ORIGINAL ARTICLE

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Association of *APOE* polymorphisms with lipid-lowering efficacy of statins in atherosclerotic cardiovascular disease

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ABSTRACT

Introduction: The apolipoprotein E (*APOE*) gene is a promising candidate for the diagnosis of hyperlipoproteinaemia and atherosclerosis. Polymorphisms in *APOE* have been reported to result in differential efficacies of statin drugs in atherosclerotic cardiovascular disease.

Methods: We classified the *APOE* genotypes of 225 patients treated with atorvastatin, and analysed the relation between genotypes and serum lipid levels.

Results: The baseline serum levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were significantly lower in carriers of *APOE* ε3 than of *APOE* ε4 genotypes. The serum levels of TC and LDL-C decreased significantly after 1 month of atorvastatin treatment. Atorvastatin has a higher significant effect in reducing serum TC and LDL-C levels in patients with the *APOE* ε4 genotype.

Conclusion: Polymorphism in the *APOE* gene is related to the efficacy of atorvastatin in reducing the serum levels of TC and LDL-C.

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Keywords: Apolipoprotein E, lipid-lowering efficacy, polymorphism, statin, total cholesterol

INTRODUCTION

Dyslipidaemia is an important cardiovascular risk factor. Statin, a hydroxymethylglutaryl coenzyme A reductase inhibitor, is widely used to reduce the risk of atherosclerotic cardiovascular disease (ASCVD).[1] It primarily inhibits the synthesis of cholesterol by lowering low-density lipoprotein cholesterol (LDL-C) levels in the body. However, a large proportion of patients fail to achieve target levels of lipids with statins, and may present with adverse reactions, such as new-onset diabetes mellitus, statin-associated muscle symptoms, and drug-induced liver injury.[2] Evidence has accumulated to show that the genetic background of the host plays a vital role in blood lipid levels and lipoproteins.[3] Studies have reported that apolipoprotein E (*APOE*) gene polymorphism is a major factor influencing the effect of statins on ASCVD.[4,5] Moreover, *APOE* was found to be involved in the regulation of lipid metabolism through various pathways, playing an important intrinsic factor in lipid levels in the body.[6] The *APOE* gene, which translates into a 299 amino acid-containing lipid-transport protein, is located on chromosome 19q13. It is expressed in various tissues, including blood, liver, brain, spleen, lung, kidney and muscles.[7] The human *APOE* has 2 main single-nucleotide polymorphisms (526C>T and 388T>C), which can form the following 3 main haploids and affect subsequent functional changes and pathological consequences: epsilon-2 (ε2) (388T-526T), epsilon-3 (ε3) (388T-526C), and epsilon-4 (ε4) (388C-526C).[8] Besides these, ε1 and ε5 are 2 rare alleles of the gene (0.1%) found in the Framingham population. The molecular basis of the *APOE* single-nucleotide polymorphisms has been attributed to the exchange of cysteine and arginine. Six polymorphic alleles carry homozygous and heterozygous genotypes, ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4.[9] The affinity for the lipoprotein receptor, and the in vivo metabolic rates are known to differ among the *APOE* gene-encoded isoforms. The stability of *APOE* isoforms has been shown to decrease in the order of ε2, ε3, then ε4, resulting in differences in blood

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CLINICAL IMPACT

What is New

- Polymorphism in *APOE* of the study population was related to lipid-lowering efficacy of atorvastatin.

