J Biomed. 2016 March; 1(1):e5417. doi: 10.17795/jbm-5417.

Published online 2016 March 21. **Research Article**

Is Apolipoprotein Genotype a Reason For the Excessive Incidence of Stroke in Persian Population?

Mohammad Reza Ghasemi,[1] Peyman Zargari,[2] Maryam Pirhoushiaran,[1] Payam Sasan nezhad,[3] Mahmoud Reza Azarpazhooh,[3] and Ariane Sadr-Nabavi[1,4,*]

[1]Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran
[2]Department of Biology, Science and Research branch, Islamic Azad University, Tehran, IR Iran
[3]Department of Neurology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, IR Iran
[4]Iranian Academic Centers for Education, Culture and Research (ACECR), Tehran, IR Iran

[*]*Corresponding author*: Ariane Sadr-Nabavi, Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran. Tel: +98-9155570305, E-mail: sadrnabavia@mums.ac.ir

Received 2016 January 19; **Accepted** 2016 January 26.

Abstract

Background: Stroke is a complex, pathologically heterogeneous disease, with varying rates of incidence and mortality worldwide. The incidence of stroke in Iran is considerably greater than that in most western countries, which may be due to the genetic background.
Objectives: This is the first case-control study in an Iranian population to assess the association between stroke subtypes and APOE 2/3/4 genotype.
Patients and Methods: APOE e4 genotypes were analyzed in 153 patients (age: 51.4 ± 13.7 years) with stroke and 153 healthy controls (age: 50.0 ± 12.2 years) matched for age and sex. Stroke subtypes were diagnosed by computed tomography scans and magnetic resonance imaging. Moreover, control subjects were matched for some stroke risk factors. The APOE genotype was detected by a multiplex tetra-primer amplification refractory mutation system (multiplex T-ARMS) polymerase chain reaction of exon 4. Data were coded and entered in SPSS Windows (version 11.5).
Results: The APOE genotype distribution in stroke patients differed significantly from that in the healthy controls (P: 0.031). Moreover, a strong association was observed between the hemorrhagic subtype and e2/e3 genotype (P = 0.001), with a moderate association with male cases and controls (P = 0.04).
Conclusions: We report that e2/e3 genotype of APOE gene may play a role particularly in hemorrhagic stroke and male sex is associated with this genotype. This difference may be a cause for the incidence of early stroke in patients in east-Iran.

Keywords: Stroke, Apolipoprotein E (APOE) Gene, Polymorphism

1. Background

Stroke is accepted as a complex, pathologically heterogeneous, multifactorial, and polygenic disease occurring due to numerous gene-gene and gene-environment interactions (1-5).

Geographically, there are varying rates of incidence and mortality of stroke worldwide, including western countries. However, according to the WHO data, the lifetime risk of stroke is one in six people (6, 7). Moreover, most stroke deaths arise in developing countries such as Iran (8-11). One of the first population-based studies on stroke conducted in the middle east region of Iran (Mashhad) reported that the incidence of stroke in Iran is considerably greater than that in most Western countries; in particular, ischemic stroke occurs approximately a decade earlier than that in other countries (11).

The major environmental risk factors of stroke are high blood pressure (the primary cause), atherosclerosis, diabetes, obesity, and hyperlipidemia. However, several studies show that genetic determinants play an important role in susceptibility to stroke (12, 13).

The excessive incidence of stroke in Iran may be due to the genetic background. In addition, family history and studies of twins propose a genetic component to be a risk factor for the primary etiologic types of stroke, ischemic stroke (IS) and hemorrhagic stroke (11, 14-18). Despite numerous studies on the genetic risk factors (including a candidate gene polymorphism case-control approach and analysis of candidate genes and functional consequences) of human stroke, there remains a lack of population-based studies on gene polymorphisms in the developing countries, especially in Iran.

Apolipoprotein E gene (apoE for protein, APOE for gene), including exon 4, is one of the most widely studied genes in stroke. Its protein product, apoE, is a 34 kDa plasma glycoprotein primarily synthesized in the liver and involved in lipid metabolism and transportation. APOE

gene is also significantly expressed in the brain. This gene is polymorphic with three common isoforms, E2 (Cys112/Cys158), E3 (Cys112/Arg158), and E4 (Arg112/Arg158), encoded by the alleles 2, 3, and 4, respectively, giving rise to six genotypes, with the genotype 3/3 occurring in about one-half to two-thirds of people in most populations (19-27). The levels of total cholesterol (TC) and LDL-cholesterol in individuals who carried apoE 4 and 2 alleles are higher than the levels in those with the 3 allele 28.

