specific allele (Shanmugam et al., 1993).

Statistical analysis

The statistical analysis was performed by STATA 11.1 (College Station, TX, USA). Student's *t*-test was used to find out the significance between the groups for age, systolic blood pressure (SBP), diastolic blood pressure (DBP), total lipid profile, serum creatinine and was expressed as mean ± standard deviation (SD). Hardy–Weinberg equilibrium for ACE genotypes was tested by 'Chi square test' in each group. Multiple regression analysis was performed for measuring the relationship between the groups for factors such as age, gender, blood pressure TGL, TCL, ACE (I/D), diabetes, hypertension, smoking and alcohol as possible risk factors. Statistical significance was considered at $p < 0.05$.

Results

The demographical and clinical parameters of patients and controls were given in Table 1. Blood pressure, serum creatinine and lipid profile were significantly higher among IS patients than controls. ACE (I/D) genotype frequencies were in line with Hardy–Weinberg equilibrium for both IS patients and healthy controls. The differences in the ACE (I/D) genotypic and allelic frequencies between the IS patients and controls were presented in Table 2. A higher frequency of ACE 'II' genotype (29% vs. 16.58%; OR = 2.055; $p = 0.004$) and 'I' (57% vs. 48.45%; OR = 1.411; $p = 0.018$) allele was noticed among IS patients than controls. The frequency of 'D' allele was significantly lower among IS patients than controls (43% vs. 51.55%; OR = 0.709; $p = 0.018$).

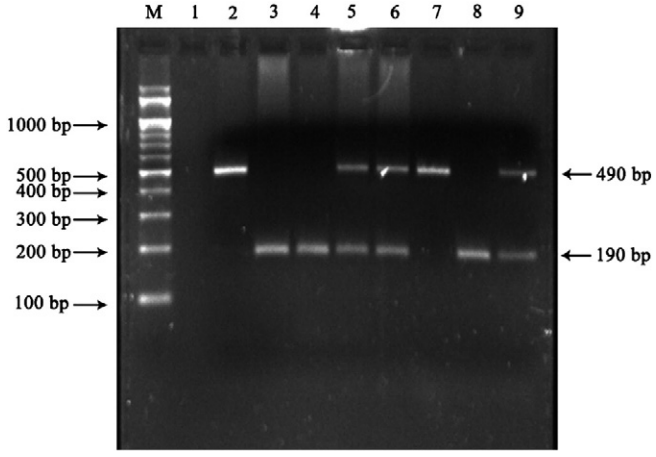

Fig. 1. Agarose gel electrophoresis, showing the amplification pattern of ACE (I/D) gene polymorphism. M represents the 100 bp ladder. Lane 1 represents the negative control. Lanes 2 and 7 show II genotype (490 bp product), Lanes 5, 6, and 9 show ID genotype (490 and 190 bp products) and Lanes 3, 4 and 8 show DD genotypes (190 bp product).

When we stratified the data by sex, the frequency was significantly increased for ACE 'II' genotype (OR = 2.044; $p = 0.014$) and 'I' allele (OR = 1.531; $p = 0.011$) and lowered for 'D' (OR = 0.653; $p = 0.011$) allele in IS male patients compared to respective controls. Among IS patients >50 years of age, a statistically significant association was observed for ACE 'II' genotype (OR = 2.288; $p = 0.006$) and 'I' allele (OR = 1.395; $p = 0.054$) than the controls of same age group. Further, the ACE 'II' genotype (OR = 2.767; $p = 0.003$) and 'I' allele (OR = 1.613; $p = 0.016$) showed very strong association only in IS male patients >50 years of age (Table 3). Such age specific association was not observed for female patients >50 years. However, 'D' allele was significantly lower among IS male patients >50 years of age than control males (OR = 0.62; $p = 0.016$). When the data was stratified for age and gender in subjects <50 years, we did not notice any difference (both in pooled and sex stratified groups). Thus, age specific association for ACE 'II' genotype and 'I' allele was significant only in male patients >50 years of age.

