Original Article

An analysis of the correlation between the human apolipoprotein E gene polymorphism and lipoprotein-associated phospholipase A2

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Contributions: (I) Conception and design: WG Yin; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: P Zhou, XH Zhou, YM Liu, C Chen, SX Xuan; (V) Data analysis and interpretation: B Cheng, JD Lin, LQ Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: Atherosclerosis is one of the most common cardiovascular and cerebrovascular diseases. This study aimed to explore the correlation between gene polymorphism of human apolipoprotein E (ApoE) and lipoprotein-associated phospholipase A2 (Lp-PLA2).

Methods: A total of 220 patients with atherosclerotic cardiovascular disease who were treated in our hospital from June 2016 to March 2017 were enrolled in this study and assigned as the atherosclerotic cardiovascular disease group and 193 patients who were treated contemporaneously in our hospital but had no atherosclerotic cardiovascular disease were enrolled and assigned as the control group. Gene polymorphism of ApoE was detected by PCR-fluorescent probe technique and the level of Lp-PLA2 was detected by ELISA.

Results: There were a total of 5 genotypes of ApoE in these two groups, which were E2/3, E3/3, E3/4, E2/4, and E4/4. E2/2 was not found in any of the patients. E3/3 made up the majority in both groups. There was no significant difference between the proportion of genotypes and frequencies of alleles in the two groups (P>0.05). There was no difference between LP-PLA2 among the different genotypes in these two groups (P>0.05).

Conclusions: We cannot conclude that ApoE gene polymorphism is related to atherosclerotic cardiovascular and cerebrovascular diseases. And it cannot be concluded that ApoE gene polymorphism is related to Lp-PLA2 level.

Keywords: Apolipoprotein E; gene polymorphism; lipoprotein-associated phospholipase A2; atherosclerosis; correlation

Submitted Jan 06, 2020. Accepted for publication Apr 02, 2020.
doi: 10.21037/cdt-20-43
View this article at: http://dx.doi.org/10.21037/cdt-20-43

Introduction

Atherosclerosis is one of the most common cardiovascular and cerebrovascular diseases (1). With the change of people's lifestyle, the mortality rate of atherosclerosis has increased yearly (2) and even if patients are treated in time, their quality of life will be greatly affected by the disease (3).

There are many causes of atherosclerosis, among which genes and heredity are important factors (4). Apolipoprotein E (ApoE) is the structural protein of many kinds of lipoproteins, it is particularly important to note that ApoE is one of the main components of low density

lipoprotein (LDL) (5), therefore, it plays an important role in the process of plasma lipid transport and metabolism and can affect the level of blood lipid and the formation of atherosclerotic lesions in varying degrees (6). The ApoE gene, located on chromosome 19, has obvious genetic polymorphism (7). There are three isomers of ApoE: E2, E3, and E4, which are encoded by three alleles: ε 2, ε 3, and ε 4 that are located at a single locus on chromosome 19. ApoE has six phenotypes, namely three homozygotes (E2/2, E3/3, E4/4) and three heterozygotes (E2/3, E2/4, E3/4) and the molecular basis is the mutual substitution of cysteine and arginine: ε 2 contains two cysteines, ε 3 is due to the replacement of one of the cysteines by arginine, ε 4 is due to the replacement of both the cysteines by arginines (8). These three isomers and six phenotypes of ApoE affect lipid metabolism due to different affinities to receptors, which accordingly affect the level of blood lipid and the formation of atherosclerotic lesions in varying degrees (9).

Plasma lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet activating factor acetylhydrolase (PAF-AH), is mainly synthesized and secreted by the inflammatory cells in atherosclerotic plaque, such as mature macrophages and lymphocytes, 80% of which is bound to LDL (10). Lp-PLA2 can oxidize and modify LDL by removing oxidized phosphatidylcholine on the surface of LDL (11), thereby produces lysophosphatidylcholine (lyso-PC) that can promote a series of endothelial inflammatory reactions (12). A previous study revealed that, as a new inflammatory marker, Lp-PLA2 was involved in all stages of atherosclerotic plaque formation (13).

