Association of *APOE* (E2, E3 and E4) gene variants and lipid levels in ischemic stroke, its subtypes and hemorrhagic stroke in a South Indian population

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HIGHLIGHTS

- APOE polymorphism (E2, E3 and E4) in certain genetic models associates with development of hemorrhagic stroke.
- In ischemic stroke subtypes association was observed with intracranial large artery, cardioembolic and lacunar stroke.
- Lipid levels of very low density lipoprotein and triglycerides were found to be associated with E2/E4 and E3/E4 genotypes in ischemic stroke.

ABSTRACT: In the present study we evaluated the association of *APOE* (E2/E3/E4) polymorphism with ischemic stroke (n=620), its subtypes and hemorrhagic stroke (n=250) in a South Indian population from Telangana. The genotypes were determined using PCR-RFLP while lipid levels were measured using commercially available kits. We found significant difference in the genotypic distribution between hemorrhagic stroke patients and controls for certain genetic models [E2/E2 vs. E2/E4; E3/E3 vs. E2/E3; E3/E3 vs. E2/E4; E4/E4 vs. E2/E3; E4/E4 vs.E2/E4 and E3 vs. E4]. However, no significant difference was observed in genotypic distribution between ischemic stroke patients and controls. On analysing the genotypic distribution between ischemic and hemorrhagic stroke patients, statistically significant difference was observed in specific genetic models [E2/E2 vs. E2/E4; E3/E3 vs. E2/E3; E3/E3 vs. E2/E4; E4/E4 vs. E2/E3 and E4/E4 vs. E2/E4]. In ischemic stroke subtypes analyzing for alleles E3 vs. E2 and E3 vs. E4, we found significant association with intracranial large artery (p=0.01), cardioembolic stroke (p=0.001 and p=0.0004) and lacunar stroke (p=0.02). Analysing the association of various genotypes with different lipid levels significant association was observed for VLDL (P=0.000) and for triglyceride (P=0.000) levels with E2/E4 and E3/E4 genotypes in ischemic stroke but not in hemorrhagic stroke. In conclusion, our results suggest that *APOE* polymorphism does seem to play a role in hemorrhagic stroke and also in the development of specific subtypes of ischemic stroke. Further, in ischemic stroke VLDL and triglycerides levels were found to be significantly associated with E2/E4 and E3/E4 genotypes.

Key words: Ischemic stroke, Haemorrhagic stroke, TOAST classification, Lipid levels, *APOE* gene

1. Introduction

Stroke is a life-changing event and the first leading cause of neurological disability [1]. It is a multifactorial, complex disease resulting from a combination of vascular, environmental and genetic factors [2]. Among the numerous genes suggested to play a role in stroke, apolipoprotein E (*APOE*) is one of the most extensively studied genes [3]. The gene encoding the protein is situated on chromosome 19q13.2 and produces a 299 amino acid polypeptide [4, 5]. Although there could be rare variants, two non-synonymous polymorphisms in this glycoprotein produce 3 alleles or isoforms which differ from each other at amino acid residues, E2 (cys112, cys158), E3 (cys112, arg158), and E4 (arg112, arg158) that give rise to 6 possible genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) with E3 being the most common allele [6].

The products from these 3 alleles are known to differ in affinity for binding to apoE and its low density lipoprotein receptors (LDL-R) and lipoproteins ultimately altering circulating levels of cholesterol [7]. Therefore, it has been suggested that *APOE* gene is a risk factor for stroke due to diversity in serum lipid profile of individuals and modulation of atherogenic lipoproteins [8, 9]. Earlier studies report E4 and E2 allele containing genotypes to be associated with increased total cholesterol and decreased cholesterol levels respectively [3]. Studies have also suggested E4 carriers to be more predisposed to risk of ischemic heart disease and atherosclerosis [10, 11]. However, with regard to stroke there have been conflicting results with negligible number of studies from India, especially from South India.

Therefore, in the present study we aimed at evaluating the association of three common variants of *APOE* gene with ischemic stroke (IS), its subtypes and hemorrhagic stroke (HS) in a South Indian population from Telangana. Further, the LDL, HDL, VLDL and triglyceride (TG) levels were also measured in both IS and HS patients in association with different genotypes.