2. Objectives

This is the first study involving a large population sample in East-Iran to assess the association between all pathological types of stroke with the APOE2/3/4 genotype. In addition, this study analyzes the cause for the incidence of stroke in younger individuals in the genetic background of East-Iran (Mashhad), which has not yet been evaluated.

3. Patients and Methods

3.1. Design of Study Population

The study population consisted of 153 stroke patients (except cryptogenic strokes). Stroke is defined as rapidly developing signs of focal or global disturbance of cerebral function lasting 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin according to the world health organization MONICA project (9, 11). We also recruited 153 age- and sex-matched healthy controls from the local population sharing the same environment. This case-control study was conducted at the Ghaem hospital (the only neurology center in Mashhad where a comprehensive neurology emergency care is provided) (11).

Demographic and other stroke data, including age, sex, hypertension, diabetes mellitus, and lipid profile, comprising low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC, and triglycerides (TG), were collected with the help of Ghaem hospital information system (HIS) software for stroke patients. The study was approved by the ethics committee of Mashhad University of Medical Sciences (MUMS).

In addition, every patient with stroke was examined by an expert stroke neurologist to confirm the diagnosis. Neuroimaging was used to classify patients with a definite first-ever stroke into IS and hemorrhage stroke (ICH and SAH) groups. Subtyping into ischemic and hemorrhagic strokes was performed by computed tomography (CT) scans and magnetic resonance imaging (MRI).

A subject was classified as having arterial hypertension if diagnosed previously with hypertension or if the systolic or diastolic blood pressure was 140 mm Hg or 90 mm Hg, respectively. Subjects were classified as having diabetes mellitus if they were already diagnosed with diabetes mellitus or if their fasting plasma glucose level was $\geq$ 7.0 mmol/L (126 mg/dL). Ischemic heart disease was established on the basis of past medical history, review of ECGs, and other relevant clinical information. Case ascertainment was also performed on the basis of the study by Azarpazhooh et.al. (11).

3.2. Genotyping

A total of 5 mL of venous blood was collected in an EDTA tube, and DNA was extracted using the PrimePrep genomic DNA isolation kit (catalog No. K-2000; Genet Bio). APOE genotyping was performed according to the study by Yang et al. (23). in a single reaction tube with six primers consisting of two common primers and two specific primers for each of two single nucleotide polymorphism (SNP) sites by multiplex tetra-primer amplification refractory mutation system (multiplex T-ARMS) polymerase chain reaction (23); however, for the exact detection of this polymorphism, we analyzed e2 and e4 SNPs by three primer ARMS and found almost 10% possible misassignment of the genotype.

The reaction mixture (25 μL/well) was then added, containing 10 mmol/L Tris-HCl (pH 8.3), 2.0 mmol/L MgCl2, 10 mM dNTP mixture, 8% dimethyl sulfoxide (DMSO), 10 pmol of each primer (APOE primers), 1 U Taq DNA polymerase (Genetbio), and 0.1% Triton X-100 (Table 1). The cycling conditions were denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 40 seconds, and extension at 72°C for 40 seconds, with a final extension step of 7 minutes at 72°C. The PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and viewed under ultraviolet light (Figures 1, 2, and 3). The banding patterns and primer sequencing are shown in Table 2, Table 3, and Figure 4.