Although many studies have respectively revealed that ApoE and Lp-PLA2 are involved in the occurrence and development of atherosclerosis, there are few studies on the association between ApoE gene polymorphism and Lp-PLA2, which, however, was analyzed in the present study.

Methods

Study subjects

A total of 413 patients who visited the People's Hospital in Qingyuan City from June 2016 to June 2019 were randomly selected. There were 220 patients in the atherosclerotic cardiovascular and cerebrovascular disease group (experimental group). The inclusion criteria were patients with atheromatous plaques, including cerebral infarction, cerebrovascular accident, stroke, coronary heart disease or other types of atherosclerotic cardiovascular and cerebrovascular diseases. The exclusion criteria were patients with cardiac insufficiency. Among these patients, 130 were male and 90 were female and the age of these patients ranged within 29–91 years old, with an average age of 66.8±11.8 years old. There were 193 patients in the non-atherosclerotic cardiovascular and cerebrovascular disease group (control group) and all these patients came from the outpatients in the same period as that of the atherosclerotic cardio-cerebrovascular disease group. These patients had no stroke confirmed by computed tomography (CT)/magnetic resonance imaging (MRI). Among these patients, 107 were male and 86 were female and the age of these patients ranged within 30–93 years old, with an average age of 58.0±13.6 years old. This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of The Sixth Affiliated Hospital of Guangzhou Medical University & Qingyuan People's Hospital. All patients provided signed informed consent.

Study methods

Specimen collection

At 12–14 hours after fasting, 4 mL of the elbow vein blood was withdrawn and sub-packed, 2 mL of it was placed in an EDTA anticoagulation tube and centrifuged at 1,500×g for 10 minutes, then the supernatant was collected and sent for testing for Lp-PLA2; the remaining 2 mL of it was placed in an EDTA anticoagulation tube and preserved at –20 ℃ for detecting ApoE genotypes.

Detection of ApoE gene polymorphism

A Lab-Aid 824 nucleic acid extraction Mini kit (Xiamen Zeesan Biotechnology Co., Ltd.) was used to extract DNA according to the reagent instructions. An ApoE gene detection kit (Wuhan Youzhiyou Medical Technology Co., Ltd.) was used to amplify the target gene on a Cfx-96 fluorescence quantitative polymerase chain reaction (PCR) instrument (bole, USA) and detect its fluorescence intensity. Primer sequences: upstream primer: 5'-ACAGAATTCGCCGGCCCTGGTAC-3', downstream primer: 5'-TAAGCTT'GGCACGCZTGTCCAAGGA-3'. The reaction system included: PCR buffer, dNTPs, specific primers and probes, internal standard primers and probes, Taq enzyme, UNG enzyme and the total volume was 25 μL. The reaction conditions of cDNA amplification via PCR were as follows: treated with Uracil-N-glycosylase (UNG) at 37 ℃ for 10 minutes, then pre-denatured at 95 ℃ for 5 minutes and at 95 ℃ for 15 seconds, then at 60 ℃ for

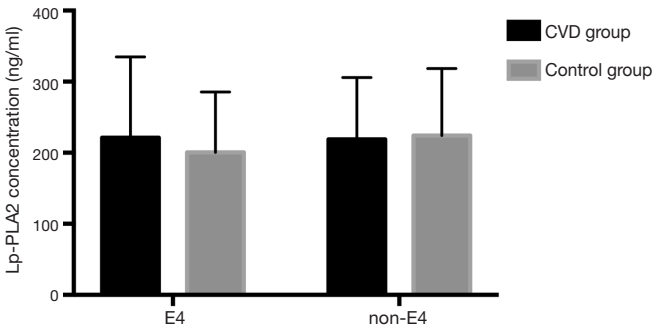

Figure 1 Comparison of Lp-PLA2 concentration distribution between ApoE E4 and non- E4 in the experimental group and the control group ($\bar{x}$±s, ng/mL).

