Am J Transl Res 2021;13(6):6772-6777
www.ajtr.org /ISSN:1943-8141/AJTR0130391

Original Article

The relationship between ApoE gene polymorphism and the efficacy of statins controlling hyperlipidemia

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Received January 19, 2021; Accepted February 21, 2021; Epub June 15, 2021; Published June 30, 2021

Abstract: Objective: To explore the relationship between ApoE gene polymorphism and clinical efficacy of statins on lipidemia. Methods: Peripheral venous blood was obtained from 220 patients with hyperlipidemia who were admitted to the outpatient department of our hospital. The potential relationship between ApoE gene polymorphism and clinical effect of statins was analyzed. Results: In the three isomers (E2, E3, E4) of ApoE, expression level of ApoE protein in ApoE4 gene carriers was significantly different from that in E2 or E3 gene carriers (both $P<0.05$). At the same time, both the decrease rate of total cholesterol (TC) in blood lipid and low density lipoprotein cholesterol (LDL-C) and the rise rate of high density lipoprotein cholesterol (HDL-C) in ApoE4 carriers after taking statins were much lower than those in non-ApoE4 patients ($P<0.05$). Conclusion: ApoE gene polymorphism is associated with hyperlipidemia and has certain influence on the clinical efficacy of statins in treatment of hyperlipidemia.

Keywords: ApoE4, gene polymorphism, statins, clinical efficacy, hyperlipidemia

Introduction

As the carrier of cholesterol, human apolipoprotein E (ApoE) plays an important role in the process of lipid metabolism [1, 2]. Statins have been widely used for nearly 20 years as a classic drug to regulate lipid metabolism in clinical practice through lowering cholesterol and reducing the incidence of cardiovascular and cerebrovascular acute adverse events. However, in clinical practice, the dosage of statins for lipid-lowering effect has obvious individual differences. For example, the dosage taken by white people is twice that of Chinese people [3-6]. Therefore, the study of pharmacogenomics is of great significance to further improve the therapeutic effect of statins. However, different studies have different conclusions about the effects of ApoE polymorphism on the lipid controlling effect of statins, and most previous studies were regional. Some literatures have pointed out that ApoE gene is not directly related to the clinical effects of statins, but there also existed conflicting research conclusions [7-9]. There were studies in China that confirmed that ApoE is related to the efficacy of statins, but the conclusions were not always unanimous [10, 11]. In this study, we explore the relationship between the ApoE gene polymorphism and the effect of statins on lipid metabolism, in order to provide more insights into the clinical effect of statins in the treatment of dyslipidemia.

Materials and methods

General materials

220 patients with hyperlipidemia who were admitted to the outpatient department of our hospital during June 2019 to June 2020 were selected as our research objects. Inclusion criteria: 1. Patients aged over 18 years; 2. The patients who suffered from cardiovascular and cerebrovascular diseases and are accompanied with hyperlipidemia after medical or surgical treatment that needs long-term lipid control; 3. Patients with good compliance; 4. The selected patients are not related to each other; 5. The patients without previous opioid

Table 1. Single nucleotide polymorphism (SNP) and changes of amino acids

SNP	Rs429358	Rs7412
Allele		
ε2	TGC	TGC
ε3	TGC	CGC
ε4	CGC	CGC
Gene phenotype (amino acid sites)	112	158
E2	Cys	Cys
E3	Cys	Arg
E4	Arg	Arg

Note: Cys: Cysteine; Arg: Arginine; SNP: Single nucleotide polymorphism.

medication history. Exclusion criteria: 1. Patients with severe hepatic and renal insufficiency; 2. Patients with severe hypo-function of heart and lung; 3. Patients with malignancy; 4. Patients who are related by blood; 5. Patients with poor control of diabetes; 6. Patients with endocrine dysfunction; 7. Patients with allergy to statins. All the subjects were informed of the study and have signed the letter of consent and this study was approved by the Ethics Committee of our hospital.

Methods

Usage and dosage of statins: Atorvastatin 10 mg/d (Specifications: 20 mg/tablet, Pfizer Pharmaceutical Co., Ltd., China) or Rosuvastatin 5 mg/d (AstraZeneca China Pharmaceutical Company, Specifications: 5 mg/tablet) was taken for at least one month (four weeks), which is a course of treatment. About 3-5 mL peripheral venous blood was added into anticoagulant tube, and then put the tube into centrifuge (LW450 × 1800) for centrifugation at 3000 r/min for 10 minutes. Then, the supernatant was separated and stored at -80°C. The serum ApoE protein level was detected by immunoturbidimetry. One month after taking the medicine, peripheral venous blood was drawn to check the blood lipid levels after fasting for 12 hours. The main indexes include total cholesterol, high-density lipoprotein and low-density lipoprotein.