2. Materials and methods

2.1. Subjects

Six hundred and twenty IS patients (males: females=434:186) and 250 HS patients (males: females=167:83) presenting with new stroke evaluated in the neurology department of Nizam's Institute of Medical Sciences, Hyderabad (Telangana, India) between September

2007 and December 2014 were included in the study. The study was approved by the ethical committee of the study hospital and by the institutional ethics committee. All the patients were examined by a qualified stroke neurologist. IS and HS were differentiated by computed tomography (CT) scans and magnetic resonance imaging (MRI). All the patients underwent CT scan as well as MRI. Patients with major cardiac, hepatic, renal, endocrinological disorders, skeletal disorders and cancerous diseases were excluded from the study. IS was classified into subtypes according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification [12]. As control group 620 healthy individuals (males: females=428:192) matched for sex and age were recruited from the same demographic area. The controls had no clinical evidence of any cerebrovascular disease. Information on demographic features and risk factors was collected using a structured questionnaire. Hypertension, alcohol use, diabetes and smoking were defined as reported previously [13]. Subjects included in the study were above the age group of 18 years and all the samples were collected only after obtaining the written informed consent.

2.2. DNA isolation and genotyping

Two ml of venous blood was collected in EDTA coated tubes and genomic DNA was extracted using standard phenol-chloroform method. The E2, E3 and E4 polymorphism in *APOE* gene was analysed using PCR-RFLP technique [14].

2.3. Plasma lipid measurements

Three ml of blood samples for the measurement of lipid levels were obtained from subjects (7 am and 11 am), after at least 12 hrs of fasting and plasma was separated and stored at -80°c till further analysis. Plasma levels of HDL, LDL, VLDL and TG were estimated by commercially available kits supplied by ERBA Manheim (Germany) and an ERBA autoanalyzer.

2.4. Statistical Analysis

Hardy–Weinberg equilibrium was tested and association between genotypes with IS, its subtypes and HS was examined by Odds ratio (OR) with 95% confidence interval (CI) and chi-square analysis using OpenEPI6 software. Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. The LDL, HDL, VLDL and TG levels in patients and controls were compared by student's t-test.

The relationship between LDL, HDL, VLDL and TG levels with *APOE* genotypes was evaluated by ANOVA and confirmed by post-hoc analysis with multiple comparisons. Statistical significance was defined as $p<0.05$.

1. Results

Six hundred and twenty IS patients, 620 age and sex matched controls and 250 HS patients from the same demographic area were included in the study. All the patients belonged to a South Indian population from Telangana. The clinical characteristics of IS patients, HS patients and controls have been given in Table 1. Mean age was 49.4 years for IS patients, 54.8 years for HS patients and 49.1 years in controls. Risk factor profile of IS patients revealed hypertension in 57.9%, diabetes in 44.7%, smoking in 43.8%, alcohol use in 33.1% and family history of stroke in 26.8% subjects. In the control group 32.1% had hypertension, 28.8% were diabetic, 31.9% smokers, 25.8% were alcohol users and 7% had a family history of stroke. Profiles of patients for the various risk factors in HS revealed hypertension in 58%, diabetes in 51%, smoking in 60%, alcohol use in 54% and family history of stroke in 7% of patients.

The genotypic distribution and allelic frequencies of *APOE* variants in IS patients, HS patients and controls have been given in Table 2. Analysis for the different genotypic models for IS patients with controls revealed no significant difference. However, for HS patients significant association was observed for various genetic models [E2/E2 vs. E2/E4 $\chi2=5.7$; p=0.01, OR= 0.1(95% CI; 0.02-0.7)]; [E3/E3 vs. E2/E3 $\chi2=6.7$; p=0.009, OR= 2.8(95% CI; 1.2-6.3)]; [E3/E3 vs. E2/E4 $\chi2=30.9$; p=0.0001, OR=0.08(95% CI; 0.03-0.2)]; [E4/E4 vs. E2/E3 $\chi2=5.6$; p=0.01, OR= 4.2(95% CI; 1.2-14.1)]; [E4/E4 vs.E2/E4 $\chi2=9.0$; p=0.002, OR= 0.1(95% CI; 0.03-0.5)] and [E3 vs. E4 $\chi2=4.7$; p=0.02, OR= 0.7(95% CI; 0.5-0.9)] (Table 3). On analysing the genotypic models for IS patients with HS patients, statistically significant difference was observed for [E2/E2 vs. E2/E4 $\chi2=3.9$; p=0.04, OR = 0.2(95% CI; 0.03-1.04)]; [E3/E3 vs. E2/E3 $\chi2=5.7$; p=0.01, OR= 2.6(95% CI; 1.2-5.9)]; [E3/E3 vs. E2/E4 $\chi2=25.5$; p=0.0001, OR= 0.1(95% CI; 0.04-0.3)]; [E4/E4 vs. E2/E3 $\chi2=4.9$; p=0.02, OR= 3.8(95% CI; 1.1-13.0)] and [E4/E4 vs. E2/E4 $\chi2=6.6$; p=0.009, OR= 0.2(95% CI; 0.04-0.6)] (Table 4).