Clinical Implications

- A standard-dose statin alone may be insufficient for *APOE* ε4 carriers in atherosclerotic cardiovascular disease. There is clinical importance for the detection of *APOE* genotypes to evaluate lipid-lowering effect of statins.

lipid levels among individuals. Interestingly, isomers of *APOE* have been associated with susceptibility to different diseases. For instance, ε2 has been associated with increased plasma levels of total cholesterol (TC) and triglyceride (TG), and is a risk factor for type III hyperlipoproteinaemia, whereas ε4 carriers generally have higher plasma concentrations of TG and LDL-C and an increased risk of ASCVD.[10] Studies have also shown *APOE* ε2 to be associated with prolonged survival compared with *APOE* ε3, while mortality risk was increased in ε4 carriers.[11] Of note, ε3 (79%) is the most common allele in the population of Europe and Asia, whereas ε4 (13.3%) and ε2 (7.3%) are less frequently observed.[12,13]

Although there have been several studies on the role of *APOE* polymorphism in lipid-lowering therapy using statins, other studies have reached inconsistent conclusions.[14] The relationship between *APOE* genotypes and statin efficacy remains controversial and inconclusive. In this study, we investigated a possible correlation between *APOE* polymorphism and the lipid-lowering efficacy of atorvastatin.

METHODS

Study design and participants

The study was conducted according to guidelines established in the Declaration of Helsinki, and approved by the Human Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University. We enrolled 225 coronary heart disease patients in Inner Mongolia from September 2017 to December 2018. Written informed consent was obtained from all subjects. This was a retrospective, double-blind, randomised, single-centre study designed to analyse the relationship between blood lipid levels and *APOE* genotypes of patients on atorvastatin (Lipitor) (Viatris Inc, Canonsburg, US) for 1 month. Mean age of patients was 63.7 years (standard deviation=11.2). Major exclusion criteria included severe liver damage, renal failure, heart failure and malignancy. The detection of *APOE*, using the gene-chip method, was completed before the enrolled patients commenced treatment with atorvastatin 20mg daily. Venous blood samples were collected after 12 hours of fasting. Metabolic indicators such as TG, TC, LDL-C and high-density lipoprotein cholesterol (HDL-C) were measured. After 1 month, the indices were reviewed.

Laboratory analysis

Total genomic DNA was extracted from 2mL of peripheral venous blood (in tubes containing EDTA anticoagulant) and stored at -80 degree Celsius until thawed for analysis. Using Baio R-Hyb automated hybridisation instrument (Shanghai BaiO Technology Company Ltd., Shanghai, China), a commercial *APOE* gene detection kit and analysing equipment, gene chips placed in the BaiO BE-2.0 Gene Chip Readerware were analysed. Genotyping was carried out by polymerase chain reaction-based ligase detection reaction using PerkinElmer GeneAmp 9600 PCR System (PerkinElmer, Shanghai, China). Accuracy of genotyping was ensured using duplicate samples and negative controls.

Statistical analysis

Continuous variables were expressed as mean (standard deviation) or as median (interquartile range) for non-normally distributed variables. Categorical variables were expressed as frequency and percentage. Data normality was determined using the Kolmogorov-Smirnov test. Changes in the levels of lipids, with respect to baseline, were assessed using the Student's t-test for normally distributed variables and the Mann-Whitney U test for non-parametric variables. Analysis of variance (ANOVA) testing was performed to analyse the differences across *APOE* genotypes, and Kruskal-Wallis test was used for multiple comparisons. Statistical analysis was performed using SPSS Statistics software version 22 (IBM Corp, Armonk, US). A *P* value of 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the 225 participants who received treatment with atorvastatin is shown in Table 1. Excluding non-genetic tests and duplicate

samples, 225 samples were tested for gene distribution frequency (Table 2). The number of ε2, ε3 and ε4 carriers was 24, 170 and 31, respectively, excluding patients with incomplete data on blood lipid levels. In the distribution of *APOE* alleles, we observed that ε3 had the highest proportion (75.56%). The distribution of ε2 and ε4 were found to be 10.67% and 13.78%, respectively. Among *APOE* genotypes ε3/ε3 was the most highly distributed (73.33%), followed by ε2/ε3 (10.22%) and ε3/ε4 (13.33%). The least common were ε2/ε2 (0.44%) and ε4/ε4 (0.44%).

