Medica Hospitalia
Journal of Clinical Medicine
Med Hosp 2022; vol 9 (2) : 154–161

Original Article

Apolipoprotein E Polymorphism and Carotid Intima Medial Thickness Progression in Post Ischemic Stroke Patient

Aditya Kurnianto, Dodik Tugasworo, Retnaningsih, Yovita Andhitara, Rahmi Ardhini, Jethro Budiman

Faculty of Medicine, Diponegoro University, Semarang, Indonesia

p-ISSN: 2301-4369 e-ISSN: 2685-7898
https://doi.org/10.36408/mhjcm.v9i2.750

Accepted: June 21th, 2022
Approved: July 5th, 2022

Author Affiliation:
Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Author Correspondence:
Aditya Kurnianto
Prof. H. Soedarto, S.H. Street, Tembalang, Semarang, Central Java 50725, Indonesia

E-mail Address:
adityakurniantosaraf2020@yahoo.com

Abstract

Background : Apolipoprotein E (APOE) gene is believed to associate with cholesterol level, a risk factor of ischemic stroke. CIMT (carotid intima-media thickness) can be used to determine the degree of atherosclerosis. Increased CIMT may predict ischemic stroke recurrence. This study aimed to determine association between increased CIMT in post ischemic stroke patients and APOE genotype.
Methods : This was an epidemiological prospective study involving 71 post ischemic stroke patients (1 month from onset), admitted from 2012 to 2013. CIMT was examined with carotid duplex ultrasound at 1st, 6th, and 12nd month after stroke onset. APOE gene polymorphism was examined using HRM (high-resolution melting) which is a simple method, accurate, and sensitive for genotyping.
Results : We found 5 APOE gene variation categories, i.e. E2E3, E2E4, E3E3, E3E4, and E4E4. The most common allele was E3 and genotype groups E3E3 was the majority of the population. E2E4 allele had the highest CIMT level among others, in the 1st month, 6th month, and 12nd month after stroke, with no association with hypertension, diabetes, and hypercholesterolemia. E3E3 allele was most often associated with hypertension, diabetes mellitus, dyslipidemia, and hyperhomocysteinemia.
Conclusion : The results showed that APOE genotype E2E4 may independently constitute risk factor for atherosclerosis progression (CIMT) in post ischemic stroke patients. While the E3E3 genotype was often associated with hypertension, diabetes mellitus, dyslipidemia, and hyperhomocysteinemia. Our results suggest that APOE E4 was not an important risk factor for carotid atherosclerosis in post ischemic stroke patient.

Keywords : APOE, atherosclerosis, CIMT, post ischemic stroke

INTRODUCTION

Stroke is the third leading cause of mortality and the first leading cause of morbidity, with approximately 80% of strokes are ischemic stroke.[1–4] Intracranial atherosclerosis is believed to become a cause of ischemic stroke in 14–40% cases.[1,2,5–8] Genetic factors are thought to play a part in the disease vulnerability of certain ethnic.[8–10] Preventive action can be performed if an individual is known to have genes that cause susceptibility to atherosclerosis and stroke.[9,11–13] Various studies have been conducted to search for candidate genes involved in the occurrence of stroke. Most research has focused on genes that have a part in the pathology of atherosclerosis through lipid metabolism, that is apolipoprotein E (ApoE).[13–17] The ApoE gene that located in chromosome 19q13 and has three regular alleles, i.e. epsilon (ε)2, ε3, and ε4; encodes three isoforms of ApoE, i.e E2, E3, and E4. Third allele will produce six genotypes ε2 / ε2, ε3 / ε3, ε4 / ε4, ε4 / ε3, ε4 / ε2, ε2 / ε3 which encodes ApoE. ε3 allele is the most commonly found in the entire population.[4,14,15,18] APOE2 has a weak association with very low density lipoprotein (VLDL) and low density lipoprotein (LDL), as well as more associated with high density lipoprotein (HDL) when compared to ApoE3, while ApoE4 is associated with VLDL and LDL, and less associated with HDL.[14,15,18,19] ApoE gene polymorphism is responsible as much as 4–8% of the variation of total cholesterol and LDL cholesterol in Caucasian.[14,20] Interaction between ApoE genotype and environment, such as age, smoking, and obesity have a part in the onset of atherosclerosis and cerebrovascular diseases.[14,15,21–24]

Intima-media thickness (IMT) of the carotid arteries (CIMT) which can be measured using ultrasound may indicate the presence of atherosclerosis, even at an early stage so it can be used as a predictor of stroke. Several studies have demonstrated that the ApoE gene have an effect on carotid artery IMT, but other studies have failed to show that relationship.[25–34] ApoE was also found to be associated with the formation of carotid plaque.[29,31,33,34] A meta-analysis conducted at 22 publication of research shows that there is a relationship between ApoE with IMT of the carotid artery, thus increasing the likelihood of aterothrombotic stroke.26 This study aimed to determine the relationship between increased CIMT in post ischemic stroke patients and APOE genotype.