Examining the association of *APOE* polymorphism with IS subtypes, we found significant association with intracranial large artery (ILA) [p=0.01, OR= 2.1(95% CI; 1.1-3.9)] and cardioembolic stroke (CE) [p=0.001, OR= 0.4(95% CI; 0.2-0.7)] for E3 vs. E2. While that for E3 vs. E4 significant association was found with ILA [p=0.01, OR= 1.6(95% CI; 1.1-2.4)], lacunar stroke [p=0.02, OR= 0.6(95% CI; 0.4-0.9)] and CE [p=0.0004, OR= 0.5(95% CI; 0.3-0.7)] (Table 5). Analysing the association of various lipid levels revealed significant association for E2/E4 and E3/E4 genotypes with VLDL [P=0.000] and triglyceride [P=0.000] levels in IS but not in HS (Table 6).

4. Discussion

Impact of *APOE* gene has been demonstrated in both cerebrovascular and cardiovascular diseases (CVD) and it has subsequently emerged as one of the major genes influencing stroke through regulation of lipoprotein metabolism [15, 16]. Isoforms of *APOE* have been reported to be associated with variations in plasma cholesterol levels with E4 allele exerting a higher influence than allele E3. Studies on genetic polymorphisms of *APOE* have reported E4 allele as a risk factor for CVD while a few do not report any such association [17]. Therefore, its role still remains elusive in stroke and hence in the present work we elucidated the genotypic and allelic variation of *APOE* gene in IS, its subtypes and HS.

Investigations on association of this gene with stroke have reported a number of positive associations however, there are reports that state negative association in Italian and Scottish cohorts for both IS and HS [18-20]. Studies in population like Chinese Han subjects have revealed E4/E4 genotype carriers to have a 2.1 fold risk of cerebral infarction as compared to E3/E3 carriers [21]. Another study comprising of habitual smokers reports, E4/E3 genotypes to develop stroke and suggests unfavourable genotypic combinations along with environmental factors to synergistically influence the development of IS [22]. Similarly, studies among Northern Han Chinese suggests an independent role for hypertension and E2/E3/E4 alleles in IS risk [23]. However, no significant association of *APOE* gene polymorphism was reported in the Guangxi Han population but a subsequent meta-analysis revealed a significant association for the genetic models E2/E4 vs. E3/E3, E3/E4 vs. E3/E3, E4/E4 vs. E3/E3 and allele E4 vs. E3 [24]. The present study revealed no such association with IS but certain genetic combinations (E2/E2 *vs.* E2/E4, E3/E3 *vs.* E2/E3 and E3/E3 *vs.* E2/E4, E4/E4 *vs.* E2/E3 and E4/E4 *vs.* E2/E4) and alleles (E3 *vs.* E4) revealed significant

association among HS patients. Further, we found significant difference for genetic models (E2/E2 *vs.* E2/E4, E3/E3 *vs.* E2/E3, E3/E3 *vs.* E2/E4, E4/E4 *vs.* E2/E3 and E4/E4 *vs.* E2/E4) between IS and HS.