60 seconds, and the fluorescence signal was collected at the end of this stage, a total of 40 cycles were performed. Finally, the CFX Manager software was used to analyze the fluorescence intensity curve.

Detection of the plasma Lp-PLA2 level

An Lp-PLA2 quantitative assay kit for human plasma was used and the level of Lp-PLA2 in plasma was detected by enzyme-linked immunosorbent assay (ELISA). The normal reference values are as follows: <175 ng/mL is normal and >175 ng/mL is abnormal (14).

Analysis of Lp-PLA2 concentration distribution difference between ApoE E4 and non-E4 in both experimental group and control group

The phase diagram of Lp-PLA2 concentration distribution between ApoE E4 and non-E4 in the experimental and control group is presented in *Figure 1*. In the control group, the median of Lp-PLA2 in patients with allele E4 was 121.56 ng/mL and the median of Lp-PLA2 in patients without allele E4 was 198.5 ng/mL, the difference was not statistically significant (t=–0.97, P=0.3356); in the experimental group, the median of Lp-PLA2 in patients with allele E4 was 182.3 ng/mL and the median of Lp-PLA2 in patients without allele E4 was 186.4 ng/mL, the difference was not statistically significant (t=–0.16, P=0.8707).

Statistical analysis

All data were processed using statistical software SPSS 22.0. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± SD). The intergroup comparison was conducted using an analysis of variance. The comparison of genotype proportion and allele frequency between the groups was conducted using an χ^2 test.

Results

Comparison of ApoE genotype and allele between the experimental group and the control group

As shown in *Figure 2*, in the experimental group, the frequency of ApoE genotype distribution was E3/3 (66.36%) > E3/4 (19.09%) > E2/3 (9.55%) > E2/4 (3.64%) > E4/4 (0.9%) > E2/2 (0.45%); and in the control group, it was E3/3 (64.77%) > E3/4 (16.58%) > E2/3 (14.51) > E2/4 (1.16%) > E4/4 (0.52%). The E3/3 genotype was the most common in both groups. The frequencies of ApoE alleles were 80.68% and 80.31% (ε 3), 12.27% and 10.62% (ε 4), and 7.05% and 9.07% (ε 2) in the experimental and control groups. The difference in genotype proportion (χ^2=329, P=0.604) and allele frequency (χ^2=1.559, P=0.459) between the two groups were not statistically significant.

Comparison of the Lp-PLA2 level among patients with different genotypes in the experimental group

As shown in *Figure 3*, the level of Lp-PLA2 in patients with different genotypes in the experimental group was as follows: 223.1±96.7 ng/mL (E2/3), 218.6±86.0 ng/mL (E3/3), E3/4 227.2±118.2 ng/mL (E3/4), 187.6±103.6 ng/mL (E2/4), and 230.6±6.9 ng/mL (E4/4), respectively. An analysis of variance revealed that the difference in the Lp-PLA2 level among the genotypes was not statistically significant (P>0.05).

Comparison of the Lp-PLA2 level in patients with different genotypes in the control group

As shown in *Figure 3*, in control group, the level of Lp-PLA2 was as follows: 194.8±100.5 ng/mL (E2/3), 231.4±92.3 ng/mL (E3/3), 208.8±90.5 ng/mL (E3/4), and 159.0±32.5 ng/mL (E2/4), respectively and no patient with E4/4 was found. An analysis of variance revealed that the difference in the Lp-PLA2 level among the genotypes was not statistically significant (P>0.05).