METHODS

This was an epidemiological prospective observational cohort study involving 71 patients with post–ischemic stroke subject (1 month from onset) admitted in the Neurology Clinic of Kariadi Hospital during 2012 to 2013. Inclusion criterias were (1) Aged 45–90 years, (2) Experiencing atherosclerosis and post ischemic stroke proved by brain CT and carotid doppler, (3) First stroke event, (4) No heart disease (ECG within normal limits or IHD without clinical symptoms), (5) Stroke onset was not more than one month. (6) Willing to participate in the study. Exclusion criterias were: (1) Bedridden, (2) unconscious. Drop out criterias were: (1) The patient didn't come in a 2 consecutive CIMT examination, (2) Suffering from hemorrhagic transformation, (3) Patient died, (4) Patient resign. CIMT was examined using carotid duplex ultrasound siemens plane soniline omnia no FBE 0322 at 1st, 6th, and 12nd month after stroke onset, all assessments were performed by the same person (the person who assess CIMT was blinded with other results). CIMT is defined as the distance between lumen-intima and media-adventitia, both right internal carotid artery and left measured 3 cm proximal and 1 cm distal to the bifurcation. CIMT was measured at the beginning of the study and measured again after 6 months and 12 months. Thickening of the intima media thickness measured using carotid Doppler. It was said to be thicken when >1mm. APOE gene polymorphism was examined using HRM (high-resolution melting) which is a simple method, accurate, and sensitive for genotyping. Examination were done in Singapore. The risk factors of atherosclerosis in the subjects (hypertension, diabetes mellitus, hypercholesterolemia, high LDL, hypertrigliseridemia, low HDL, hyperuricemia, and hyperhomocyisteinemia) were also evaluated in this research.

This research has been getting informed consent from patient and Ethical Clearance of Health Research Ethics Committee of the Faculty of Medicine Diponegoro University and has received permission from the Director of the Dr. Kariadi Hospital. The number of ethical clearance was 377/EC/FK/RSDK/2012. Selection bias, information bias, and cofounding was avoided by applying inclusion and exclusion criteria in each subject by at least two examiners and CIMT measurement was measured by neurovascular specialist who was blinded with other results.

RESULT

Figure 1 showed Consolidated Standards of Reporting Trials (CONSORT) flow diagram of reporting observational study. This research has been carried out within a period of 24 months, from January 2012 to December 2013. In the early stages we obtained 155 subjects who met the inclusion and exclusion criterias. In the 12-month study period there were 83 subjects loss of follow-up so that the remaining 72 subjects who finished the procedure. Loss of follow-up was caused by the patients who died, the people who do not proceed to the examination because they do not get a referral from a family doctor, the patients who did not continue the investigation because they do not want to

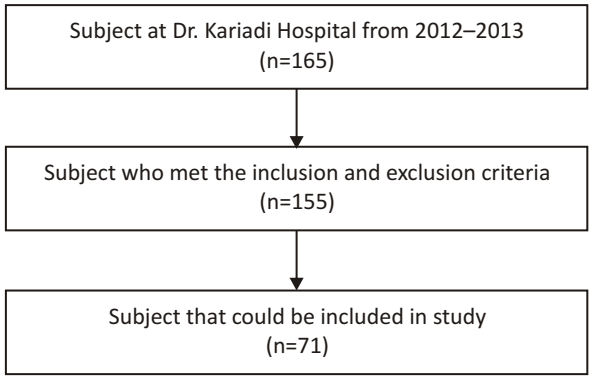

Figure 1. CONSORT flow diagram

TABLE 1
The frequency of APOE allele and genotype

APOE allele	Frequency
E2	11 (7.7%)
E3	102 (71.8%)
E4	29 (20.5%)
E2E3	6 (8.3%)
E2E4	5 (6.9%)
E3E3	39 (54.2%)
E3E4	18 (25.0%)
E4E4	3 (4.2%)