Simultaneously studying the association of this gene with IS subtypes, significant association was observed with ILA, lacunar and CE. Study in a Taiwanese population suggested a probable association between E3/E4 genotype and lacunar infarct but not with atherothrombotic infarcts [25]. Findings by Paternoster et al. (2008) also show a clear association with Carotid Intima-Media Thickness (CIMT) with a possible association with large artery IS [26]. In contrast to these reports, a recent finding states E4 allele to be associated with intracranial atherosclerosis among men [27]. Study in a Japanese population suggests E2 allele carriers having a risk factor for atherothrombosis, intracerebral hemorrhage (ICH) and cardioembolism and E4 carriers at risk of atherothrombosis, ICH and subarachnoid haemorrhage (SAH) [28]. Contrary to all these findings, our study however, is the first report to explain association in subtypes based on widely accepted TOAST classification of stroke. This effort in our suggestion would be helpful in explaining the IS epidemiology and it's variation on a more global standard.

Studies with respect to HS are comparatively lesser but have substantially discussed the role of different clinical and genetic factors in contributing to its development. Chowdhury et al. (2001) in their findings in a Bangladeshi population report E3/E4 genotype and E4 carriers as risk factors for cerebral thrombosis and E2 carriers at risk of HS. This study also documents HDL levels to show weaker but positive association with E3/E4 genotype in IS patients [29]. Haplotype studies on this gene suggest independent association of E4 allele with lobar ICH but not with nonlobar ICH. Another study reports male subjects with E2/E3 genotype to have a higher risk for deep intracerebral hemorrhage (SDICH) than those bearing E3/E3 genotype [30, 31]. Similarly Biffi et al. (2010) in their findings report allele E2 and E4 as independent risk factors for lobar ICH and an association between E4 allele and deep ICH [32]. Studies involving recurrent and nonrecurrent ICH patients also report E2 and E4 alleles to be significantly related to recurrent ICH [33].

Few other studies also suggest sex-dependent effect of E3/E4 genotype in men and E2/E3 among women in contributing to increased mortality in stroke independent of serum

cholesterol levels [34]. A study by Luthra et al. (20020 suggests E4 allele, TG, age and hypertension to be predictors for stroke development among Asian Indians [17]. Association between E4 allele, LDL and IS was reported by Kang et al. (2006). However, there was no significant difference found in the lipid concentrations and distribution of *APOE* genotypes between large artery atherosclerosis (LAA) and small artery occlusion (SAO) [35]. They also report LDL concentrations to be significantly higher in IS group as compared to controls and LDL concentration to be lower among E2 carriers than in E3 and E4 alleles but with no difference between E4 and E3 carriers. Studies in a population of European ancestry too showed positive dose-response association with LDL and IS [36]. Our study on the other hand revealed significant association for VLDL and TG levels with E2/E4 and E3/E4 genotypes for IS. This variation in association of genotypes with lipid levels of two major stroke types seems interesting and requires detailed study with equal sample size for better conclusion. However, a biological explanation can be found in terms of atherosclerosis development where IS seems to be more influenced by plaque deposition and accounts for a major percentage of stroke attack than HS which is more due to high blood pressure causing rupture of blood vessels.

Recent studies involving only ICH cases report E4 allele to be associated with higher risk among Asians and Caucasians [37]. Similarly a meta-analysis carried out by Sudlow et al. (2006) found a significant association of E4 allele bearers with IS and SAH but not with ICH. However, genotypes with E2 were found to be associated with ICH whereas E4 genotypes associated strongly with large artery IS subtype among Asian subjects as compared to the Whites [38]. A simultaneous meta-analysis by Banerjee et al. 2006 reports marginally statistical significant association of E4 allele with stroke [39]. Similarly, recent studies among Chinese suggest E4 allele to be associated with an increased risk of developing cerebral infarction [40, 41]. Further, studies also report E4 carriers to have cerebral perfusion impairment in early brain injury following SAH and E2 or E4 carriers to experience microbleeds more frequently than E3/E3 carriers [42, 43].

Conclusion

In conclusion, our study reveals that *APOE* (E2, E3 and E4) gene polymorphism in certain genotypic combinations contributes to the risk of HS. Additionally, in IS subtypes E2 and E4 alleles associated significantly with ILA, CE and lacunar. This finding may be on

account of high prevalence of ILA, CE and lacunar subtypes as compared to the other two subtypes in our patient samples [44]. Further, a significant association of E2/E4 and E3/E4 genotypes with VLDL and TG levels was observed among IS patients.

Acknowledgement: Authors gratefully acknowledge the financial assistance received from ICMR, India for this study.