3.3. Statistical analysis

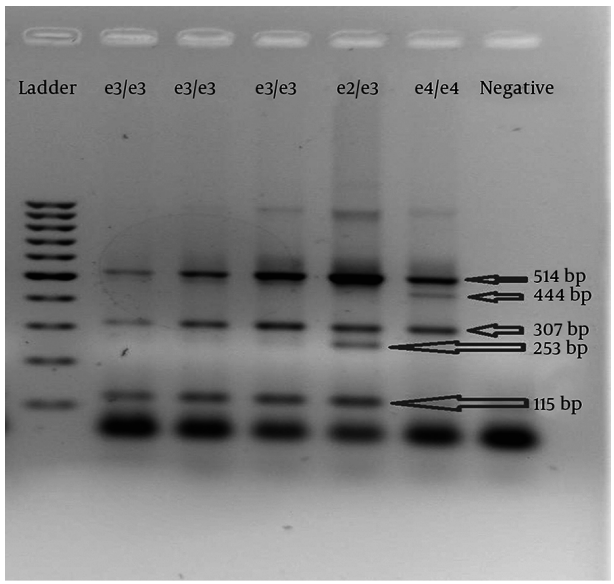
Data on qualitative characteristics are expressed as percent values or absolute numbers as indicated, and quantitative characteristics are expressed as means (SD). In addition, quantitative data were normalized using one-sample Kolmogorov-Smirnov test. Comparisons between groups were made using χ^2 test or Fisher's exact test (nominal data) and student's t-test or Mann-Whitney test (interval data). A value of P = 0.05 was considered as statistically significant. Analyses were adjusted for age, sex, and additionally for some stroke risk factors (hypertension, diabetes, DM, ischemic heart attack, LDL, HDL, triglycerides, and TC). Hardy-Weinberg equilibrium was tested by the χ^2 method.

Table 1. Demographic Data and Risk Factor Profile in Stroke Patients and Their Controls[z,a]

	Cases, n = 153	Controls, n = 154	P Value
Age	51.4 (13.7)	50.0 (12.2)	0.357[b]
Gender, No.			0.853[c]
Female	93	92	
Male	60	62	
Hypertension, No.			0.214[c]
Yes	72	23	
No	81	38	
IHD, No.			0.012[c]
Yes	28	3	
No	125	58	
Diabetes mellitus, %	39 (25.5)	41 (26.6)	0.821[c]
LDL	126.8 (34.6)	124.9 (31.8)	0.622[b]
HDL	40.8 (9.5)	41.6 (8.9)	0.431[b]
TG	139.6 (93.0)	144.1 (81.2)	0.658[b]
T-C	185.2 (46.0)	192.6 (39.3)	0.135[b]

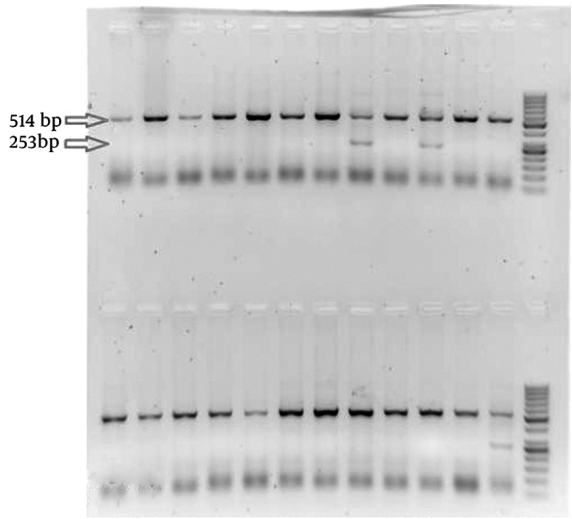
[z]Abbreviations: SD, standard deviation; T-C, total cholesterol; TG, triglyceride.
[a]Values are expressed as mean (SD) unless otherwise indicated.
[b]Student t-test.
[c]χ^2 test.

Figure 1. (1 - 5) Bands are for a Multiplex Tetra-Primer Amplification Refractory Mutation System (Multiplex T-ARMS) Polymerase Chain Reaction



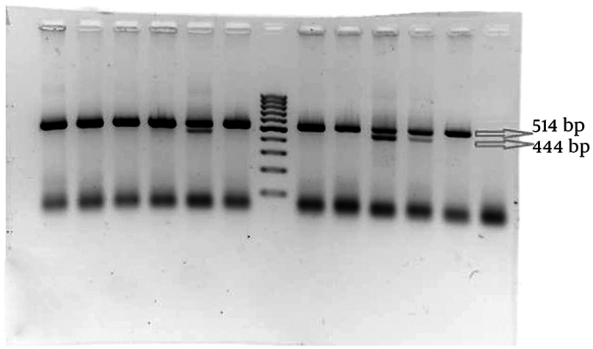
Ladder 100 bp fermentase, agarose gel 3%, ethidium bromide staining.