We then performed a comparison of lipid levels among the 3 groups of ε2, ε3 and ε4 by variance analysis. The levels of TC and LDL-C in the ε3 genotype were significantly lower than those in the ε4 genotype; there was no statistical difference among the different genotypes for levels of TG and HDL-C (Fig. 1).

The number of samples that were ultimately followed up was 78. Paired Student's t-tests were used to group the blood lipids and genotypes, after treatment. There was a significant decrease in the levels of TC (4.5 versus 3.99mmol/L, 0.51mmol reduction) and LDL-C (2.68 vs 2.34mmol/L, 0.34mmol/L reduction), $P<0.05$. However, there was no statistical difference observed in the levels of TG (2.00 vs 1.74mmol/L) and HDL-C (1.04 vs 0.92mmol/L) (Fig. 2).

We further evaluated the effect of different *APOE* genotypes in lowering lipid levels in patients during follow-up. There were 8 patients with ε2, 60 patients with ε3, and 10 patients with ε4; statins was found to be more effective in lowering the levels of TC and LDL-C in *APOE* ε4 (Table 3) (Fig. 3). Baseline characteristics were also compared among *APOE* genotypes, there were no statistically significant differences in age, gender and comorbidities of study participants (Table 4).

Table 1. Baseline characteristics of patients in study

Characteristics	N=225
Age, mean (SD), years	63.7 (11.2)
Male, no. (%)	127 (56.4)
Female, no. (%)	98 (43.6)
Hypertension, no. (%)	93 (40.8)
Type 2 diabetes, no. (%)	34 (14.9)
Coronary heart disease, no. (%)	97 (42.5)

SD: standard deviation

Table 2. Distribution of *APOE* genotypes in patients

Isoform	No. of patients (%)	Genotype	No. (%)
ε2	24 (10.67)	ε2/ε2	1 (0.44)
		ε2/ε3	23 (10.22)
ε3	170 (75.56)	ε2/ε4	5 (2.22)
		ε3/ε3	165 (73.33)
ε4	31 (13.78)	ε3/ε4	30 (13.33)
		ε4/ε4	1 (0.44)

DISCUSSION

Our study indicated that ε3 exhibited the highest distribution frequency (75.56%), followed by ε4 (13.78%) and ε2 (10.67%). Additionally, the proportion of ε3/ε3 individuals was higher, consistent with the findings of previous studies.[15,16]

The basal values of TC and LDL-C significantly vary across the genotypes. Specifically, ε4 carriers in our study population have relatively higher levels of TC and LDL-C. The APOE protein is known to be a high-affinity ligand for receptors of the LDL family, the impact of *APOE* on the metabolism of lipoproteins is thought to be largely the result of an effect on receptor-binding activity by *APOE*.[1]

The 3 alleles—ε2, ε3 and ε4—differ in their amino-acid sequences, resulting in functional differences in the affinity of LDL receptors in binding. The *APOE* ε2 allele has been reported to have a lower binding affinity than that of the ε3 and ε4 alleles, resulting in decreased hepatic levels of very low-density lipoprotein and chylomicron remnant clearance, thus reducing the uptake of postprandial lipoprotein particles. Furthermore, it could be postulated that

Table 3. Reduction in lipid levels with atorvastatin use for each *APOE* genotype

	Genotype		
Lipid	*APOE* ε2	*APOE* ε3	*APOE* ε4
TC, %	68.41	86.85	93.74
TG, %	91.94	95.37	96.19
LDL-C, %	93.41	96.44	96.96
HDL-C, %	92.64	95.84	90.91

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride

Table 4. Age, sex and comorbidity for each *APOE* genotype

Characteristics	***APOE* ε2**	***APOE* ε3**	***APOE* ε4**	***P* value**
Age, mean (SD), years	64.5 (12.9)	63.3 (11.4)	65.6 (9.2)	0.56
Male, no. (%)	11 (45.8)	102 (60.0)	14 (45.2)	0.16
Hypertension, no. (%)	11 (45.8)	69 (40.6)	13 (41.9)	0.89
Type 2 diabetes, no. (%)	6 (25.0)	23 (13.5)	5 (16.1)	0.49
Coronary heart disease, no. (%)	10 (41.7)	73 (42.9)	14 (45.2)	0.96