Discussion

In view of the fact that the Lp-PLA2 may be related to the

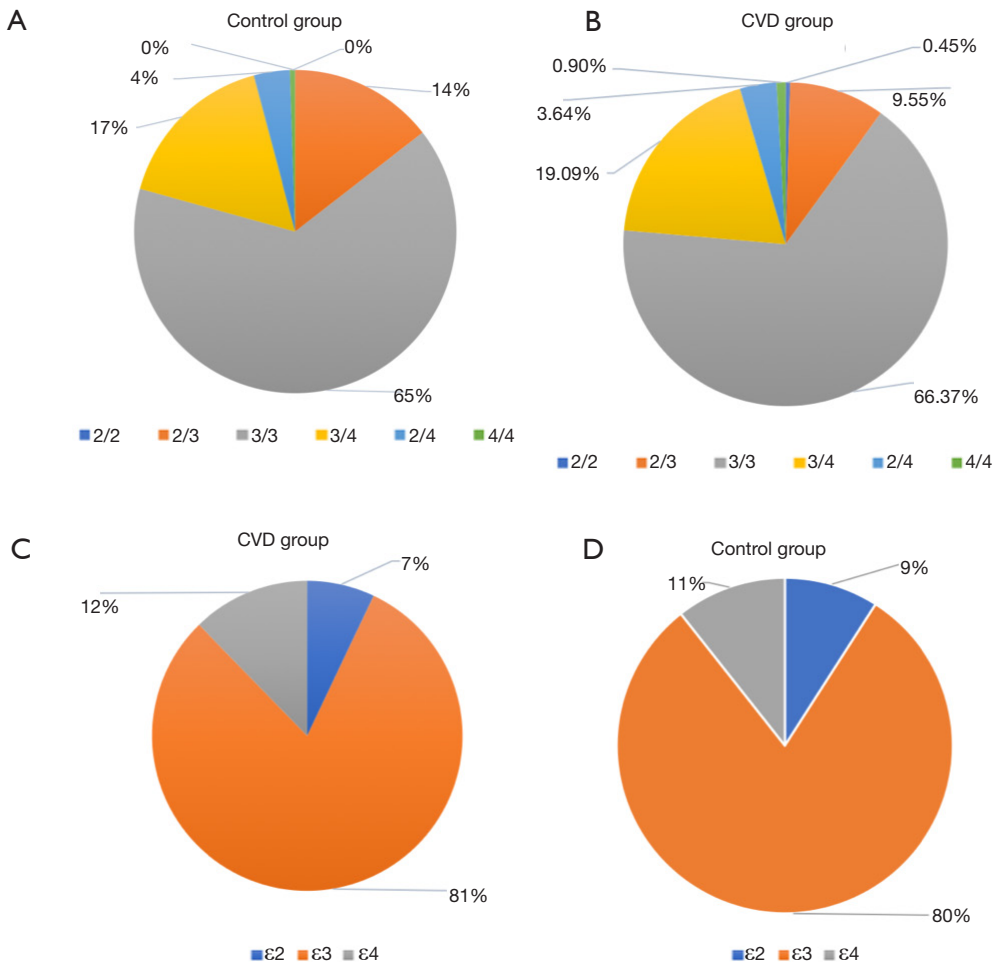

Figure 2 Comparison of ApoE genotype and allele between the experimental group and the control group (%). (A) ApoE genotype in Control group; (B) ApoE genotype in CVD group; (C) ApoE allele in CVD group; (D) ApoE allele in Control group.

formation of atherosclerosis, the purpose of this study was to determine whether ApoE gene polymorphism affects the plasma level of Lp-PLA2, in order to figure out the role of Lp-PLA2 in AS and to provide a reference basis for clinical diagnosis and treatment. A previous study revealed that the distribution of six genotypes of the ApoE gene was uneven in a normal population, the distribution frequency of E3/3 was the highest in the general population, the frequency was mostly more than 60%, the heterozygotes (E2/3, E3/4) with ApoE3 was in the middle, accounting for approximately 30%, the frequency of E2/2, E4/4 and E2/4 was the lowest, and the sum of the three was generally no more than 10%, while the most common allele in the population was ε 3 (15). The results of the present study revealed that in the experimental and control group, the frequency of ApoE3/3 genotype was the highest, accounting for 66.36% and 64.77%, respectively and the frequency of ε3 was also the highest in the alleles of ApoE, accounting for 80.68% and 80.31%, respectively. These findings are consistent with the results of other epidemiological studies.