Genotyping of ApoE: Whole blood genomic DNA of the selected patients was extracted using the whole blood DNA Extraction Kit (FireGen medical technology (Jiangsu) Co., Ltd., China) and was then amplified through polymerase chain reaction (PCR). The main primer sequences are listed as follows: primer 1 (5'-ACAGAATYCGCCGGGGGGCGTCGTAG-3), primer 2 (5'-TTAGCTTGCAGCGGCTGTACCACCG-3'). After amplification, the genotypes of ApoE were detected by Sanger method (including three types of homozygotes: E2/E2, E3/E3, E4/E4 and three types of heterozygote: E2/E3, E3/E4, E2/E4). See the **Table 1**.

Outcome measures

Main outcome measures: the effects of ApoE gene polymorphism on lipid-lowering effect of lipid-lowering drugs; Expression frequency of ApoE (E2, E3, E4) and peripheral blood volume of ApoE; Effects of statins in ApoE4 carriers and non-carriers.

Secondary outcome measures: clinical effects of different statins.

Statistics analysis

SPSS 22.0 statistical software was used for analysis. The measurement data were in accordance with normal distribution and expressed as mean ± standard deviation ($\bar{x}$ ± sd). The selected patients were divided into three groups according to the isomers of ApoE, and were also classified into the carrying group and the non-carrying group according to whether carrying ApoE4 or not. Independent sample t test was used for comparison between two groups and univariate analysis of variance combined with post LSD-t pairwise comparison was used for more than three groups. Whether lipid targets were achieved was taken as dependent variables, and the variables that showed between-group difference with $P<0.05$ were used as the independent variables for binary logistic regression analysis. Logistic regression analysis was carried out by conditional progressive forward method. The enumeration data were expressed as cases (n, %) and were compared by chi square test. $P<0.05$ means that the difference was statistically significant.

Results

The demographic characteristics of the subjects included in this study

The average age of the population included in this study was 64.8±5.5 years old, with more males than females. Other complications and medication profiles were shown in **Table 2**.

Table 2. Basic clinical information of samples in this study (($\bar{x}$ ± sd) or (n, %))

Clinical features	n=220
Age (mean; years)	64.8±5.5
Gender (male/female)	162/58
Smoking history (n)	131 (59.54)
Drinking history (n)	107 (48.63)
Diabetes (n)	82 (37.27)
Atorvastatin/Rosuvastatin (n)	119/101 (54.09/45.91)
Coronary disease (n)	89 (40.45)
Apoplexy (n)	74 (33.63)

Table 3. Genotyping frequency of ApoE genotypes (n, %)

Types	n (%)
E2/E2	6 (2.73)
E2/E3	18 (8.18)
E2/E4	11 (5.00)
E3/E3	128 (58.18)
E3/E4	47 (21.36)
E4/E4	10 (4.55)

Table 4. Comparison of serum levels of ApoE in different isomers of ApoE ($\bar{x}$ ± sd)

Isomers	Cases	ApoE (mg/L)	F	P
E2	35	44.9±2.0[a]	117.766	0.000
E3	193	42.1±1.9[b]		
E4	68	38.8±2.1		

Note: E2 vs. E4, [a]P<0.05; E3 vs. E4, [b]P<0.05.

Frequency of ApoE

The results showed that the frequency of E3/E3 was the highest and that of E2/E2 was the lowest in ApoE genotypes. See **Table 3** for details.

Comparison of serum levels of ApoE in different isomers of ApoE

The results of this study showed that the ApoE level of E4 carriers was the lowest and that of E2 carriers was the highest (all P<0.05), indicating that ApoE4 might be closely related to hypercholesterolemia. See **Table 4** for details.

Comparison of lipid-lowering effects of strains between ApoE4 carriers and non-ApoE4 carriers

The results showed that the reduction rate of TC, LDL-C and the increase rate of HDL-C in non-ApoE4 carriers were all much higher than those in ApoE4 carriers (P<0.05), preliminarily indicating that ApoE4 affects the clinical effect of statins. See **Figures 1-3** for details.