One hundred and seventy five (87.5%) patients were clinically diagnosed as patients with large vessel disease (LVD) and twenty five (12.5%) patients with small vessel disease (SVD). Of 175 patients with LVD, 28.57% (n, 50) had 'II' genotype, 56% (n, 98) had 'ID' and 15.43% (n, 27) had 'DD' genotype. The frequencies of 'II' genotype (OR = 1.713; $p < 0.043$) and 'I' (OR = 1.382; $p = 0.039$) allele was significantly higher in LVD patients compared to controls. On the contrary the 'D' allele was significantly lower in LVD patients (OR = 0.724; $p = 0.039$) than controls. When we stratified the LVD by sex, the 'I' allele frequency was significantly higher among LVD male patients compared to control males (OR = 1.473; $p = 0.030$). No such association of ACE genotype or allele was observed for LVD female patients (n, 38) and SVD patients (n, 25; pooled). Further, the frequencies of 'DD' genotype ($p = 0.048$) and the 'D' allele ($p = 0.030$) were significantly lower in LVD male patients than control males (Table 4).

Considering the co-occurrence of other ailments of IS patients, 49.50% (n, 99) had hypertension and 49.50% (n, 99) had type 2 diabetes mellitus (T2DM) and 32.5% (n, 64) had both hypertension and T2DM. The ACE 'II' genotype (OR = 2.925; $p = 0.005$) and 'I' allele (OR = 1.576; $p = 0.030$) were significantly higher among the IS hypertension (ISHT) patients than the controls (Table 5). A significantly lower frequency of ACE 'DD' genotype was observed among IS-Diabetic (ISDM) patients (28.26% vs. 15.15; OR = 0.453; $p = 0.034$) (Table 6) and IS patients with hypertension than the controls (OR = 0.635; $p = 0.030$) (Table 5).

Risk factors for stroke

Multiple logistic regression analysis was performed for independent variables such as age, sex, TGL, TCL, SBP and DBP (data not shown). In both LVD and SVD, SBP (OR = 1.05; $p = 0.001$), TGL (OR = 1.01; $p = 0.001$) and TCL (OR = 1.01; $p = 0.033$) were found to be an independent risk factor. In the present study smoking (OR = 5.72; p = 0.053) and diabetes (OR = 2.95; p = 0.041) were identified as a strong predictor for LVD than SVD (OR = 0.17; p = 0.053 and OR = 0.34; p = 0.041 respectively).

Table 1
Demographical characteristics of the study population.

Demographic data	Patients (n = 200)	Controls (n = 193)	*p* value
Age (years) mean ± SD	57.5 ± 13.8	56 ± 13.2	NS
Male/female distribution	156/44	144/49	NS
Smokers	32.6 (51)	–	–
Non-smokers	67.3 (105)	–	–
Alcohol	33.33 (52)	–	–
Non-alcohol	66.66 (104)	–	–
Systolic BP (mm Hg)	140.9 ± 23.8	123 .6 ± 14.15	**0.001***
Diastolic BP (mm Hg)	89.2 ± 14.3	82.8 ± 8.33	**0.001***
Glucose (mg/dl)	154.1 ± 64.8	106.2 ± 15.8	**0.001***
Total cholesterol (mg/dl)	207.8 ± 71.3	170.4 ± 31.9	**0.001***
Triglycerides (mg/dl)	195.4 ± 82.1	150.9 ± 29.6	**0.001***
HDL cholesterol (mg/dl)	43.7 ± 10.5	37.2 ± 6.1	**0.001***
LDL cholesterol (mg/dl)	130 ± 68.6	97.4 ± 31.7	**0.001***
Serum creatinine (mg/dl)	1.1 ± 0.4	0.9 ± 0.2	**0.001***

* Highly significant.

Table 2
ACE (I/D) genotype and allele frequencies in IS patients and controls.