TABLE 2
Mean CIMT based on ApoE allele variation

Apo E allele	Mean CIMT (mm)		
	1st mo	6th mo	12nd mo
E2/E3 (n=6)	0.70 ± 0.12	0.79 ± 0.16	0.92 ± 0.23
E2/E4 (n=5)	0.76 ± 0.27	0.95 ± 0.28	1.07 ± 0.30
E3/E3 (n=39)	0.71 ± 0.26	0.82 ± 0.27	0.93 ± 0.28
E3/E4 (n=18)	0.72 ± 0.20	0.85 ± 0.25	0.94 ± 0.26
E4/E4 (n=3)	0.65 ± 0.22	0.76 ± 0.20	0.94 ± 0.21

Keterangan : *data terdistribusi normal (p>0,05)

come to the hospital only for this research, the patients still came to the hospital but refused to continue the examination because it was considered inconvenient or wasting time, patients who did not continue the examination because resettled outside the city or their address were unclear, so that from the 155 study subjects who meet the inclusion and exclusion criteria remaining 72 subjects that met the procedure regularly. From this 72 subjects, only 71 (98,6%) gene samples that can be examined, 1 sample was damaged.

We found 5 gene variation categories, i.e E2E3, E2E4, E3E3, E3E4, and E4E4. The most common allele was E3 (71,8%).

Subjects with E2/E4 allele had the highest CIMT among others, and so the mean CIMT in 1st, 6th, and 12nd month exanimation. In the end of this study, in 12nd

TABLE 3
APOE polymorphisms and atherosclerosis risk factors

Apo E allele	Hypertension		Diabetes mellitus		Hypercholesterolemia		High LDL	
	Yes	No	Yes	No	Yes	No	Yes	No
E2/E3 (n=6)	1 (16.7%)	5 (83.3%)	1 (16.7%)	5 (83.3%)	2 (33.3%)	4 (66.7%)	2 (33.3%)	4 (66.7%)
E2/E4 (n=5)	0 (0.0%)	5 (100%)	0 (0.0%)	5 (100.0%)	0 (0.0%)	5 (100.0%)	1 (20.0%)	4 (80.0%)
E3/E3 (n=39)	16 (41.0%)	23 (59.0%)	4 (10.3%)	35 (89.7%)	15 (38.5%)	24 (61.5%)	11 (28.2%)	28 (71.8%)
E3/E4 (n=18)	5 (27.8%)	13 (72.2%)	1 (5.6%)	17 (94.4%)	7 (38.9%)	11 (61.1%)	4 (22.2%)	14 (77.8%)
E4/E4 (n=3)	1 (33.3%)	2 (66.7%)	0 (0.0%)	3 (100.0%)	1 (33.3%)	2 (66.7%)	1 (33.3%)	2 (66.7%)

Apo E allele	Hypertrigliseridemia		Low HDL		Hyperuricemia		Hyperhomocyisteinemia	
	Yes	No	Yes	No	Yes	No	Yes	No
E2/E3 (n=6)	0 (0.0%)	6 (100.0%)	5 (83.3%)	1 (16.7%)	0 (0.0%)	6 (100.0%)	5 (83.3%)	1 (16.7%)
E2/E4 (n=5)	2 (40.0%)	3 (60.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)	5 (100.0%)	2 (40.0%)	3 (60.0%)
E3/E3 (n=39)	8 (20.5%)	31 (79.5%)	28 (71.8%)	11 (28.2%)	2 (5.1%)	37 (94.9%)	24 (61.5%)	15 (38.5%)
E3/E4 (n=18)	1 (5.6%)	17 (94.4%)	11 (61.1%)	7 (38.9%)	2 (11.1%)	16 (88.9%)	12 (66.7%)	6 (33.3%)
E4/E4 (n=3)	1 (33.3%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	0 (0.0%)	3 (100.0%)	2 (66.7%)	1 (33.3%)

month exanimation, subjects with E2/E3 allele had the lowest CIMT compared to others.

Correlation between ApoE allele variation and atherosclerosis risk factors will show in table 3.

E2E4 allele had no association with hypertension, diabetes, and hypercholesterolemia. E3E3 allele was most often associated with hypertension, diabetes mellitus, dyslipidemia, and hyperhomocysteinemia.