Figure 2. Confirmation of 253 bp Band by Bidirectional Amplification Refractory Mutation System (-ARMS) Polymerase Chain Reaction



Ladder 50 bp fermentase, agarose Gel 2%, ethidium bromide staining

Figure 3. Confirmation of 444 bp Band by Bidirectional Amplification Refractory Mutation System (-ARMS) Polymerase Chain Reaction



Ladder 100 bp fermentase, agarose Gel 2%, ethidium bromide staining

Figure 4. Location of Primer Schematically

The expected proportion was measured for each of the six genotypes, including e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4, and the proportion for each allele, including e2, e3,

Table 2. Multiplex T-ARMS PCR Primers (23)

Primers	Sequences
Common outer primers (FO)	5-ACTGACCCCGGTGGCGGAGGA-3
Common outer primers (RO)	5-CAGGCGTATCTGCTGGGCCTGCTC-3
Inner primers at codon 112 (FI)	5-GGCGCGGACATGGAGGACGgGC-3
Inner primers at codon 112 (RI)	5-GCGGTACTGCACCAGGCGGCCtCA-3
Inner primers at codon 158 (FII)	5-CGATGCCGATGACCTGCAGAcGC-3
Inner primers at codon 158 (RII)	5-CCCGGCCTGGTACACTGCCAGtCA-3

Table 3. Multiplex T-ARMS PCR Product Size in a Single Reaction Tube

Alleles Pair of Primer	Product Size, bp
E2	
FO-RI	115
FO-RII	253
E3	
FO-RI	115
FII-RO	307
E4	
FI-RO	444
FII-RO	307
Control	514

and e4, was also measured, in cases and controls. The risk of stroke was analyzed in terms of the subgroups and sex. Data analysis was performed by SPSS software package (version 11.5).

4. Results

Tables 4 and 5 show the distribution of apoE genotypes and alleles according to sex and stroke subtypes, respectively. The 153 stroke patients were grouped as follows: ischemic 77 (50.3%), hemorrhagic 51 (33.3%), and other 25 (16.3%), including 60 (39.2%) males and 93 (60.8%) females. The e2/e3/e4 polymorphism was selected due to the majority of polymorphisms being strongly linked in most of the population studies. To clarify the influence of genetic background on the risk of stroke, we also analyzed the relationship among two primary etiologic subgroups of stroke, sex, and age. A statistically significant difference was observed between the case and control group. Based on the APOE gene e2/e3/e4 polymorphism genotypes, an association was observed between the genotypes of APOE gene e2/e3polymorphism and all the stroke types. In addition, there was a strong association between the genotypes of APOE gene e2/e3 genotype and hemorrhagic stroke ($P = 0.001$). The association of APOE e2/e3 polymorphism with age of male stroke patients was also analyzed as separate groups. The difference between genotype distributions was also statistically significant. In other words, we found that the e2/e3 genotype was an independent risk factor for hemorrhagic stroke in males among the Persian population.

5. Discussion

The present study has demonstrated serendipitous results. APOE e2/e3 polymorphism may play a role in Iranian patients with stroke (east-Iran), a fact contradicted in few previous studies, especially studies on western stroke patients. Such contradictions may be due to undetermined sample sizes, mistaken stroke classification, different and no wide age ranges, inaccurate analysis of genotype (mistyping), or not matching the cases and controls in terms of environmental risk factors. We believe that there are several advantages in our study. First, according to the statistical formula, we determined a sample size that involved all the subtypes of stroke, and our sample size is also one of the largest in this area. Second, we diagnosed and classified stroke subjects by serial CT or MRI findings; in addition, an expert stroke neurologist examined the patients for confirmation, case ascertainment was performed according to the Mashhad stroke incidence study (MSIS), and misclassification was prevented by not using hospital discharge or death records. Third, we used wider age ranges so as to assess the age-dependent association with stroke. Fourth, we analyzed APOE e2/e3/e4 polymorphisms based on several laboratory studies [we used ARMS for single SNPs to prevent errors arising from multiplex tetra-primer amplification refractory mutation system (multiplex T-ARMS)]. Finally, we matched the cases and controls in terms of common stroke risk factors such as diabetes mellitus, age, sex, and lipid profile.