Genotype/allele	HC (n = 193)[a] (n = 144)[b] (n = 49)[c]	IS (n = 200)[a] (n = 154)[b] (n = 46)[c]	OR	95% CI	χ^2	*p* value
II	16.58 (32)[a]	29.00 (58)[a]	2.05	1.229–3.445	7.892	**0.004***
	17.36 (25)[b]	29.87 (46)[b]	2.04	1.137–3.688	5.899	**0.014***
	14.29 (07)[c]	26.09 (12)[c]	2.07	0.660–6.621	1.280	0.203
ID	63.73 (123)	56.00 (112)	0.72	0.473–1.108	2.131	0.124
	59.02 (85)	55.19 (85)	0.86	0.526–1.390	0.303	0.559
	77.55 (38)	58. 70 (27)	0.41	0.153–1.094	3.080	0.076
DD	19.69 (38)	15.00 (30)	0.72	0.412–1.257	1.199	0.232
	23.61 (34)	14.94 (23)	0.57	0.303–1.062	3.082	0.076
	08.16 (04)	15.21 (07)	2.02	0.480–8.991	0.567	0.346
I	48.45 (187)	57.00 (228)	1.41	1.054–1.888	5.430	**0.018***
	46.87 (135)	57.47 (177)	1.53	1.094–2.144	6.277	**0.011***
	53.06 (52)	55.43 (51)	1.10	0.597–2.029	0.033	0.772
D	51.55 (199)	43.00 (172)	0.71	0.530–0.948	5.430	**0.018***
	53.13 (153)	42.53 (131)	0.65	0.466–0.914	6.277	**0.011***
	46.94 (46)	44.57 (41)	0.91	0.493–1.675	0.033	0.772

IS — Ischemic Stroke; HC — Healthy Controls.
[a] Pooled.
[b] Males.
[c] Females.
* Highly significant.

Genotype — stroke correlation

Logistic regression analysis of genetic models was carried out dominant (II + ID vs. DD), recessive (II vs. (DD + ID) and additive (II vs. DD) modes of inheritance. The results of the recessive and co-dominant model revealed a significant association (OR = 2.06; 95% CI — 1.23–4.46; $p = 0.003$ and OR = 0.65; 95% CI — 0.43–0.99; $p = 0.034$ respectively) with IS (Table 7). Co-dominant model revealed a significant association (OR — 0.65; 95% CI —

Table 3
ACE (I/D) genotype and allele frequencies in >50 years IS patients and controls.

Genotype/allele	HC (n = 140)[a] (n = 104)[b] (n = 36)[c]	>50 years (n = 146)[a] (n = 113)[b] (n = 33)[c]	OR	95% CI	χ^2	*p* value
II	15.00 (21)[a]	28.77 (42)[a]	2.29	1.226–4.293	7.105	**0.006***
	13.46 (14)[b]	30.10 (34)[b]	2.77	1.319–5.868	7.753	**0.003***
	19.44 (07)[c]	24.14 (08)[c]	1.33	0.367–4.829	0.036	0.772
ID	66.43 (93)	55.48 (81)	0.63	0.379–1.046	3.151	0.069
	63.46 (66)	53.98 (61)	0.68	0.378–1.207	1.633	0.170
	75.00 (27)	60.61 (20)	0.51	0.161–1.609	1.047	0.301
DD	18.57 (26)	15.75 (23)	0.82	0.423–1.586	0.226	0.535
	23.08 (24)	15.93 (18)	0.63	0.303–1.312	1.344	0.229
	5.56 (2)	15.15 (5)	3.04	0.465–24.716	0.846	0.247
I	48.21 (135)	56.51 (165)	1.40	0.990–1.967	3.616	**0.054***
	45.19 (94)	57.08 (129)	1.61	1.084–2.401	5.660	**0.016***
	56.94 (41)	54.54 (36)	0.98	0.438–1.879	0.013	0.864
D	51.79 (145)	43.49 (127)	0.72	0.508–1.010	3.616	**0.054***
	54.81 (114)	42.92 (97)	0.62	0.417–0.923	5.660	**0.016***
	43.06 (31)	45.45 (30)	1.10	0.532–2.284	0.013	0.864

HC — Healthy Controls.
[a] Pooled.
[b] Males.
[c] Females.
* Highly significant.

Table 4
ACE (I/D) genotype and allele frequencies in LVD patients and controls.