It also shows in our article, the identification of APOE polymorphisms gene rs429358 using qPCR-HRM, which will be explained further in fig. 2 and qPCR-HRM analysis for APOE rs429358polymorphism screening which will be shown in fig. 3.

DISCUSSION

ApoE Gene has three alleles variations; E2, E3 and E4. The presence of variant alleles were caused by SNP (single nucleotide polymorphism) at amino acid positions of 112 and 158. Both the SNP in the SNP database were registered as rs429358 and rs7412.35,36

This study were conducted with sequencing and confirmed with HRM examination which is a new method with high speed and accuracy. E3 allele is an allele most often found in the normal population.[4,14,15,18] This study found that the E3 (n = 104; 73.2%) is the allele with highest frequency in post-ischemic stroke patients. This is in contrast with the results of Saidi *et al.* (2007) which showed the finding of the high frequency of E4 allele in 213 stroke patients, the possible explanation of this is due to the ethnic differences in study subjects.[27] There were E4 / E4 alleles at codon 130 of ApoE gene found in 3 subjects of this study (4.2%). Cysteine is an amino acid with sulfihidril group (-SH) which was polar (hydrophilic) and functions as a component of the allele surface structure. Cysteine change to arginine which were basic (having -NH2) can cause changes in the structure of ApoE.[4,14,15,18,37,38] Distribution of allele E3 / E3 at mostly suffered from hypertension (41.0%). Related previous studies showed that hypertension was an independent risk factor for CIMT progression.[39,40] Conversely, E2 / E4 allele had no hypertension. E3 / E3 allele also had the most diabetes mellitus subjects in the study while alleles E2 / E4 had no diabetes mellitus. None of the who had subject alleles E2 / E4 in the study were suffering from hypercholesterolemia. Hypercholesterolemia acquired most often in allele E3 / E3 and most rarely in allele E2 / E4. The highest mean of LDL levels obtained in patients with E3 / E3, 11 (28.2%). This is in contrast to other studies stating that the E4 allele was related with high level of LDL.[14,15,18,19] Allele combinations are estimated to cause changes in the structure of apolipoprotein E, which causes a change in its function as well. Further study is needed in thehealthy control population and functional testing mutation alleles in p.Cys130Arg to determine its pathogenetic