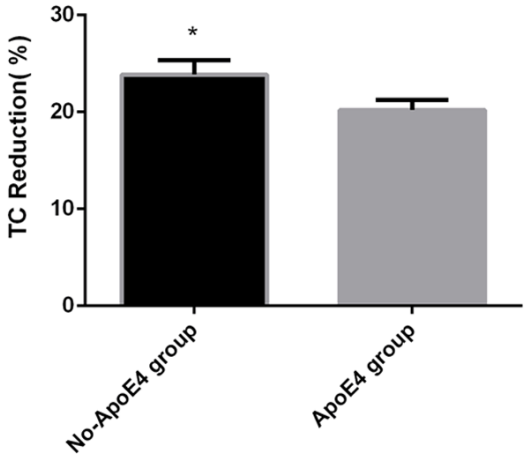

Figure 1. The reduction rate of TC. Compared with ApoE4 group, *P<0.05. TC: total cholesterol.

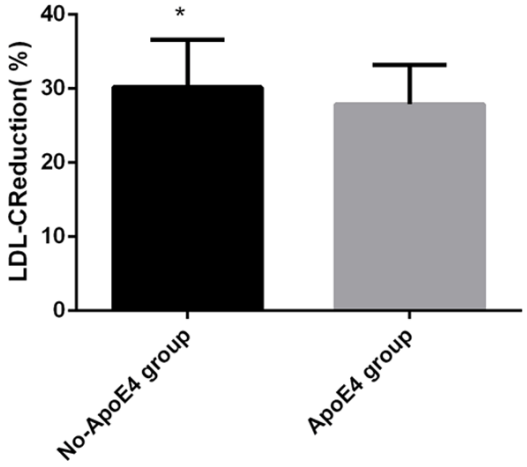

Figure 2. The reduction rate of LDL-C. Compared with ApoE4 group, *P<0.05. LDL-C: low density lipoprotein cholesterol.

Comparison about the effects of statins on blood lipid control

The results of this study showed that there was no significant difference between the effect of atorvastatin and rosuvastatin on clinical blood lipid control (P>0.05). See **Table 5** for details.

Effects of ApoE gene polymorphism on lipid regulation of statins

In this study, the influencing factors of lipid-lowering drugs were analyzed. A ≥25% decrease in

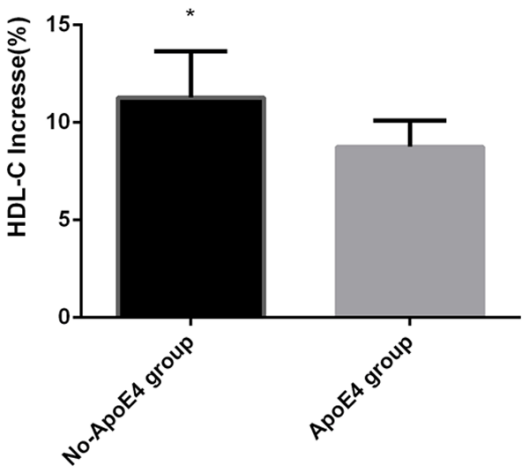

Figure 3. The increase rate of HDL-C. Compared with ApoE4 group, *P<0.05. HDL-C: high density lipoprotein cholesterol.

Table 5. Comparison about different clinical lipid regulating drugs of statins ($\bar{x}$ ± sd)

Groups	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Atorvastatin	3.79±0.54	1.97±0.32	1.03±0.21
Rosuvastatin	3.87±0.41	1.90±0.34	1.05±0.22
t	1.124	0.122	0.498
P	0.262	1.551	0.678

Note: TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.

LDL concentration was considered as effective and a <25% decrease was considered as invalid. The results showed that gender and drug type had no influence on the treatment effects of statins, while E4 gene carrying, smoking history and drinking history were the main influencing factors of clinical efficacy of statins. See **Tables 6**, **7** for details.

Discussion

Cardiovascular and cerebrovascular diseases remain the system diseases with the highest mortality in the world, and atherosclerosis is the one of the important cardiovascular and cerebrovascular diseases. The main pathophysiological changes of atherosclerosis are inflammatory activation and lipid deposition. Therefore, controlling of the lipid level is of great significance in the prevention and treatment of cardiovascular and cerebrovascular diseases [12, 13].