Genotype/allele	HC (n = 169)[a] (n = 130)[b] (n = 39)[c]	LVD (n = 175)[a] (n = 137)[b] (n = 38)[c]	OR	95% CI	χ^2	*p* value
II	18.93 (32)[a]	28.57 (50)[a]	1.71	1.003–2.931	3.883	**0.043***
	19.23 (25)[b]	28.47 (39)[b]	1.67	0.098–3.086	2.636	0.086
	17.95 (07)[c]	28.95 (11)[c]	1.86	0.564–6.269	0.758	0.291
ID	59.17 (100)	56.00 (98)	0.88	0.559–1.378	0.236	0.586
	55.38 (72)	56.20 (77)	1.03	0.619–1.726	0.000	0.903
	71.79 (28)	55. 26 (21)	0.49	0.168–1.382	1.615	0.159
DD	21.90 (37)	15.43 (27)	0.65	0.362–1.166	1.965	0.130
	25.39 (33)	15.33 (21)	0.53	0.276–1.021	3.581	**0.048***
	10.26 (04)	15.79 (06)	1.64	0.363–7.752	0.147	0.517
I	48.52 (164)	56.57 (198)	1.38	1.012–1.188	4.153	**0.039***
	46.92 (122)	56.57 (155)	1.47	1.032–2.103	4.594	**0.030***
	53.85 (42)	56.58 (43)	1.18	0.563–2.217	0.032	0.749
D	51.48 (174)	43.43 (152)	0.72	0.530–0.988	4.153	**0.039***
	53.08 (138)	43.43 (119)	0.68	0.475–0.969	4.594	**0.030***
	46.15 (36)	43.42 (33)	0.90	0.451–1.776	0.032	0.749

LVD — Large Vessel Disease.
HC — Healthy Controls.
[a] Pooled.
[b] Males.
[c] Females.
* Highly significant.

0.43–0.99; $p = 0.034$). Further, we calculated the 'degree of dominance' (h) test to find out the deviation of heterozygous state from the risk of disease. The analysis revealed that the degree of dominance (h) was <1 for the heterozygous state (I/D) and implied its level of closeness to the disease risk conferred by homozygous II state.

Discussion

The results of our study revealed a significant association of ACE 'II' genotype and 'I' allele with IS. Contrary to the previous reports, 'II' genotype and 'I' allele frequencies were significantly high, in both pooled and male IS cases. No clear mechanism was elucidated so far for the association of II genotype and I allele with IS. However, substance p (a neuropeptide) a substrate for ACE was shown to be implicated in the inflammatory response. This 'substance p' was found in intra cerebral tissue and vascular endothelium, which suggests a role in endothelial metabolism and vascular tone. Further, the plasma ACE activity (coronary) was found to be unrelated to intracerebral ACE activity. Previous studies in other ethnic populations have documented a strong association of 'DD' genotype and 'D' allele (Catto et al., 1996; Kalita et al., 2011; Munshi et al., 2008; Sharma, 1998) while others revealed no association with IS (Gao et al., 2006; Karagiannis et al., 2004; Markus et al., 1995; Pera et al., 2006; Pfohl et al., 1998; Tascilar et al., 2009; Tuncer et al., 2006). Presumably, these observed variations could be attributed to the differences in ethnic composition of study subjects and study design (Szolnoki, 2005).

Table 5
ACE (I/D) genotype and allele frequencies in IS patients with Hypertension (ISHT) and Controls.

Genotype/Allele	ISHT (n = 99)	Control (n = 89)	OR	95% CI	χ2	*p* value
II	31.31 (31)	13.48 (12)	2.925	1.318-6.583	7.465	**0.005***
ID	57.58 (57)	70.79 (63)	0.56	0.292-1.073	2.994	0.069
DD	11.11 (11)	15.73 (14)	0.67	0.265-1.684	0.513	0.394
I	60.10 (119)	48.88 (87)	1.576	1.025-2.423	4.325	**0.03***
D	39.90 (79)	51.12 (91)	0.635	0.413-0.975	4.325	**0.03***

ISHT - Ischemic Stroke with Hypertension
* Highly Significant

Table 6
ACE (I/D) genotype and allele frequencies in IS patients with Diabetes Mellitus (ISDM) and Controls.