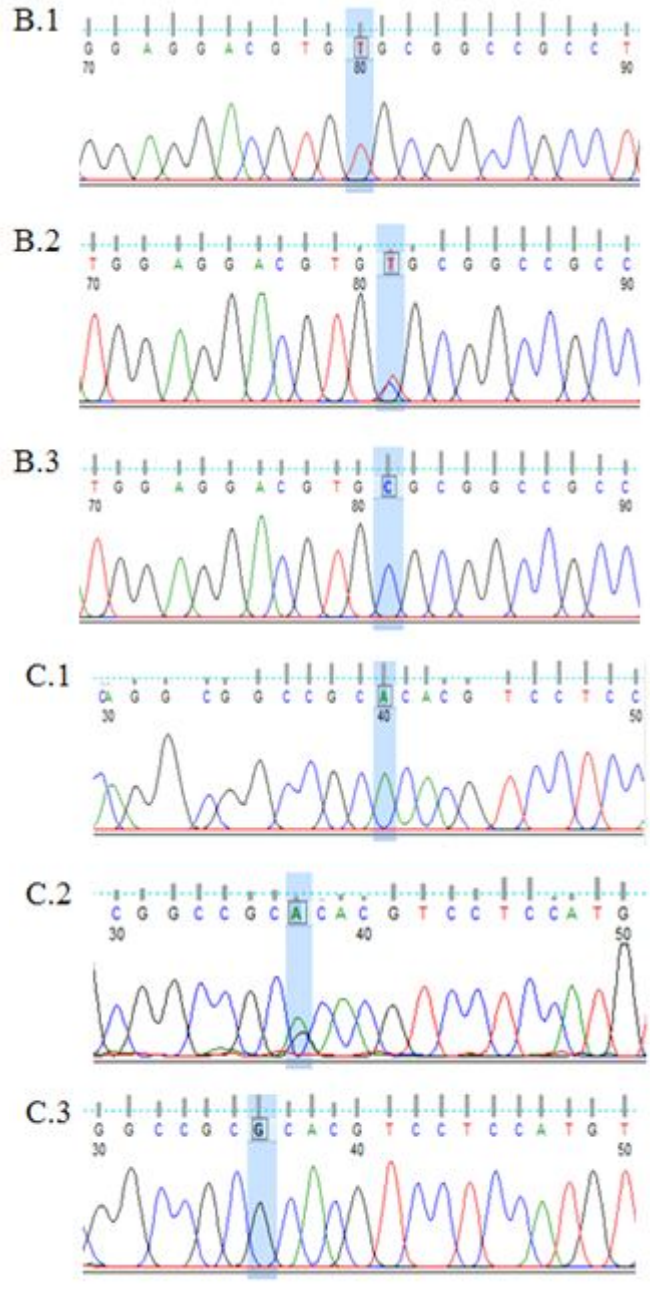

Figure 2. Identification of APOE polymorphisms gene rs429358 using qPCR-HRM
B. Forward sequences: (B.1) Homozygotes TT (wildtype), (B.2) HeterozygotesTC dan (B.3) Homozygotes CC
C. Reverse sequences: (C.1) Homozygotes TT (wildtype), (C.2) Heterozygote TC dan (C.3) Homozygotes CC

effects.[13,41]

It is known to have strong association between the incidence of type 2 diabetes mellitus and the incidence of atherosclerosis in cerebral arteries.[42,43] Examination of fasting blood glucose (FBG) and post prandial blood glucose (PPBG) in the 1st month investigation found the average 104 ± 20.18 and 46.56 ± 142.9. Means that the blood sugar levels in subjects still in the normal range. This condition can affect the association between ApoE gene polymorphism with incidence of DM. We need further study to understand association between diabetes and ApoE gene polymorphism.[14,15,21–24,42,43]

In this study, the ApoE allele E3 / E3 suffered most hypertriglyceridemia 8 (20.5%). This controversy also found in other studies. Dallongevile *et al*. (1999) suggests that ApoE alleles career has higher risk of hypertriglyceridemiathan other alleles. However, Ivanova, *et al*. (2012) stated that E4 allele career are at

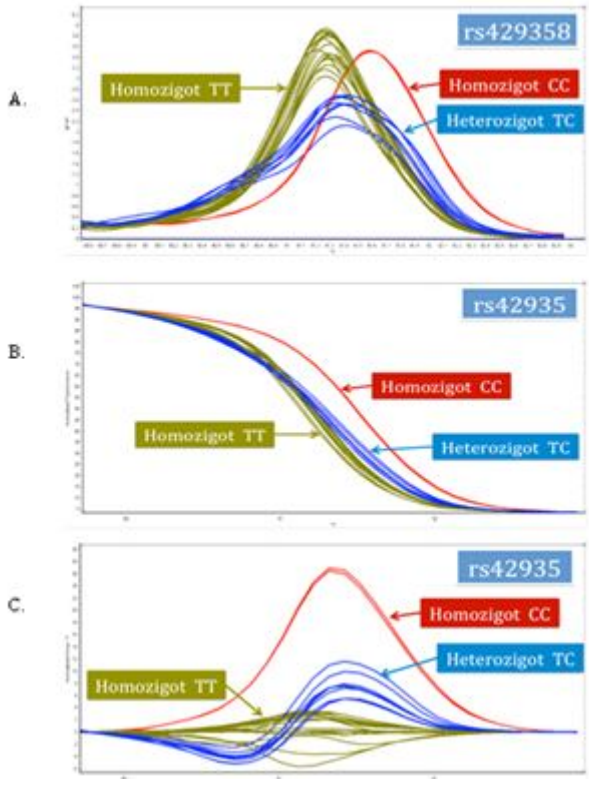

Figure 3. qPCR-HRM analysis for APOE rs429358 polymorphism screening:
green color shows TT homozygotes, blue color shows heterozygotes TC and red color shows CC homozygotes.

higher risk of hypertriglyceridemia.[44,45]

On the condition of hyperuricemia, disorders of lipid metabolism will lower uric filtration by the kidneys. This condition may increase the levels of uric acid in the blood that causes hiperuricaemia. APOE2 allele causes changes in the receptor capture point Apolipoprotein E with heparan sulfate proteoglycans. These changes cause the catabolism of triglyceride-rich lipoproteins in APOE2 career. Wu *et al*. (2014) also reported that APOE2 career have a greater risk of hyperuricemia than other alleles. In this study hiperuresemia not commonly found, allele E3 / E3 and E3 / E4 respectively 2 subjects (11.1%) have the most higher uric acid level. Further research is needed on ischemic stroke patients with hyperuricemia with a larger number of samples to look at the role of ApoE gene polymorphisms.[46,47]