As the cornerstone of lipid-lowering drugs, statins have been in the forefront of clinical treatment and prevention of cardiovascular and cerebrovascular diseases [14, 15]. Statins mainly reduce the risk of cardiovascular and cerebrovascular events such as coronary syndrome and myocardial infarction through reducing the level of LDL. Statins inhibit the activity of HMG-CoA reductase by competitive selection and further suppress the synthesis of cholesterol, thus achieving effective clinical cholesterol lowering effect. At the same time, the expression of LDL receptor is up-regulated, which enhances the degradation of LDL by hepatocytes, and finally achieve the therapeutic effect of lipid-lowering drugs [16].

Studies have shown that statins mainly metabolize through cytochrome P450 enzymes, and the influencing factors of P450 drugs can all have an important impact on the clinical therapeutic effects of statins [17]. At present, studies have confirmed that ApoE is closely related to the metabolism of statins, which mainly affects the binding capacity of LDL receptor and its metabolism in liver, thus participating in lipid metabolism [18]. In addition, ApoE gene polymorphism has also been preliminarily confirmed to be associated with blood lipid levels. However, the previous research conclusions are inconsistent, which may be caused by the differences of race and region. Therefore, our present study explored the potential relationship between the clinical efficacy of statins and ApoE in this region, which is of great significance for improving the study of the whole population.

Gene analysis showed that two SNPs of ApoE gene rs7412 and rs429358 produced three alleles E2/E3/E4 and six genotypes. Different genotypes lead to the changes of corresponding amino acids and the quality of corresponding encoded proteins, which lead to individual differences in therapeutic effects of medicine [19]. Previous studies have confirmed that E3 gene is the most common gene among these alleles, and E4 gene carriers have a higher risk of cardiovascular and cerebrovascular diseases than others, which may be related to the influence of ApoE4 gene on ApoE metabolism [19]. The results of our study showed that the E3 genotype was the most commonly seen genotype in 220 subjects, which was in line with the epidemiological survey all over the

Table 6. Analysis of influencing factors of lipid regulating effect of statins (n)

Influencing factors	Effective	Invalid	χ^2	P
Male/female	110/41	52/17	0.695	0.154
Atorvastatin/rosuvastatin	79/40	65/36	0.099	0.752
E4 gene carrying	22	44	7.630	0.006
Smoking history	90	31	8.556	0.003
Drinking history	78	29	8.593	0.003
Coronary disease	44	35	1.139	0.286
Apoplexy	38	36	0.427	0.513

Table 7. Logistic analysis about influencing factors related to lipid regulating effect of statins

Influencing factors	β	P	OR	OR (95% CI)	
				Lower limit	Upper limit
E4 carriers	1.237	<0.001	3.448	1.219	4.677
Smoking history	0.698	0.024	2.011	1.258	2.934
Drinking history	0.856	0.031	2.353	1.06	2.451

Note: OR: odds ratio; CI: confidence interval.

world. It also showed that the apolipoprotein content in peripheral blood of ApoE4 gene carriers was lower than that of other genes [20, 21], which further confirmed the conclusions from previous literature that E4 gene was involved in the physiological process of lipid disorder [22].

In our present study, we analyzed the clinical effects of different ApoE genotypes and statins and the results showed that the ApoE4 gene carriers had both lower reduction rate of TG and LDL and lower increase rate of HDL than the non-ApoE4 gene carriers. The reason may be due to the fact that ApoE4 gene binds to its downstream receptor, which reduces the metabolism of apolipoprotein and the clearance efficiency of blood lipid, resulting in lipid residue in the body and ultimately affecting the clinical therapeutic effect of statins. At the same time, the logistic analysis also pointed out that ApoE4 was the influencing factor of clinical statins, which confirmed that different genotypes of ApoE gene (E4 gene reduced the clinical lipid-lowering effect) affected the clinical therapeutic effect of statins [23].

In conclusion, different subtypes of ApoE gene may affect the therapeutic effect of statins. However, the present study is a single center study with small sample size, which needs to be further confirmed by large-scale clinical trials of multi center cooperation. More importantly, a more comprehensive and accurate study of gene is the necessary prerequisite for translating the effect of E4 gene on lipid metabolism into clinical application. Finally, whether the effects of smoking and drinking history on the lipid-lowering efficacy of statins are related to ApoE4 gene needs further research.

Disclosure of conflict of interest

None.

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