Genotype/Allele	ISDM (n = 99)	Control (n = 92)	OR	95% CI	$\chi 2$	*p* value
II	23.23 (23)	20.65 (19)	1.163	0.554-2.445	0.065	0.728
ID	61.61 (61)	51.09 (47)	1.537	0.830-2.849	1.744	0.148
DD	15.15 (15)	28.26 (26)	0.453	0.209-0.976	4.115	**0.034***
I	54.04 (107)	46.20 (85)	1.369	0.897-2.091	2.044	0.152
D	45.96 (91)	53.80 (99)	0.73	0.478-1.115	2.044	0.152

ISDM - Ischemic Stroke with Diabetes Mellitus
* Highly Significant

The contribution of ACE activity to the development of cerebrovascular disease remained elusive. In the present study, 'D' allele was observed to be higher in healthy controls than in IS patients. A recent meta-analysis revealed 'D' allele as a low-penetrance susceptibility marker for IS (Zhang et al., 2012). Contrary to our finding, Munshi et al. (2008) have reported that ACE 'ID' and 'DD' genotypes with an elevated incidence of stroke in south Indian population. Similarly, another study from North Indian population suggested that ACE 'DD' genotype was significantly higher in IS patients than in controls (Kalita et al., 2011). However, study by Prabhakar et al. (2014) reported no such association for 'DD' genotype and 'D' allele in south India, particularly in IS with small vessel disease (SVD). Further, a lack of association of 'DD' genotype and 'D' allele in earlier studies on Turkish IS patients lends support to our finding (Dikmen et al., 2006; Tuncer et al., 2006). Such contradictory reports were documented for various populations (Pera et al., 2006; Saidi et al., 2009; Um et al., 2003). According to Catto et al. (1996), low plasma ACE activity may be the marker for stroke risk. Our results revealed an association of ACE 'II' genotype and 'I' allele with LVD, the most frequent subtype of IS in south India. Thus, based on the results of our study and previous studies it is possible to hypothesize that either 'DD' genotype or 'D' allele dependent increase in ACE activity (under certain pathophysiological condition) and subsequent lowering of plasma ACE level (by unknown mechanism) prior to a stroke event or 'II' genotype or 'I' allele dependent inherent low level of circulating ACE may be attributed to the IS. However, a prospective case control study would be required to confirm such a hypothesis.

In the present study, significant increase in serum triglycerides, TC, HDL and LDL are found as a significant risk factor for IS. Our result is in accordance with a previous study (Hachinski et al., 1996) and it was reported earlier that hypertension is the important risk factor for stroke, diabetes and cardiovascular diseases (Jiang et al., 2009). Since, the frequency of hypertension and diabetes among IS patients in the present study was found to be equal (49.5%), it is possible to conclude that hypertension and diabetes are two important factors for the development of stroke in south Indian population. Diabetes probably predisposes to the development of stroke by its contribution to the atherogenesis in the cerebral vessels (Lavy et al., 1973). Smoking can accelerate the effect of the ACE I/D gene polymorphism on the risk of blood pressure, hypertension, coronary artery disease and the risk of mortality at a younger age (Schut et al., 2004). Multiple logistic regression analysis revealed that smoking and diabetes were the two important independent risk factors for LVD form of IS. Smoking was reported as a major cause of stroke in several populations and smokers are four

Table 7
Analysis of genotypes as risk factor for ischemic stroke.

Study models	OR	95% CI	*p* value
Whole group (n = 200)			
II + ID vs. DD[a]	1.31	0.75–2.29	0.318
II vs. DD[b]	2.29	1.14–4.60	0.109
II vs. (DD + ID)[c]	2.06	1.23–3.46	**0.003***
ID vs. (DD + II)[d]	0.65	0.43–0.99	**0.034***

[a] Dominant effect of the I allele.
[b] Additive effect of the I allele.
[c] Recessive effect of the I allele.
[d] Co-dominant model.
* Highly significant.

times more likely to have a stroke compared with non-smokers (Aldoori and Rahman, 1998; Bonita et al., 1999). Cigarette smoking is known to contribute to the development of carotid artery atherosclerosis (Dempsey and Moore, 1992; Hankey, 1999). Few studies demonstrated the gender specific comparison with ACE gene. Influence of RAS genotype on the ACE plasma concentrations and molar ratio of Ang-II to Ang I–VII has a greater gender dimorphism. The gene regulation of RAS, ACE concentration and circulating levels of Ang-II were affected by testosterone (Bos et al., 2008; Danser et al., 1998; Reyes-Engel et al., 2006). Thus, the present study emphasized the pathological role of 'II' genotype and 'I' allele in males >50 years of age. Further, this male specific association could be attributed to the risky behaviors of males such as cigarette smoking and alcohol consumption.