The highest mean homocysteine level were seen in ApoE gene alleles E3 / E3 24 (61.5%). Consistent with previous studies, this study also showed that levels of hyperhomocysteinemia became an independent risk factor for ischemic stroke.[48,49]

The results showed that the average CIMT was thicker in subjects with homozygous genes E2/E4 compared with others, both in the examination of 1 month, 6 months, and 12 month.E2 / E4 alleles that was found in five subjects (6.9%) seem to have the least amount of risk factors (hyperhomocysteinemia and HDL) but has an CIMT value of the thickest on the measurement of 1 month, 6 months, and 12 month. This is consistent with previous studies showed that the Apo E4 allele is associated with pathological thickening of the CIMT is not dependent on plasma lipid levels.[26–34,50] APOE2 seems to be protective allele against the fibrous cap atheroma (atherosclerosis). Some studies indicate that APOE genotype independently associated with atherosclerosis, plasma lipid levels and other risk factors.[25–34]

Alleles E2 / E4 have the thickest CIMT among others in 1 month, 6 months and 12 months examination. In the study, E2 / E4 alleles was not associated with other risk factors except HDL (100%). This suggests that the E2 / E4 have a possibility to become an independent risk factor in this study. Alleles E2 / E3 have the lowest CIMT value when compared to other variants. This is consistent with previous studies that showed that the E2 allele is protective.[26–34,50] Almost all risk factors alone or together are the in this study, allele E3 / E3 was the most common allele. This is consistent with the literature that says that E3 / E3 genotype is most often found in the population that is considered as normal allele.[4,14,15,18]

CONCLUSION

The results showed that APOE genotype E2E4 may independently constitute risk factor for atherosclerosis progression (CIMT) in post ischemic stroke patients. While the E3E3 genotype was often associated with hypertension, diabetes mellitus, dyslipidemia, and hyperhomocysteinemia. Our results suggest that APOE E4 is not an important risk factor for carotid atherosclerosis in post ischemic stroke patient. Further research is needed with the larger sample size with diverse demographic variances about the relationship of APOE polymorphism and CIMT.