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Table 1

Characteristics	Ischemic stroke Patients (n=620)	Controls (n=620)	p value (Ischemic stroke *vs.* Controls)	Haemorrhagic stroke Patients (n=250)	p value (Hemorrhagic stroke *vs.* Controls)	p value (Ischemic stroke *vs.* Hemorrhagic stroke)
Age	49.4 (17.4)	49.1 (16.9)		54.8 (3.3)		
Male:female	434:186	428:192		167:83		
Systolic Bp(mmHg) (Mean±S.D)	141.8 (17.1)	126.7 (16.2)	=0.0001	145.3 (16.8)	=0.0001	=0.006
Diastolic Bp(mmHg) (Mean±S.D)	88.2 (20.6)	78.7 (16.2)	=0.0001	88.6 (12.5)	=0.0001	=0.77
Total Cholesterol (Mean±S.D)	198.2 (40.7)	195.8 (45.9)	=0.33	177.3 (34.2)	=0.0001	=0.0001
Triglycerides (Mean±S.D)	179.1(39.9)	138.2 (44.3)	=0.0001	127.9 (33.8)	=0.001	=0.0001
Random Glucose (Mean±S.D)	130.3(7.4)	119.03(21.9)	=0.0001	131.3 (6.4)	=0.0001	=0.06
HDL cholesterol (Mean±S.D)	50.4(20.9)	59.3(23.3)	=0.0001	74.1 (14.1)	=0.0001	=0.0001
Hypertension	57.9%	32.1%	=0.00	58%	=0.00	=0.00
Diabetes	44.7%	28.8%	=0.00	51%	=0.00	=0.00
Smokers	43.8%	31.9%	=0.00	60%	=0.00	=0.00
Alcohol use	33.1%	25.8%	=0.005	54%	=0.00	=0.00
Family history of stroke	26.8%	7%	=0.00	7%	=0.07	=0.00

Clinical characteristics of ischemic stroke patients, hemorrhagic stroke patients and controls

Age, systolic BP, diastolic BP, total cholesterol, high density lipoprotein (HDL) cholesterol, random glucose and triglycerides are given as mean (SD). *p* values were calculated using Students' paired t-test (SPSS 18).

Table 2

Distribution of APOE (E2, E3 and E4) genotypes and allelic frequencies in ischemic stroke patients, hemorrhagic stroke patients and controls

Group	Genotypes							Alleles			
	E2/E2	E3/E3	E4/E4	E2/E3	E2/E4	E3/E4	Total	E2	E3	E4	Total
Ischemic stroke %	5 (0.8)	431 (69.5)	12 (2.0)	46 (7.4)	6 (1.0)	120 (19.3)	620	62 (0.05)	1028 (0.83)	150 (0.12)	1240
Control %	5 (0.8)	436 (70.3)	12 (2.0)	50 (8.0)	4 (0.7)	113 (18.2)	620	64 (0.05)	1035 (0.84)	141 (0.11)	1240
Hemorrhagic stroke %	3 (1.2)	172 (68.8)	7 (2.8)	7 (2.8)	19 (7.6)	42 (16.8)	250	32 (0.06)	393 (0.79)	75 (0.15)	500

Table 3

Chi-square, p-value and odds ratio (*APOE* gene) for ischemic stroke patients with controls and haemorrhagic stroke patients with controls