Further analysis of data revealed that the diabetes is the second most independent risk factor next to smoking. Previous studies indicated that diabetes per se increases the likelihood of severe carotid atherosclerosis (Folsom et al., 1994; O'Leary et al., 1992). When we carried out genetic model based risk analysis, we did not find any associations in dominant and additive models. However, analysis of recessive model (II vs. (ID + DD) revealed significant disease risk (OR = 2.06; 95% CI = 1.23–3.46; $p = 0.003$). Thus, it is concluded that homozygous 'II' genotype has showed greater risk for stroke than homozygous 'DD' genotype in our population. However, the limitation of our study is the absence of data on plasma ACE level of IS patients and its correlation to genetic data. World Health Organization (WHO) estimated that by 2050, 80% of stroke cases in the world would come from low and middle income countries such as India and China. In conclusion, our data revealed a significant association of ACE 'II' genotype and 'I' allele with male IS patients in south India. However, more prospective studies with a larger sample size are required to ascertain the role of ACE 'II' genotype in the aetiopathogenesis of ischemic stroke in south India.

Acknowledgments

The financial support by various funding agencies of the Govt. of India (DBT, DRDO, UGC) to the corresponding author (KB) is thankfully acknowledged. The authors were grateful to the various central facilities such as CAS, CEGS, NRCBS, DBT-IPLS, DST-CEFC, and DST-PURSE at Madurai Kamaraj University. We sincerely thank all the patients and paramedical staffs of hospitals from where we collected clinical specimen.

References

Adams Jr., H.P., Bendixen, B.H., Kappelle, L.J., Biller, J., Love, B.B., Gordon, D.L., et al., 1993. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial: TOAST: Trial of Org 10172 in Acute Stroke Treatment. Stroke 24, 35–41.
Aldoori, M.I., Rahman, S.H., 1998. Smoking and stroke: a causative role. BMJ 317, 962–963.
Bonita, R., Duncan, J., Truelsen, T., Jackson, R.T., Beaglehole, R., 1999. Passive smoking as well as active smoking increases the risk of acute stroke. Tob. Control 8, 156–160.
Bos, M.J., Schuit, S.C., Koudstaal, P.J., Hofman, A., Uitterlinden, A.G., Breteler, M.M., 2008. Variation in the estrogen receptor alpha gene and risk of stroke: the Rotterdam Study. Stroke 39, 1324–1326.
Brass, L.M., Isaacsohn, J.L., Merikangas, K.R., Robinette, C.D., 1992. A study of twins and stroke. Stroke 23, 221–223.
Britov, A.N., Bystroya, M.M., 2003. New guidelines of joint National Committee (USA) on prevention, diagnosis and management of hypertension from JNC V 1 JNCv11. Kardiologiia 43, 93–97.
Catto, A., Carter, A.M., Barret, J.H., Stickland, M., Bamford, J., Davies, J.A., et al., 1996. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke 27 (3), 435–440.
Costerousse, O., Allegrini, J., Lopez, M., Alhenc-Gelas, F., 1993. Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. Biochem. J. 290 (Pt 1), 33–40.
Dalal, P., Bhattacharjee, M., Vairale, J., Bhat, P., 2007. UN millennium development goals: can we halt the stroke epidemic in India? Ann. Indian Acad. Neurol. 10, 130–136.
Danser, A.H., Derkx, F.H., Schalekamp, M.A., Hense, H.W., Riegger, G.A., Schunkert, H., 1998. Determinants of interindividual variation of renin and prorenin concentrations: evidence for a sexual dimorphism of (pro)renin levels in humans. Hypertension 16, 853–862.
Dempsey, R.J., Moore, R.W., 1992. Amount of smoking independently predicts carotid artery atherosclerosis severity. Stroke 23, 693–696.
Dhar, S., Ray, S., Dutta, A., Sengupta, B., Chakrabarti, S., 2012. Polymorphism of ACE gene as the genetic predisposition of coronary artery disease in Eastern India. Indian Heart J. 64 (6), 576–581.
Dikmen, M., Gunes, H.V., Degirmenci, I., Ozdemir, G., Basaran, A., 2006. Are the angiotensin-converting enzyme gene and activity risk factors for stroke? Arq. Neuropsiquiatr. 64, 211–216.
Ehlers, M.R.W., Riordan, J.F., 1989. Angiotensin-converting enzyme: new concepts concerning its biological role. Biochemistry 28, 5311–5316.
Elbaz, A., Amarenco, P., 1999. Genetic susceptibility and ischaemic stroke. Curr. Opin. Neurol. 12, 47–55.
Folsom, A.R., Eckfeldt, J.H., Weitzman, S., Ma, J., Chambless, L.E., Barnes, R.W., et al., 1994. Relation of carotid artery wall thickness in diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) study investigators. Stroke 25, 66–73.
Gao, X., Yang, H., ZhiPing, T., 2006. Association studies of genetic polymorphism, environmental factors and their interaction in ischemic stroke. Neurosci. Lett. 398, 172–177.