SD: standard deviation

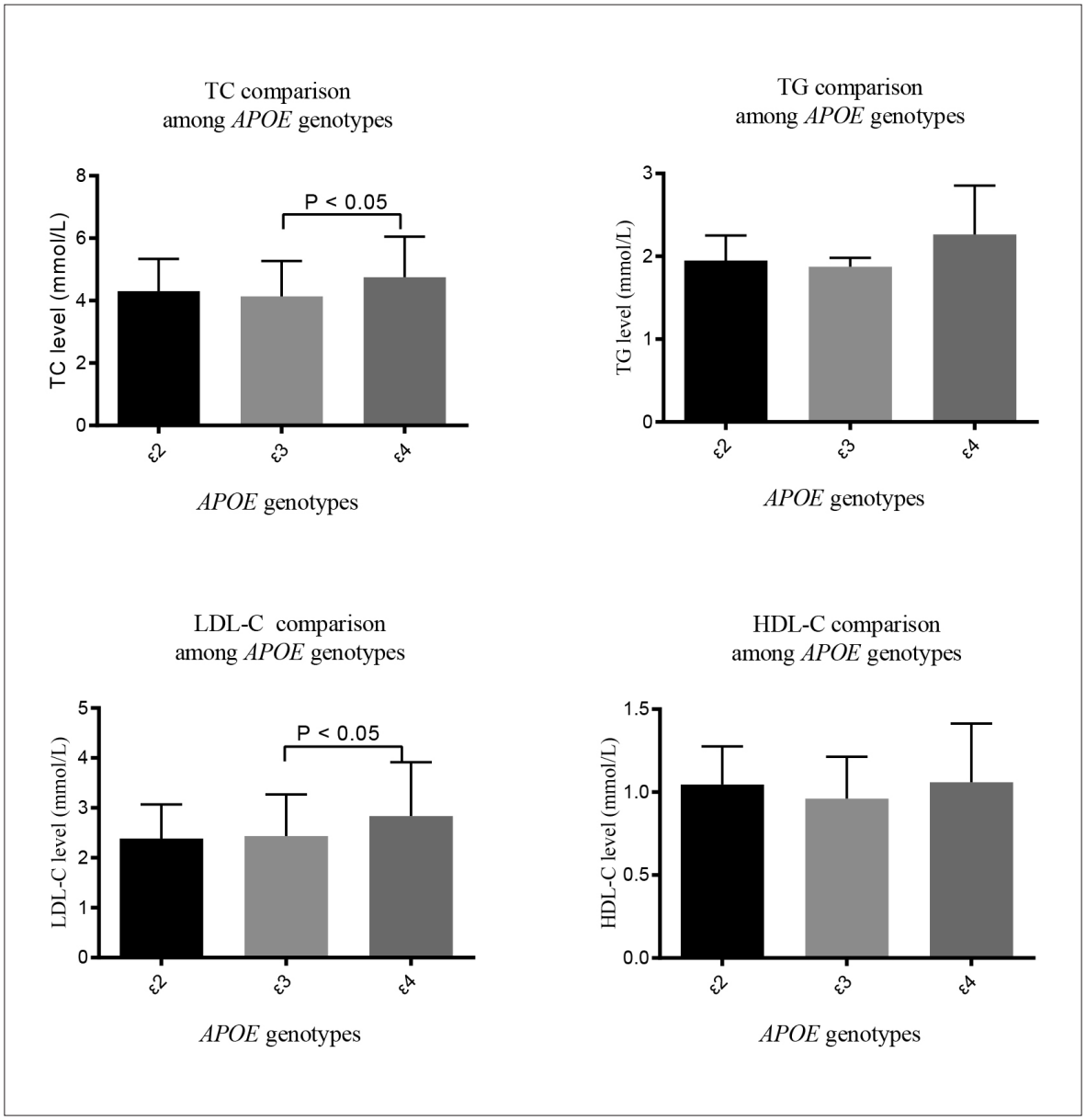

Fig. 1. Relationship between lipid levels (TC, TG, LDL-C and HDL-C) and ε2, ε3 and ε4 isoforms before treatment with atorvastatin.
HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.