Stroke, an abrupt onset of a focal neurological deficit secondary to a vascular event lasting more than 24 hours, is a heterogeneous complex disease. It is categorized as either ischemic (caused by embolisms or thrombosis) or hemorrhagic (caused primarily by spontaneous rupture of a blood vessel or aneurysm).

Apolipoprotein E (Apo E) is a normal constituent of plasma chylomicrons, very low-density lipoprotein (VLDL), and HDL in humans (26). Liver is the major source of apoE; is synthesized by the adrenal glands, the kidney, and monocytes;and is also present in the cerebrospinal fluid (27-29).

The distribution of APOE e2/e3 genotype varies in male and female patients and is approximately influenced by

Table 4. ApoE GENOTYPE and Allele Frequencies in Patients With all Type of Stroke, Sex Group and Controls[a]

	Cases	Controls	P Value	Male Cases	Male Controls	P Value	Female Cases	Female Controls	P Value
Genotype distribution			0.031			0.040			0.590
Total	153	154		60	62		93	92	
e2/e2	0 (0)	3 (1.9)		0 (0)	3 (4.8)		0 (0)	0 (0)	
e3/e3	120 (78.4)	124 (80.5)		44 (73.3)	47 (75.8)		76 (81.7)	7 (83.7)	
e4/e4	2 (1.3)	0 (0)		1 (1.7)	0 (0)		1 (1.1)	0 (0)	
e2/e3	17 (11.1)	7 (4.5)		9 (15.0))	2 (3.2)		8 (8.6)	5 (5.4)	
e2/e4	0 (0)	1 (0.6)		0 (0)	1 (1.6)		0 (0)	0 (0)	
e3/e4	14 (9.2)	19 (12.3)		6 (10)	9 (14.5)		8 (8.6)	10 (10.9)	
Allele distribution			0.816			0.916			0.710
Total	306	308		120	124		186	184	
e2	17 (5.6)	14 (4.5)		9 (7.5)	9 (7.3)		8 (4.3)	5 (2.7)	
e3	271 (88.6)	274 (89.0)		103 (85.8)	105 (84.7)		168 (90.3)	169 (91.8)	
e4	18 (5.9)	20 (6.5)		8 (6.7)	10 (8.1)		10 (5.4)	10 (5.4)	

[a] Values are expressed as No. (%).

Table 5. ApoE Genotype and Allele Frequencies in Patients With Subtype of Stroke and in Their Controls[a]

	Ischemic Stroke	Controls	P Value	Hemorrhagic Stroke	Controls	P Value	Others Stroke	Controls	P Value
Genotype distribution			0.685			0.001			0.367
Total	77	154		51	154		25	154	
e2/e2	0 (0)	3 (1.9)		0 (0)	3 (1.9)		0 (0)	3 (1.9)	
e3/e3	62 (80.5)	124 (80.5)		39 (76.5)	124 (80.5)		19 (76.0)	124 (80.5)	
e4/e4	0 (0)	0 (0)		1 (2.0)	0 (0)		1 (4.0)	0 (0)	
e2/e3	6 (7.8)	7 (4.5)		10. (19.6)	7 (4.5)		1 (4.0)	7 (4.5)	
e2/e4	0 (0)	1 (0.6)		0 (0)	1 (0.6)		0 (0)	1 (0.6)	
e3/e4	9 (11.7)	19 (12.3)		1 (2.0)	19 (12.3)		4 (16.0)	19 (12.3)	
Allele distribution			0.912			0.069			0.348
Total	154	308		102	308		50	308	
e2	6 (3.9)	14 (4.5)		10 (9.8)	14 (4.5)		1 (2.0)	14 (4.5)	
e3	139 (90.3)	274 (89.0)		89 (87.3)	274 (89.0)		43 (86.0)	274 (89.0)	
e4	9 (5.8)	20 (6.5)		3 (2.9)	20 (6.5)		6 (12.0)	20 (6.5)	

[a] Values are expressed as No. (%).

sex. A slightly significant statistical association is also observed between e2/e3 genotypes in the male population (P = 0.04). Moreover, this association is strong in hemorrhagic subtypes (P = 0.001).