REFERENCES

1. Donkor ES. Stroke in the 21st Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. Stroke Res Treat. 2018;1–10.
2. Katan M, Luft A. Global burden of stroke. Semin Neurol. 2018;38:208–11.
3. Hankey GJ, Macleod M, Gorelick PB, Chen C, Caprio FZ, Mattle H. Warlow's Stroke Practical Management 4th edition. New Jersey: Wiley Blackwell; 2019.
4. Gilroy J. Basic Neurology 3rd Edition. New York: McGraw-Hill; 2000.
5. Louis ED, Mayer SA, Noble JM. Merritt's Neurology 14th edition. Philadelphia: Lippincott Williams and Wilkins (LWW); 2021.
6. Ropper AH, Samuels MA, Klein JP, Prasad S. Adam and Victor's principless of neurology. USA: McGraw-Hill Education; 2019.
7. Caplan LR. Caplan's Stroke A Clinical Approach 5th Edition. Cambridge: Cambridge University Press; 2016.
8. Kim JS, Caplan LR, Wong KSL. Intracranial Atherosclerosis. West Sussex: Blackwell Publishing; 2008.
9. Jing L, Su L, Ring BZ. Ethnic Background and Genetic Variation in the Evaluation of Cancer Risk: A Systematic Review. PLoS One. 2014;9(6):1–11.
10. Markus HS. Stroke Genetics. Oxford: Oxford University Press; 2003.
11. Markus HS. Unravelling the genetics of ischaemic stroke. PLoS Med. 2010 Mar;7(3):1–5.
12. Rao R, Tah V, Casas JP, Hingorani A, Whittaker J, Smeeth L, *et al*. Ischaemic stroke subtypes and their genetic basis: a comprehensive meta-analysis of small and large vessel stroke. Eur Neurol. 2009;61(2):76–86.
13. Tamam Y, Tasdemir N, Toprak R, Tamam B, Iltumur K. Apolipoprotein E genotype in patients with cerebrovascular diseases and its effect on the disease outcome. Int J Neurosci. 2009;119(7):919–35.
14. Ganaie HA, Biswas A, Bhattacharya AP, Pal S, Ray J, Das SK. Association of APOE Gene Polymorphism with Stroke Patients from Rural Eastern India. Ann Indian Acad Neurol. 2020;23(4):504–9.
15. Konialis C, Spengos K, Iliopoulos P, Karapanou S, Gialafos E, Hagnefelt B, *et al*. The APOE E4 Allele Confers Increased Risk of Ischemic Stroke Among Greek Carriers. Adv Clin Exp Med Off organ Wroclaw Med Univ. 2016;25(3):471–8.
16. Kostulas K, Brophy VH, Moraitis K, Manolescu A, Kostulas V, Gretarsdottir S, *et al*. Genetic profile of ischemic cerebrovascular disease and carotid stenosis. Acta Neurol Scand. 2008 Sep;118(3):146–52.
17. Hagberg JM, Wilund KR, Ferrell RE. APO E gene and gene-environment effects on plasma lipoprotein-lipid levels. Physiol Genomics. 2000 Dec;4(2):101–8.
18. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis. 1988;8(1):1–21.
19. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet. 1985 Mar;37(2):268–85.
20. Jarvik GP, Goode EL, Austin MA, Auwerx J, Deeb S, Schellenberg GD, et al. Evidence that the apolipoprotein E-genotype effects on lipid levels can change with age in males: a longitudinal analysis. Am J Hum Genet. 1997 Jul;61(1):171–81.
21. Talmud PJ. Gene-environment interaction and its impact on coronary heart disease risk. Nutr Metab Cardiovasc Dis. 2007 Feb;17(2):148–52.
22. Marques-Vidal P, Bongard V, Ruidavets J-B, Fauvel J, Hanaire-Broutin H, Perret B, *et al*. Obesity and alcohol modulate the effect of apolipoprotein E polymorphism on lipids and insulin. Obes Res. 2003 Oct;11(10):1200–6.
23. Meer IM, Witteman JCM. Apolipoprotein E genotype, smoking, and cardiovascular disease. J Hypertens. 2002;20:2327-9. J Hypertens. 2002;20:2327–9.
24. Zerba KE, Ferrell RE, Sing CF. Genotype-environment interaction: apolipoprotein E (ApoE) gene effects and age as an index of time and spatial context in the human. Genetics. 1996 May;143(1):463–78.
25. Abboud S, Viiri LE, Lütjohann D, Goebeler S, Luoto T, Friedrichs S, *et al*. Associations of apolipoprotein E gene with ischemic stroke and intracranial atherosclerosis. Eur J Hum Genet. 2008 Aug;16(8):955–60.
26. Paternoster L, Martínez González NA, Lewis S, Sudlow C. Association between apolipoprotein E genotype and carotid intima-media thickness may suggest a specific effect on large artery atherothrombotic stroke. Stroke. 2008 Jan;39(1):48–54.
27. 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 Off J Natl Stroke Assoc. 2007;16(4):160–6.
28. Wohlin M, Sundström J, Lannfelt L, Axelsson T, Syvänen AC, Andrén B, *et al*. Apolipoprotein E epsilon4 genotype is independently associated with increased intima-media thickness in a recessive pattern. Lipids. 2007 May;42(5):451–6.
29. Debette S, Lambert J-C, Gariépy J, Fievet N, Tzourio C, Dartigues J-F, *et al*. New insight into the association of apolipoprotein E genetic variants with carotid plaques and intima-media thickness. Stroke. 2006 Dec;37(12):2917–23.
30. Volcik KA, Barkley RA, Hutchinson RG, Mosley TH, Heiss G, Sharrett AR, *et al*. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. Am J Epidemiol. 2006 Aug;164(4):342–8.
31. Beilby JP, Hunt CCJ, Palmer LJ, Chapman CML, Burley JP, McQuillan BM, *et al*. Apolipoprotein E gene polymorphisms are associated with carotid plaque formation but not with intima-media wall thickening: results from the Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Stroke. 2003 Apr;34(4):869–74.
32. Elosua R, Ordovas JM, Cupples LA, Fox CS, Polak JF, Wolf PA, *et al*. Association of APOE genotype with carotid atherosclerosis in men and women: the Framingham Heart Study. J Lipid Res. 2004 Oct;45(10):1868–75.
33. Doliner B, Dong C, Blanton SH, Gardener H, Elkind MS V, Sacco RL, *et al*. Apolipoprotein E Gene Polymorphism and