Glader, C.A., Boman, J., Saikku, P., Stenlund, H., Weinehall, L., Hallmanns, G., et al., 2000. The proatherogenic properties of lipoprotein (a) may be enhanced through the formation of circulating immune complexes containing *Chlamydia pneumoniae* specific IgG antibodies. Eur. Heart J. 21 (8), 639–646.
Goldstein, L.B., Adams, R., Becker, K., Furberg, C.D., Gorelick, P.B., Hademenos, G., et al., 2001. Primary prevention of ischemic stroke: a statement for healthcare professionals from the Stroke Council of the American Heart Association. Circulation 103, 163–182.
Hachinski, V., Graffagnino, C., Beaudry, M., Bernier, G., Buck, C., Donner, A., et al., 1996. Lipids and stroke; a paradox resolved. Arch. Neurol. 53, 303–308.
Hankey, G.J., 1999. Smoking and risk of stroke. J. Cardiovasc. Risk 6, 207–211.
Inanir, A., Basol, N., Karakus, N., Yigit, S., 2013. The importance of association between angiotensin-converting enzyme (ACE) Gene I/D polymorphism and diabetic peripheral neuropathy. Gene 530 (2), 253–256.
Jiang, X., Sheng, H., Li, J., Xun, P., Cheng, Y., Huang, J., et al., 2009. Association between renin–angiotensin system gene polymorphism and essential hypertension: a community-based study. J. Hum. Hypertens. 23 (3), 176–181.
Kalita, J., Somarajan, B.I., Kumar, B., Mittal, B., Misra, U.K., 2011. A study of ACE and ADD1 polymorphism in ischemic and hemorrhagic stroke. Clin. Chim. Acta 412, 642–646.
Karagiannis, A., Balaska, K., Tziomalos, K., Tokalaki-Nikolaidou, L., Papayeoryiou, A., Zamboulis, C., 2004. Lack of an association between angiotensin-converting enzyme gene insertion/deletion polymorphism and ischaemic stroke. Eur. Neurol. 51, 148–152.
Lavy, S., Melamed, E., Cahane, E., Carmon, A., 1973. Hypertension and diabetes as risk factors in stroke patients. Stroke 4 (5), 751–759.
Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., et al., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2095–2128.
Markus, H.S., Barley, J., Lunt, R., Bland, J.M., Jeffery, S., Carter, N.D., et al., 1995. Angiotensin-converting enzyme gene deletion polymorphism. A new risk factor for lacunar stroke but not carotidatheroma. Stroke 26 (8), 1329–1333.
McGeer, E.G., Singh, E.A., 1992. Angiotensin-converting enzyme in cortical tissue in Alzheimer's and some other neurological diseases. Dementia 3, 299–303.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Munshi, A., Sultana, S., Kaul, S., Reddy, B.P., Alladi, S., et al., 2008. Angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischemic stroke in a South Indian population. J. Neurol. Sci. 272, 132–135.
Narain, Y., Yip, A., Murphy, T., Brayne, C., Easton, D., Evans, J.G., et al., 2000. The ACE gene and Alzheimer's disease susceptibility. J. Med. Genet. 37 (9), 695–697.
O'Leary, D.H., Polak, J.F., Kronmal, R.A., Kittner, S.J., Bond, M.G., Wolfson Jr., S.K., et al., 1992. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. Stroke 23, 1752–1760.
Pera, J., Slowik, A., Dziedzic, T., Wloch, D., Szczudlik, A., 2006. ACE I/D polymorphism in different etiologies of ischemic stroke. Acta Neurol. Scand. 114, 320–322.
Pfohl, M., Fetter, M., Koch, M., Barth, C.M., Rüdiger, W., Häring, H.U., et al., 1998. Association between angiotensin I-converting enzyme genotypes, extracranial artery stenosis, and stroke. Atherosclerosis 140 (1), 161–166.