Genotypes	OR (95% CI)	χ2	p-value
Ischemic stroke with controls			
E2/E2 *vs.* E3/E3	1.0 (0.3 – 3.5)	0.0003	=0.9
E2/E2 *vs.* E4/E4	1 .0 (0.2 – 4.4)	0	=0.9
E3/E3 *vs.* E4/E4	0.1 (0.4 – 2.2)	0.0007	=0.9
E2/E2 *vs.* E2/E3	1.1 (0.3 – 4.0)	0.01	=0.9
E2/E2 *vs.* E2/E4	0.6 (0.1 - 4.0)	0.2	=0.9
E2/E2 *vs.* E3/E4	0.9 (0.3 - 3.3)	0.01	=0.9
E3/E3 *vs.* E2/E3	1.1 (0.7 - 1.6)	0.11	=0.7
E3/E3 *vs.* E2/E4	0.7 (0.2 – 2.4)	0.42	=0.5
E3/E3 *vs.* E3/E4	0.9 (0.7 – 1.2)	0.23	=0.7
E4/E4 *vs.* E2/E3	1.0 (0.4 - 2.7)	0.03	=0.9
E4/E4 *vs.* E2/E4	0.7 (0.1 – 3.0)	0.3	=0.6
E4/E4 *vs.* E3/E4	0.9 (0.4 – 2.2)	0.02	=0.9
Alleles			
E2 *vs.* E3	0.9 (0.7 - 1.4)	0.02	=0.9
E2 *vs.* E4	0.9 (0.6 – 1.4)	0.12	=0.7
E3 *vs.* E4	0.9 (0.7 – 1.2)	0.3	=0.6
Haemorrhagic stroke with controls			
E2/E2 *vs.* E3/E3	1.5 (0.3 – 6.4)	0.33	=0.6
E2/E2 *vs.* E4/E4	1.0 (0.1 – 5.6)	0.001	=0.9
E3/E3 *vs.* E4/E4	0.7 (0.3 – 1.7)	0.6	=0.4
E2/E2 *vs.* E2/E3	4.3 (0.8 – 21.9)	3.4	=0.06
E2/E2 *vs.* E2/E4	0.1 (0.02 – 0.7)	5.7	=0.01
E2/E2 *vs.* E3/E4	1.6 (0.4 – 7.0)	0.4	=0.52
E3/E3 *vs.* E2/E3	2.8 (1.2 – 6.3)	6.7	=0.009
E3/E3 *vs.* E2/E4	0.08 (0.03 – 0.2)	30.9	=0.0001
E3/E3 *vs.* E3/E4	1.1 (0.7 – 1.6)	0.08	=0.8
E4/E4 *vs.* E2/E3	4.2 (1.2 – 14.1)	5.6	=0.01
E4/E4 *vs.* E2/E4	0.1 (0.03- 0.5)	9.0	=0.002
E4/E4 *vs.* E3/E4	1.5 (0.5 – 4.2)	0.8	=0.3
Alleles			
E2 *vs.* E3	1.3 (0.8 – 2.0)	1.5	=0.2
E2 *vs.* E4	0.9 (0.6 – 1.6)	0.05	=0.8
E3 *vs.* E4	0.7 (0.5 - 0.9)	4.7	=0.02

Table 4

Chi-square, p-value and odds ratio (*APOE* gene) for ischemic stroke patients with haemorrhagic stroke patients

Genotypes	OR (95% CI)	χ2	p-value
E2/E2 *vs.* E3/E3	0.7 (0.16 – 2.8)	0.3	=0.5
E2/E2 *vs.* E4/E4	0.9 (0.17 – 5.3)	0.001	=0.9
E3/E3 *vs.* E4/E4	0.7 (0.3 – 1.7)	0.6	=0.4
E2/E2 *vs.* E2/E3	3.9 (0.8 – 20.2)	2.9	=0.08
E2/E2 *vs.* E2/E4	0.2 (0.03 – 1.04)	3.9	=0.04
E2/E2 *vs.* E3/E4	1.7 (0.4 – 7.4)	0.5	=0.47
E3/E3 *vs.* E2/E3	2.6 (1.2 – 5.9)	5.7	=0.01
E3/E3 *vs.* E2/E4	0.1 (0.04 – 0.3)	25.5	=0.0001
E3/E3 *vs.* E3/E4	1.1 (0.7 – 1.6)	0.4	=0.5
E4/E4 *vs.* E2/E3	3.8 (1.1 – 13.0)	4.9	=0.02
E4/E4 *vs.* E2/E4	0.2 (0.04- 0.6)	6.6	=0.009
E4/E4 *vs.* E3/E4	1.6 (0.6 – 4.5)	1.0	=0.3
Alleles			
E2 *vs.* E3	1.3 (0.8 – 2.1)	1.7	=0.18
E2 *vs.* E4	1.0 (0.6 – 1.7)	0.01	=0.9
E3 *vs.* E4	0.7 (0.5 – 1.0)	3.1	=0.07

Table 5

APOE (E2, E3 and E4) genotypic and allelic frequencies in ischemic stroke patients classified according to TOAST classification