Subclinical Carotid Atherosclerosis: The Northern Manhattan Study. J stroke Cerebrovasc Dis Off J Natl Stroke Assoc. 2018 Mar;27(3):645–52.
34. Qin X, Li J, Wu T, Wu Y, Tang X, Gao P, *et al*. Overall and sex-specific associations between methylation of the ABCG1 and APOE genes and ischemic stroke or other atherosclerosis-related traits in a sibling study of Chinese population. Clin Epigenetics. 2019 Dec;11(1):189.
35. Giau V Van, Bagyinszky E, An SSA, Kim SY. Role of apolipoprotein E in neurodegenerative diseases. Neuropsychiatr Dis Treat. 2015;11:1723–37.
36. Zhen J, Huang X, Van Halm-Lutterodt N, Dong S, Ma W, Xiao R, *et al*. ApoE rs429358 and rs7412 Polymorphism and Gender Differences of Serum Lipid Profile and Cognition in Aging Chinese Population. Front Aging Neurosci. 2017;9:248.
37. Yamauchi K, Kawakami Y. The redox status of cysteine thiol residues of apolipoprotein E impacts on its lipid interactions. Biol Chem. 2020 Apr;401(5):617–27.
38. Frieden C. ApoE: the role of conserved residues in defining function. Protein Sci. 2015 Jan;24(1):138–44.
39. Chen L, Yang Q, Ding RUI, Liu DAN, Chen Z. Carotid thickness and atherosclerotic plaque stability, serum inflammation, serum mmp-2 and mmp-9 were associated with acute cerebral infarction. Exp Ther Med. 2018;16(6):5253–7.
40. Ren L, Shi M, Wu Y, Ni J, Bai L, Lu H, –. Correlation between hypertension and common carotid artery intima-media thickness in rural China: a population-based study. J Hum Hypertens. 2018 Sep;32(8–9):548–54.
41. Dickstein DL, Walsh J, Brautigam H, Stockton SDJ, Gandy S, Hof PR. Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. Mt Sinai J Med. 2010;77(1):82–102.
42. Katakami N. Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. J Atheroscler Thromb. 2018 Jan;25(1):27–39.
43. Poznyak A, Grechko A V, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The Diabetes Mellitus-Atherosclerosis Connection: The Role of Lipid and Glucose Metabolism and Chronic Inflammation. Int J Mol Sci. 2020 Mar;21(5).
44. Ivanova R, Puerta S, Garrido A, Cueto I, Ferro A, Ariza MJ, et al. Triglyceride levels and apolipoprotein E polymorphism in patients with acute pancreatitis. Hepatobiliary Pancreat Dis Int. 2012 Feb;11(1):96–101.
45. Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J Lipid Res. 1992 Apr;33(4):447–54.
46. Wu J, Qiu L, Guo X, Xu T, Cheng X, Zhang L, *et al*. Apolipoprotein E gene polymorphisms are associated with primary hyperuricemia in a Chinese population. PLoS One. 2014;9(10):1–9.
47. Cardona F, Tinahones FJ, Collantes E, Escudero A, García-Fuentes E, Soriguer FJ. The elevated prevalence of apolipoprotein E2 in patients with gout is associated with reduced renal excretion of urates. Rheumatology (Oxford). 2003 Mar;42(3):468–72.
48. Kuller LH, Grandits G, Cohen JD, Neaton JD, Prineas R. Lipoprotein particles, insulin, adiponectin, C-reactive protein and risk of coronary heart disease among men with metabolic syndrome. Atherosclerosis. 2007 Nov;195(1):122–8.
49. Christen WG, Ajani UA, Glynn RJ, Hennekens CH. Blood levels of homocysteine and increased risks of cardiovascular disease: causal or casual? Arch Intern Med. 2000 Feb;160(4):422–34.
50. Touboul PJ, Labreuche J, Bruckert E, Schargrodsky H, Prati P, Tosetto A, *et al*. HDL-C, triglycerides and carotid IMT: A meta-analysis of 21,000 patients with automated edge detection IMT measurement. Atherosclerosis. 2014;232(1):65–71.