Prabhakar, P., De, T., Nagaraja, D., Christopher, R., 2014. Angiotensin-converting enzyme gene insertion/deletion polymorphism and small vessel cerebral stroke in Indian population. Int. J. Vasc. Med. 2014, 305–309.
Reyes-Engel, A., Morcillo, L., Aranda, F.J., Ruiz, M., Gaitan, M.J., Mayor-Olea, A., et al., 2006. Influence of gender and genetic variability on plasma angiotensin peptides. J. Renin Angiotensin Aldosterone Syst. 7, 92–97.
Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., Soubrier, F., 1990. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin. Invest. 86 (4), 1343–1346.
Rigat, B., Hubert, C., Corvol, P., Soubrier, F., 1992. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 20 (6), 1433.
Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., et al., 2008. Heart disease and stroke statistics: 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 117, e25–e146.
Rothwell, P.M., Coull, A.J., Giles, M.F., et al., 2004. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). Lancet 363, 1925–1933.
Saidi, S., Zammiti, W., Slamia, L.B., Ammou, S.B., Almawi, W.Y., et al., 2009. Interaction of angiotensin-converting enzyme and apolipoprotein E gene polymorphisms in ischemic stroke involving large-vessel disease. J. Thromb. Thrombolysis 27, 68–74.
Schut, A.F., Bleumink, G.S., Stricker, B.H., Hofman, A., Witteman, J.C., Pols, H.A., et al., 2004. Angiotensin converting enzyme insertion/deletion polymorphism and the risk of heart failure in hypertensive subjects. Eur. Heart J. 25 (23), 2143–2148.
Shahid, S., Abid, A., Mehdi, S.Q., Firasat, S., Lanewala, A., Naqvi, S.A., et al., 2012. Association of the ACE-II genotype with the risk of nephrotic syndrome in Pakistani children. Gene 493 (1), 165–168.
Shanmugam, V., Sell, K.W., Saha, B.K., 1993. Mistyping ACE heterozygotes. Genome Res. 3, 120–121.
Sharma, P., 1998. Meta-analysis of the ACE gene in ischemic stroke. J. Neurol. Neurosurg. Psychiatry 64, 227–230.
Szolnoki, Z., 2005. Evaluation of the interactions of common genetic mutations in stroke. Methods Mol. Med. 104, 241–250.
Tascilar, N., Dursun, A., Ankarali, H., Mungan, G., Ekem, S., et al., 2009. Angiotensin-converting enzyme insertion/deletion polymorphism has no effect on the risk of atherosclerotic stroke or hypertension. J. Neurol. Sci. 285, 137–141.
Tuncer, N., Tuglular, S., Kiliç, G., Sazci, A., Us, O., Kara, I., 2006. Evaluation of the angiotensin–converting enzyme insertion/deletion polymorphism and the risk of ischemic stroke. J. Clin. Neurosci. 13 (2), 224–227.
Um, J.Y., Joo, J.C., Kim, K.Y., Lee, K.M., Kim, H.M., 2003. Angiotensin converting enzyme gene polymorphism and traditional Sasang classification in Koreans with cerebral infarction. Hereditas 138, 166–171.
Zhang, Z., Xu, G., Liu, D., Fan, X., Zhu, W., Liu, X., 2012. Angiotensin-converting enzyme insertion/deletion polymorphism contributes to ischemic stroke risk: a meta-analysis of 50 case–control studies. PLoS One 7 (10).
Zhou, Y.F., Yan, H., Hou, X.P., Miao, J.L., Zhang, J., Yin, Q.X., et al., 2013. Association study of angiotensin converting enzyme gene polymorphism with elderly diabetic hypertension and lipids levels. Lipids Health Dis. 12, 187.