TOAST Classification	No. of patient	Genotype (%)						Allelic Frequencies			χ2	p value	χ2	p value
		E2/E2	E3/E3	E4/E4	E2/E3	E2/E4	E3/E4	E2	E3	E4	(E3 vs. E2)		(E3 vs. E4)	
Large artery atherosclerosis	303													
A) Intracranial large artery (%)	225	1 (0.4)	185 (82.2)	3 (1.3)	8 (3.6)	2 (0.9)	26 (11.6)	12 (0.03)	404 (0.9)	34 (0.07)	5.5	=0.01	5.9	=0.01
B) Extracranial large artery (%)	78	1 (1.3)	58 (74.3)	2 (2.6)	7 (9)	1 (1.3)	9 (11.5)	10 (0.06)	132 (0.85)	14 (0.09)	0.33	=0.56	0.7	=0.39
Small artery occlusions (%) (Lacunar)	83	1 (1.2)	50 (60.2)	1 (1.2)	4 (5)	2 (2.4)	25 (30)	8 (0.05)	129 (0.78)	29 (0.17)	0.01	=0.99	5.1	=0.02
Cardioembolism (%)	76	2 (2.6)	33 (43.4)	2 (2.6)	12 (15.9)	0	27 (35.5)	16 (0.1)	105 (0.7)	31 (0.2)	9.7	=0.001	12.5	=0.0004
Other determined etiology (%)	50	0	27 (54)	2 (4)	9 (18)	0 (10)	12 (24)	9 (0.09)	75 (0.75)	16 (0.16)	3.2	=0.07	2.4	=0.11
Undetermined etiology (%)	108	0	78 (72.2)	2 (1.9)	6 (5.5)	1 (1)	21 (19.4)	7 (0.03)	183 (0.85)	26 (0.12)	1.4	=0.23	0.03	=0.85

Table 6

Relationship of Mean±SD values of LDL, HDL, VLDL and triglyceride levels with APOE genotypes among ischemic stroke and hemorrhagic stroke patients

Ischemic stroke	Genotypes	N=620	Mean	Std. deviation	p value
LDL	E2/E2	5	131.2	8.1	
	E3/E3	431	136.6	17.8	
	E4/E4	12	132.5	7.2	
	E2/E3	46	133.3	16.2	
	E2/E4	6	134.6	7.6	
	E3/E4	120	131.7	17.3	= 0.11
HDL	E2/E2	5	49.8	4.5	
	E3/E3	431	50.7	6.8	
	E4/E4	12	50.1	6.6	
	E2/E3	46	49.4	7.8	
	E2/E4	6	49.9	8.6	
	E3/E4	120	50.7	7.9	=0.90
VLDL	E2/E2	5	22.4	4.8	
	E3/E3	431	38.8	8.1	
	E4/E4	12	36.5	12.9	
	E2/E3	46	24.7	6.8	
	E2/E4	6	48.1	3.2	
	E3/E4	120	44.5	5.7	=0.000
Triglycerides	E2/E2	5	112.8	23.4	
	E3/E3	431	194.1	40.1	
	E4/E4	12	182.8	64.2	
	E2/E3	46	124.1	33.8	
	E2/E4	6	239.8	16.2	
	E3/E4	120	222.5	28.9	=0.000
Hemorrhagic stroke	**Genotypes**	**N=250**	**Mean**	**Std. deviation**	**p value**
LDL	E2/E2	3	129.2	22.2	
	E3/E3	172	138.8	19.2	
	E4/E4	7	132.2	19.7	
	E2/E3	7	136.7	20.2	
	E2/E4	19	138.2	21.7	
	E3/E4	42	142.3	22.5	=0.73
HDL	E2/E2	3	71.6	11.9	
	E3/E3	172	73.2	18.0	
	E4/E4	7	74.8	17.3	
	E2/E3	7	73.1	19.4	
	E2/E4	19	74.7	21.2	
	E3/E4	42	76.2	18.3	=0.96
VLDL	E2/E2	3	25.1	1.9	
	E3/E3	172	27	8.7	
	E4/E4	7	25.3	6.1	
	E2/E3	7	25.6	6.6	
	E2/E4	19	25	5.3	
	E3/E4	42	26.2	4.5	=0.88
Triglycerides	E2/E2	3	125.3	9.7	
	E3/E3	172	133.4	43.7	
	E4/E4	7	126.7	30.3	
	E2/E3	7	128.1	33.1	
	E2/E4	19	124.2	26.8	
	E3/E4	42	131.2	22.8	=0.93