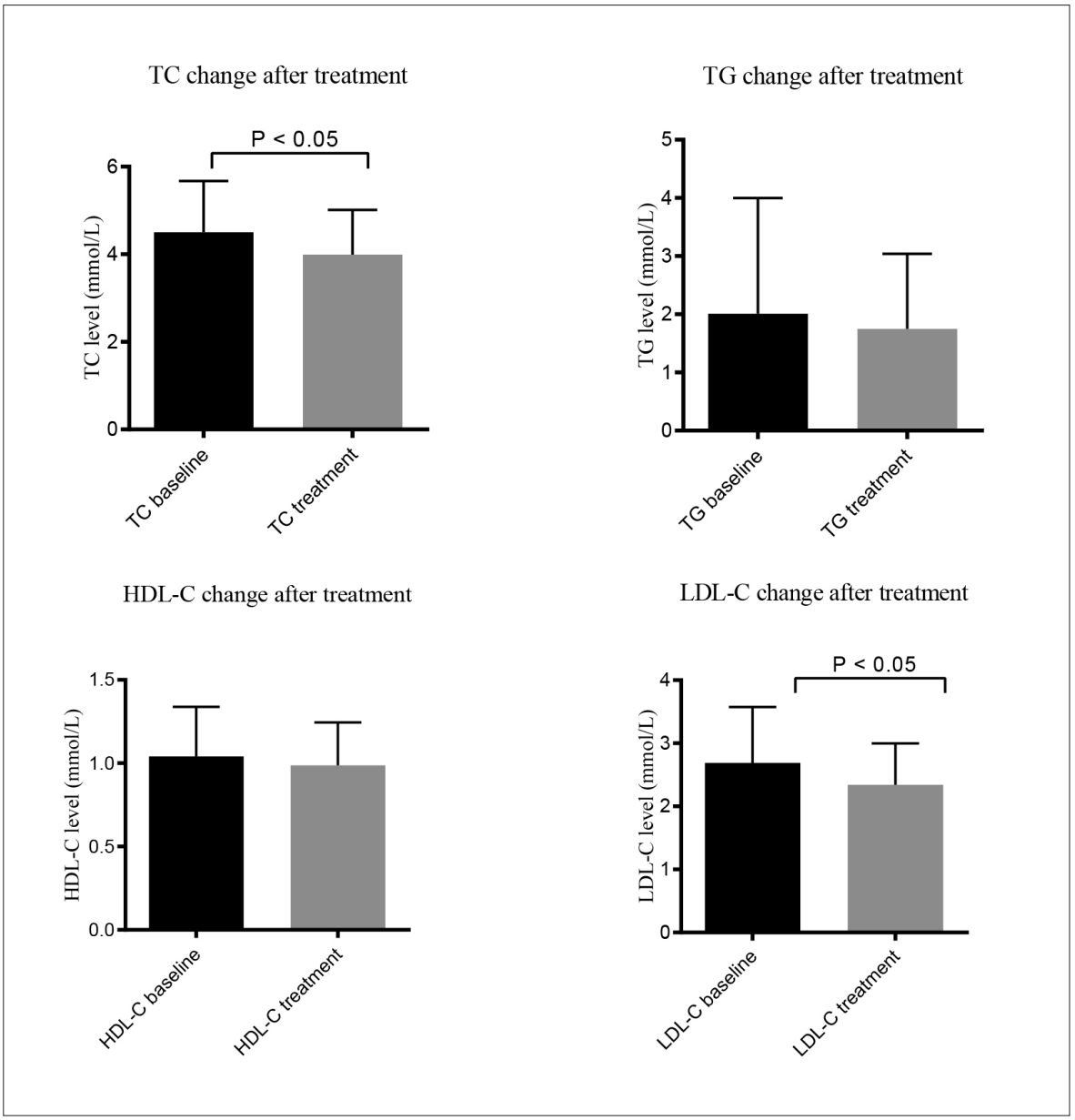

Fig. 2. Comparison of lipid levels at baseline and after treatment with atorvastatin.
HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.

