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

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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 I (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 I (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 I (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 I (OR = 2.288; p = 0.006) and I (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 I genotype and I allele compounded by factors such as smoking and diabetes among south Indian IS patients.

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

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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 (Brito and Bybro, 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.
The 16th intron of the polymorphic ACE gene was detected using following primers: forward 5′-CTG GAG ACC ACT CCC ATC TCT 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).](image-url)
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 type 2 diabetes mellitus (T2DM) and 32.5% (n, 64) had both hypertension and T2DM. Of 175 patients with LVD, 28.57% (n, 50) 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 hypertensive (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.
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 = 0.43–0.99; p = 0.034).

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

| Genotype/allele | HC (n = 193)a | IS (n = 200)a | OR | 95% CI   | χ² | 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   | 28.57 (51)b   | 2.03 | 1.137–3.688 | 5.899 | **0.014** |
|                 | 14.29 (12)c   | 26.20 (45)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 | 5.671 | 0.346   |
|                 | 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   |
|                 | 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.

- **Pooled.**
- **Males.**
- **Females.**
- *Highly significant.

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

| Genotype/allele | HC (n = 140)a | >50 years (n = 146)a | OR | 95% CI   | χ² | 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 | 0.434   |
|                 | 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 | **0.018** |
|                 | 56.94 (41)    | 54.56 (36)           | 0.98 | 0.438–1.879 | 0.013 | 0.864   |
|                 | 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 | **0.018** |
|                 | 43.06 (31)    | 45.45 (30)           | 1.10 | 0.532–2.284 | 0.013 | 0.864   |

HC — Healthy Controls.

- **Pooled.**
- **Males.**
- **Females.**
- *Highly significant.
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 between 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 intracerebral 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 4
ACE (I/D) genotype and allele frequencies in LVD patients and controls.

| Genotype/allele | HC (n = 169) | LVD (n = 175) | OR | 95% CI | χ² | p value |
|-----------------|-------------|--------------|----|--------|----|---------|
| II              | 18.93 (32)  | 28.57 (50)   | 1.71 | 1.003–2.931 | 3.883 | 0.043*  |
|                 | 19.23 (25)  | 28.47 (39)   | 1.67 | 0.998–3.086 | 2.636 | 0.086  |
|                 | 17.95 (20)  | 28.95 (11)   | 1.86 | 0.664–6.269 | 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 | 0.195  |
| 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 | 0.004* |
|                 | 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.

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*  |
|                 | 57.58 (57)    | 70.79 (63)      | 0.56 | 0.292–1.073 | 2.994 | 0.069  |
|                 | 11.11 (11)    | 15.73 (14)      | 0.67 | 0.265–1.684 | 0.513 | 0.394  |
|                 | 60.10 (119)   | 48.88 (87)      | 1.576 | 1.025–2.423 | 4.325 | 0.03*  |
|                 | 39.90 (79)    | 51.12 (91)      | 0.635 | 0.413–0.975 | 4.325 | 0.03*  |

ISHT - Ischemic Stroke with Hypertension
* 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 times more likely to have a stroke than non-smokers (World Health Organization, 2012).

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   | χ²   | 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 times more likely to have a stroke than non-smokers (World Health Organization, 2012).

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 | 1.31 | 0.75–2.29 | 0.318 |
| II vs. DD | 2.29 | 1.14–4.60 | 0.109 |
| II vs. (DD + ID) | 2.06 | 1.23–3.46 | 0.003* |
| ID vs. (DD + II) | 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 ‘I’ genotype and ‘T’ 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 ‘T’ 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.

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