In the present study, we confirmed the results of previously published studies in Japanese subjects, showing that e2/e3 genotypes of APOE gene contribute to the risk of hemorrhagic stroke; however, the role of APOE polymorphism in stroke is still being debated, particularly in this area.

Compared to another study, we found a lower percentage of e2/e3 in stroke patients, particularly hemorrhagic stroke, although large-scale studies are needed to confirm this finding in this population. We studied all the subtypes of stroke in Iranian patients for the first time based on other larger epidemiological studies such as the MSIS and the study by Azarpazhooh et al. In fact, we are planning for further studies with a large sample size, due to the genetic background in Iran.

In conclusion, this study showed that the e2/e3 genotype of APOE gene may play a role in stroke, especially hemorrhagic stroke, with an association with male sex. This is the first population-based, longitudinal study to assess the association between genotype and stroke in the Iranian population. Hence, this study is a novel pathway toward understanding the pathways underlying this multifactorial complex disorder, particularly in the Persian population.

Footnote

Funding/Support: This research was supported by Mashhad University of Medical Sciences (MUMS).

References

1. Meschia JF, Worrall BB, Rich SS. Genetic susceptibility to ischemic stroke. *Nat Rev Neurol.* 2011;**7**(7):369–78. doi: 10.1038/nrneurol.2011.80. [PubMed: 21629240].
2. Sherif M. Hamdy Genetics of Stroke Syndromes. *Egypt J Neurol Psychiat Neurosurg.* 2011;**48**(1):3–9.
3. Rubattu S, Giliberti R, Volpe M. Etiology and pathophysiology of stroke as a complex trait. *American Journal of Hypertension.* 2000;**13**(10):1139–48. doi: 10.1016/s0895-7061(00)01249-8.
4. Hassan A, Markus HS. Genetics and ischaemic stroke. *Brain.* 2000;**123** (**Pt 9**):1784–812. [PubMed: 10960044].
5. Bersano A, Ballabio E, Bresolin N, Candelise L. Genetic polymorphisms for the study of multifactorial stroke. *Hum Mutat.* 2008;**29**(6):776–95. doi: 10.1002/humu.20666. [PubMed: 18421701].
6. Seshadri S, Wolf P. Lifetime risk of stroke and dementia: current concepts, and estimates from the Framingham Study. *The Lancet Neurology.* 2007;**6**(12):1106–14. doi: 10.1016/s1474-4422(07)70291-0.
7. Seshadri S, Beiser A, Kelly-Hayes M, Kase CS, Au R, Kannel WB, et al. The lifetime risk of stroke: estimates from the Framingham Study. *Stroke.* 2006;**37**(2):345–50. doi: 10.1161/01.STR.0000199613.38911.b2. [PubMed: 16397184].
8. Paul SL, Srikanth VK, Thrift AG. The large and growing burden of stroke. *Curr Drug Targets.* 2007;**8**(7):786–93. [PubMed: 17630931].
9. Oveisgharan S, Sarrafzadegan N, Shirani S, Hosseini S, Hasanzadeh P, Khosravi A. Stroke in Isfahan, Iran: hospital admission and 28-day case fatality rate. *Cerebrovasc Dis.* 2007;**24**(6):495–9. doi: 10.1159/000110418. [PubMed: 17971627].
10. Saleheen D, Bukhari S, Haider SR, Nazir A, Khanum S, Shafqat S, et al. Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. *Stroke.* 2005;**36**(10):2275–7. doi: 10.1161/01.STR.0000182242.59466.ee. [PubMed: 16166573].
11. Azarpazhooh MR, Etemadi MM, Donnan GA, Mokhber N, Majdi MR, Ghayour-Mobarhan M, et al. Excessive incidence of stroke in Iran: evidence from the Mashhad Stroke Incidence Study (MSIS), a population-based study of stroke in the Middle East. *Stroke.* 2010;**41**(1):e3–e10. doi: 10.1161/STROKEAHA.109.559708. [PubMed: 19926844].
12. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens.* 2003;**21**(11):1983–92. doi: 10.1097/01.hjh.0000084751.37215.d2. [PubMed: 14597836].
13. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JJ, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension.* 2003;**42**(6):1206–52. doi: 10.1161/01.HYP.0000107251.49515.c2. [PubMed: 14656957].
14. van Gijn J, Rinkel GJ. Subarachnoid haemorrhage: diagnosis, causes and management. *Brain.* 2001;**124**(Pt 2):249–78. [PubMed: 11157554].
15. Sudlow C, Martinez Gonzalez NA, Kim J, Clark C. Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls. *Stroke.* 2006;**37**(2):364–70. doi: 10.1161/01.STR.0000199065.12908.62. [PubMed: 16385096].
16. Flossmann E, Schulz UG, Rothwell PM. Systematic review of methods and results of studies of the genetic epidemiology of ischemic stroke. *Stroke.* 2004;**35**(1):212–27. doi: 10.1161/01.STR.0000107187.84390.AA. [PubMed: 14684773].
17. Woo D, Sauerbeck LR, Kissela BM, Khoury JC, Szaflarski JP, Gebel J, et al. Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study. *Stroke.* 2002;**33**(5):1190–5. [PubMed: 11988589].
18. Kissela BM, Sauerbeck L, Woo D, Khoury J, Carrozzella J, Pancioli A, et al. Subarachnoid hemorrhage: a preventable disease with a heritable component. *Stroke.* 2002;**33**(5):1321–6. [PubMed: 11988610].
19. Utermann G, Langenbeck U, Beisiegel U, Weber W. Genetics of the apolipoprotein E system in man. *Am J Hum Genet.* 1980;**32**(3):339–47. [PubMed: 7386461].
20. Utermann G, Steinmetz A, Weber W. Genetic control of human apolipoprotein E polymorphism: comparison of one- and two-dimensional techniques of isoprotein analysis. *Hum Genet.* 1982;**60**(4):344–51. [PubMed: 7106770].
21. Zannis VI, Breslow JL. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. *Biochemistry.* 1981;**20**(4):1033–41. doi: 10.1021/bi00507a059.
22. Saidi S, Slamia LB, Ammou SB, Mahjoub T, Almawi WY. Association of apolipoprotein E gene polymorphism with ischemic stroke involving large-vessel disease and its relation to serum lipid levels. *J Stroke Cerebrovasc Dis.* 2007;**16**(4):160–6. doi: 10.1016/j.jstrokecerebrovasdis.2007.03.001. [PubMed: 17689412].
23. Yang YG, Kim JY, Park SJ, Kim SW, Jeon OH, Kim DS. Apolipoprotein E genotyping by multiplex tetra-primer amplification refractory mutation system PCR in single reaction tube. *J Biotechnol.* 2007;**131**(2):106–10. doi: 10.1016/j.jbiotec.2007.06.001. [PubMed: 17643539].
24. Bazrgar M, Karimi M, Fathzadeh M, Senemar S, Peiravian F, Shojaee A, et al. Apolipoprotein E polymorphism in Southern Iran: E4 allele in the lowest reported amounts. *Mol Biol Rep.* 2008;**35**(4):495–9. doi: 10.1007/s11033-007-9113-3. [PubMed: 17594534].
25. Kokubo Y, Chowdhury AH, Date C, Yokoyama T, Sobue H, Tanaka H. Age-Dependent Association of Apolipoprotein E Genotypes With Stroke Subtypes in a Japanese Rural Population. *Stroke.* 2000;**31**(6):1299–306. doi: 10.1161/01.str.31.6.1299.
26. Gao X, Yang H, ZhiPing T. Association studies of genetic polymorphism, environmental factors and their interaction in ischemic stroke. *Neuroscience letters.* 2006;**398**(3):172–7.
27. Debette S, Lambert JC, Gariepy J, Fievet N, Tzourio C, Dartigues JF, et al. New insight into the association of apolipoprotein E genetic variants with carotid plaques and intima-media thickness. *Stroke.* 2006;**37**(12):2917–23. doi: 10.1161/01.STR.0000249011.94055.00. [PubMed: 17095737].
28. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am J Hum Genet.* 1985;**37**(2):268–85. [PubMed: 3985008].
29. Mahley RW, Innerarity T. Lipoprotein receptors and cholesterol homeostasis. *Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes.* 1983;**737**(2):197–222. doi: 10.1016/0304-4157(83)90001-1.