APOE ε4 genotype could increase the affinity for binding to LDL receptors, resulting in decreased uptake of LDL-C and increased levels of circulating plasma cholesterol.[2] Differences in the levels of blood lipids, induced by genetic polymorphisms, increase the risk factors for coronary heart disease among ε4 carriers compared to those carrying other alleles.[17] Studies have shown that ε4 increases the plasma levels of TC and LDL-C, as well as the risk for atherosclerosis.[18,19] Moreover, ε4 carriers have a 40% higher risk of developing ASCVD where treatment with statins is often ineffective, whereas ε2 carriers have been reported to exhibit a higher lipid-lowering effect with statins.[20,21]

At 1 month follow-up of our patients, we observed that the levels of TC and LDL-C were significantly lowered with the same dose of statins, although there were no significant differences between the TG and HDL groups. Therefore, the advantages of statin in lowering cholesterol were consistent with those previously reported.[22] Importantly, a comparison of the lipid-lowering effect among genotypes revealed that

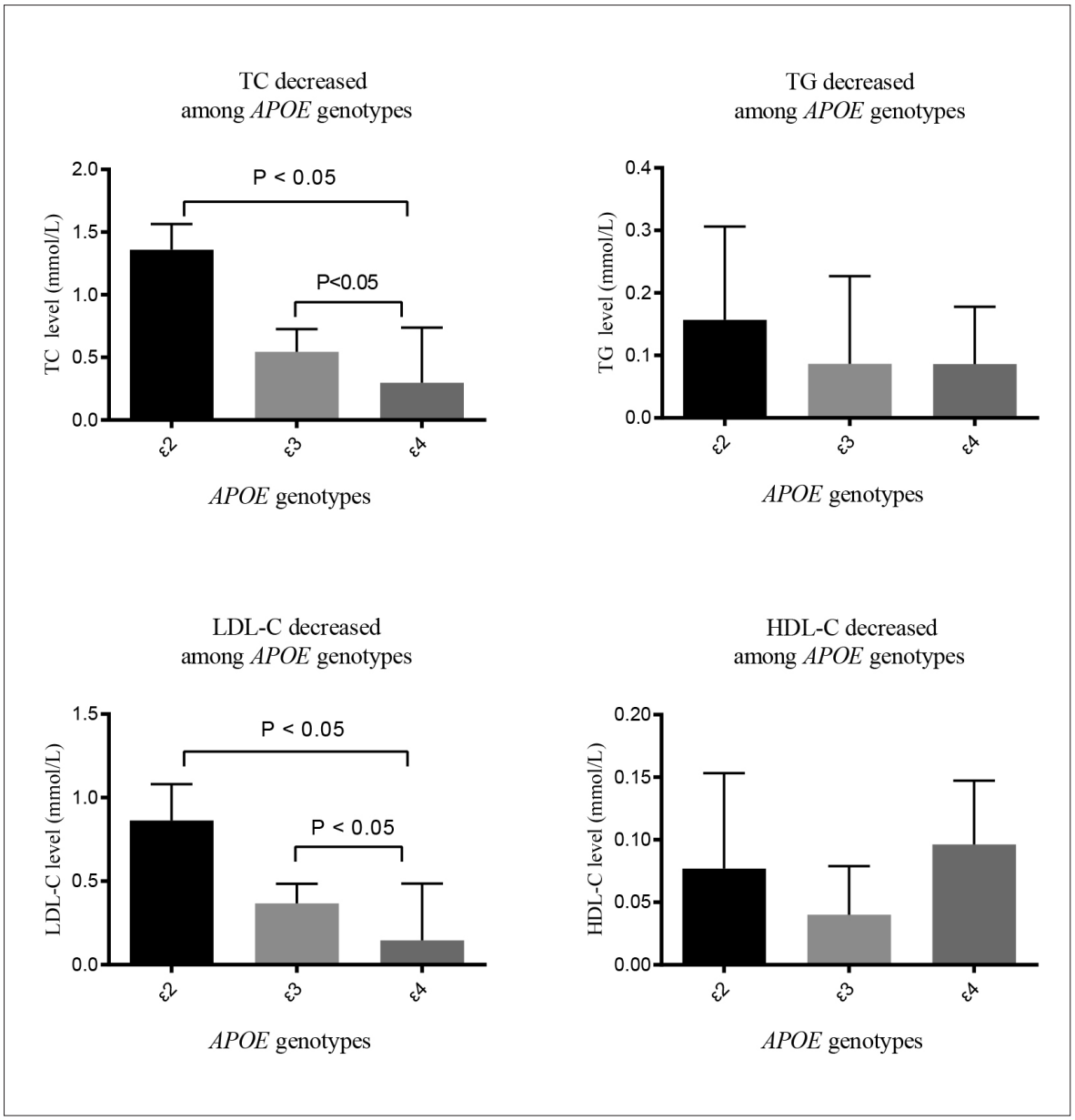

Fig. 3. Reduction of lipid levels among *APOE* genotypes with the use of atorvastatin.
HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.

ε4 carriers exhibited the smallest decrease in the levels of LDL-C and TC, consistent with findings of previous studies where the ε4 allele seemed to have an inadequate response to statin therapy compared to carriers of other alleles.[23] Age, gender and diseases such as diabetes have been reported as factors that influenced the effectiveness of statins.[24] In our study, baseline characteristics such as age, gender and diseases were similarly distributed among *APOE* genotypes, which may reduce the factors influencing the lipid-lowering efficacy of statins to an extent. Our study showed that the *APOE* ε4 allele was associated with a poor response to treatment with atorvastatin, while individuals carrying the *APOE* ε2 allele appeared to have a higher cholesterol-lowering effect from it.

CONCLUSION

The current study revealed effects of *APOE* genotypes on the lipid-lowering response of statins in patients. Due to the heterogeneity of our study population, follow-up studies should involve a larger sample size and consider the effects of age, race, drug dosage,

treatment duration, environment, and statistical method used. The results of our study suggest the lipid-lowering effect of a standard-dose atorvastatin may be insufficient for *APOE* ε4 carriers. Further studies on *APOE* genotypes and its role in lipid-lowering effect of statins is of clinical importance.