In the present study, ApoE gene polymorphism was detected in patients in the experimental and control group. The investigators revealed, in comparison, that the differences in gene composition and gene frequency between the two groups were not statistically significant (P>0.05) and could, therefore, not conclude that ApoE gene polymorphism is correlated to atherosclerotic cardiovascular and cerebrovascular diseases. The causes of the results were analyzed and the investigators considered that the reason may be that the most common allele in the population is

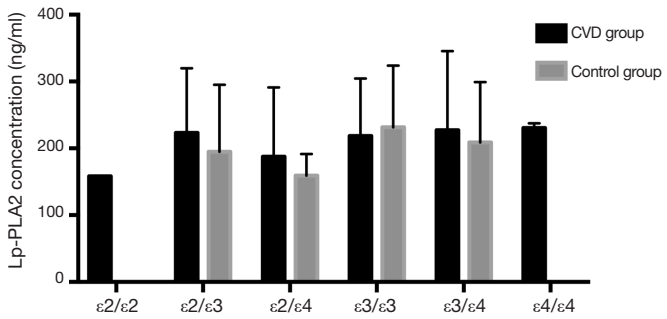

Figure 3 Comparison of the Lp-PLA2 level among patients with different genotypes in the experimental group and the control group ($\bar{x}$±s, ng/mL).

ε 3, however, the allele ε 4 causing atherosclerosis and ε 2 with an anti-atherosclerosis function has no significant distribution (15). Therefore, it is very difficult to get different results under the limited conditions of the small sample in this study, suggesting that larger patient samples are needed for analysis in the future to get a clearer conclusion.

Currently, there's no consensus on the role of Lp-PLA2 in AS. Its effects on AS plaques may depend on the type of lipoprotein particles (16,17). In the present study, the plasma level of Lp-PLA2 was also measured. The results revealed that, in both groups, the difference in the level of Lp-PLA2 among different genotypes was not statistically significant (P>0.05), that is to say, it could not conclude that the ApoE gene polymorphism is correlated to Lp-PLA2 level. Previous studies have revealed that Lp-PLA2 released into blood circulation mainly bound to apolipoprotein B-rich lipoproteins and LDL accounted for 80% of them (16,18,19). The level of Lp-PLA2 was positively correlated with the level of LDL subfractions in patients with atherosclerotic diseases (20,21). However, the present study suggests that ApoE gene polymorphism is not necessarily correlated to the Lp-PLA2 level. Therefore, the level of Lp-PLA2 may be correlated to the polymorphism of other components in LDL, such as apolipoprotein B. Since the sample size of this study was small and the individual difference in gene polymorphism is relatively large, a greater patient sample is needed for analysis in the future to get a clearer conclusion.

Conclusions

The differences in gene composition and gene frequency of ApoE gene polymorphism between patients with atherosclerotic and non-atherosclerotic cardiovascular and cerebrovascular diseases were not statistically significant. Therefore, this study could not conclude that ApoE gene polymorphism is correlated to atherosclerotic cardiovascular and cerebrovascular diseases. In both groups the difference in the level of Lp-PLA2 among different genotypes was also not statistically significant, therefore, this study could not conclude that ApoE gene polymorphism is correlated to Lp-PLA2 level.

Acknowledgments

Funding: This work was supported by Qingyuan Industrial Technology Research and Development Special Funds (No.2017A021) and Natural Science Foundation of Guangdong Province (No.2018A030307065).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at: http://dx.doi.org/10.21037/cdt-20-43). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of The Sixth Affiliated Hospital of Guangzhou Medical University & Qingyuan People's Hospital. All patients provided signed informed consent.

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Cite this article as: Yin WG, Zhou P, Zhou XH, Liu YM, Chen C, Xuan SX, Cheng B, Lin JD, Xu LQ. An analysis of the correlation between the human apolipoprotein E gene polymorphism and lipoprotein-associated phospholipase A2. Cardiovasc Diagn Ther 2020;10(3):520-525. doi: 10.21037/cdt-20-43