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REFERENCES

1. Lamprecht DG Jr, Shaw PB, King JB, et al. Trends in high-intensity statin use and low-density lipoprotein cholesterol control among patients enrolled in a clinical pharmacy cardiac risk service. J Clin Lipidol 2018;12:999-1007.
2. Adhyaru BB, Jacobson TA. Safety and efficacy of statin therapy. Nat Rev Cardiol 2018;15:757-69.
3. Karjalainen JP, Mononen N, Hutri-Kähönen N, et al. New evidence from plasma ceramides links apoE polymorphism to greater risk of coronary artery disease in Finnish adults. J Lipid Res 2019;60:1622-9.
4. M Teterina, A Geraskin, P Potapov, et al. The impact of APOC3 and APOE gene polymorphisms on response to statin therapy in acute myocardial infarction. Eur Heart J 2019;40:430.
5. Kirac D, Bayam E, Dagdelen M, et al. HMGCR and ApoE mutations may cause different responses to lipid lowering statin therapy. Cell Mol Biol (Noisy-le-grand) 2017;63:43-8.
6. Marais AD. Apolipoprotein E in lipoprotein metabolism, health and cardiovascular disease. Pathology 2019;51:165-76.
7. Raman S, Brookhouser N, Brafman DA. Using human induced pluripotent stem cells (hiPSCs) to investigate the mechanisms by which Apolipoprotein E (APOE) contributes to Alzheimer's disease (AD) risk. Neurobiol Dis 2020;138:104788.
8. Tudorache IF, Trusca VG, Gafencu AV. Apolipoprotein E—A multifunctional protein with implications in various pathologies as a result of its structural features. Comput Struct Biotechnol J 2017;15:359-65.
9. James LM, Engdahl BE, Georgopoulos AP. Apolipoprotein E: the resilience gene. Exp Brain Res 2017;235:1853-59.
10. Dose J, Huebbe P, Nebel A, et al. APOE genotype and stress response—a mini review. Lipids Health Dis 2016;15:121.
11. Wolters FJ, Yang Q, Biggs ML, et al. The impact of APOE genotype on survival: Results of 38,537 participants from six population-based cohorts (E2-CHARGE). PLoS One 2019; 14:e0219668.
12. Tuomas Kerola, Terho Lehtimäki, Mika Kähönen, et al. Statin pharmacogenomics: lipid response and cardiovascular outcomes. Current Cardiovascular Risk Reports 2010;4:150-8.
13. Bousoula E, Kolovou V, Perrea D, et al. Pharmacogenetics and statin treatment: reality or theory? Curr Vasc Pharmacol 2015; 13:616-23.
14. Zhang L, He S, Li Z, et al. Apolipoprotein E polymorphisms contribute to statin response in Chinese ASCVD patients with dyslipidemia. Lipids Health Dis 2019;18:129.
15. Han S, Xu Y, Gao M, et al. Serum apolipoprotein E concentration and polymorphism influence serum lipid levels in Chinese Shandong Han population. Medicine (Baltimore) 2016;95:e5639.
16. Wanmasae S, Sirintronsopon W, Porntadavity S, et al. The effect of APOE, CETP, and PCSK9 polymorphisms on simvastatin response in Thai hypercholesterolemic patients. Cardiovasc Ther 2017;35.
17. Satizabal CL, Samieri C, Davis-Plourde KL, et al. APOE and the association of fatty acids with the risk of stroke, coronary heart disease, and mortality. Stroke 2018;49:2822-9.
18. Lei Y, Yang G, Hu L, et al. Increased dipeptidyl peptidase-4 accelerates diet-related vascular aging and atherosclerosis in ApoE-deficient mice under chronic stress. Int J Cardiol 2017; 243:413-20.
19. Pereira LC, Nascimento JCR, Rêgo JMC, et al. Apolipoprotein E, periodontal disease and the risk for atherosclerosis: a review. Arch Oral Biol 2019;98:204-12.
20. Nawabi A, Yang M, Cai X, et al. Association of apolipoprotein E gene polymorphism with lipid profile in patients with acute coronary syndrome in Han Chinese: A critical review. World J Cardiovasc Dis 2019;9:825-45.
21. Konialis C, Spengos K, Iliopoulos P, et al. The APOE E4 allele confers increased risk of ischemic stroke among Greek carriers. Adv Clin Exp Med 2016;25:471-8.
22. Wong MWK, Braidy N, Crawford J, et al. APOE genotype differentially modulates plasma lipids in healthy older individuals, with relevance to brain health. J Alzheimers Dis 2019;72:703-16.
23. Zhang L, He S, Li Z, et al. Apolipoprotein E polymorphisms contribute to statin response in Chinese ASCVD patients with dyslipidemia. Lipids Health Dis 2019;18:129.
24. Karlson BW, Palmer MK, Nicholls SJ, et al. Effects of age, gender and statin dose on lipid levels: results from the VOYAGER meta-analysis database. Atherosclerosis 2017;265:54-9.