Association of ApoE Gene Polymorphisms With Cardio-cerebrovascular Complications in Type 2 Diabetes Mellitus in the Chinese Population

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Research article

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Abstract

Objective: To analyze and study the relationship between ApoE gene polymorphism and cardio-cerebrovascular complications in type 2 diabetes mellitus(T2DM) in the Chinese Population.

Methods: From January 2018 to January 2019, 1140 patients with type 2 diabetes admitted to the Department of Endocrinology, the Affiliated Hospital of Xuzhou Medical University were selected as the case group, including 590 patients with coronary heart disease(CHD) and 550 patients with cerebral infarction(CI), and 1198 patients with type 2 diabetes without complications during the same period were selected as the control group. General baseline data of the two groups were collected, such as gender, age, course of disease, lipid profile, HbA1C, BMI, blood pressure, carotid plaque and complications. ApoE genotypes were identified in all participants who participated in the study.

Results: This study showed that the ApoE genotypes in both the case group and the control group had the highest frequency of E3/E3. The E3/E4 genotype frequency and E4 allele frequency in the case group were higher than those in the control group (P<0.05). In the case group, the frequency of E2/E3 and E3/E4 genotypes of CI group was lower than that of CHD group, while the frequency of E3/E3 genotype was higher than that of CHD group. TC and LDL-c levels were significantly increased in patients with ApoE E3/E4 genotype(P<0.05). ApoE genotype E3/E4 was more associated with carotid plaque than E2/E3. ApoE genotype and ApoE allele were positively correlated with TC and LDL-c levels (P<0.05).Logistic regression results show that ApoE gene polymorphism is associated with cardio-cerebrovascular complications in T2DM patients. ApoE E3/E4 genotype and allele E4 may be risk factors for T2DM patients with cardio-cerebrovascular complications.

Conclusion: ApoE E3/E4 genotypes and T2DM patients carrying E4 allele have a higher risk of cardio-cerebrovascular complications than other genotypes. ApoE E2 allele has a certain protective effect , however E4 allele may be a risk factor for cardio-cerebrovascular complications in T2DM patients, and its mechanism may be related to the effect of ApoE gene on lipid metabolism.

Introduction

The global incidence of T2DM has increased rapidly in recent years due to increased life expectancy in developing countries, rising rates of obesity and westernized lifestyles. However, chronic complications of T2DM are a major cause of morbidity and mortality [1, 2]. Dyslipidemia or lipoprotein abnormality may aggravate microvascular and macrovascular complications in T2DM patients and promote atherosclerosis [3, 4]. As an important component of plasma lipoprotein, apolipoprotein plays an extremely important role in the metabolism of plasma lipoprotein, so there is a close relationship between apolipoprotein and T2DM. Apolipoprotein E (ApoE) is one of the important apolipoproteins involved in the metabolism of body lipids and the regulation of body lipids in human body. Its gene polymorphism is closely related to the level of body lipids [5]. This paper analyzed the ApoE genotype of T2DM patients with CI and CHD, and compared the blood lipid levels of patients with different ApoE phenotypes to explore the role of ApoE gene polymorphism in the occurrence and development of cardio-cerebrovascular complications of T2DM.

1. Materials And Methods

1.1 General information T2DM patients admitted to the Department of Endocrinology, the Affiliated Hospital of Xuzhou Medical University from January 2018 to January 2019 were collected. 1140 patients were selected as clinical subjects and set as case group, including 550 patients with CHD and 590 patients with CI. The related data were presented in table 1.There was no significant difference in gender and age between the two groups (P>0.05). General baseline data of the two groups were collected, such as course of disease, lipid profile, HbA1C, BMI, blood pressure, carotid plaque and complications. All subjects signed informed consent forms.

1.2 Inclusion criteria and Exclusion criteria The diagnosis of patients in the cerebral infarction group was in line with the standards of the Fourth National Conference on cerebrovascular diseases, which were confirmed by CT or MRI. According to the results of selective coronary angiography, at least two interventional physicians confirmed that the inner diameter of the main vascular lumen stenosis was ≥50%. All patients in the case group and the control group were excluded from patients with two or more cardio-cerebrovascular diseases, severe liver and kidney diseases, blood diseases, severe infection, thyroid diseases and malignant tumors, and all subjects did not take lipid-lowering drugs.

1.3 Principles of ApoE genotyping detection Genomic DNA was extracted from each blood sample by using a QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions, and DNA concentration was quantified by using a NanoDrop 2000™ spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). PCR was performed according to the following protocol: 50°C for two

minutes, pre-denaturation at 95℃ for 10minutes, followed by 40 cycles at 95℃ for 15 seconds and 64℃ for 1minute. Specific gene fragments amplified by PCR were hybridized with on-chip specific nucleic acid probes to detect specific gene locus sequences, including six ApoE gene types at sites 112 and 158 (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, E4/E4). The minimum detection limit of DNA was 5.0×10^3copies/ml, and $>5.0\times10^3$copies/ml was positive for this site[6].

1.4 Instruments and Reagents

1.4.1 Determination of lipid profile and ApoE genotyping Fasting blood was collected early in the morning on the second day of admission for both groups. 2ml peripheral venous blood was collected with EDTA-K2 anticoagulant tube. After centrifugation, plasma was separated for ApoE genotyping.ApoE genotype detection kit (gene-chip assay) was purchased from Wuhan Youzhiyou Biotechnology Co., LTD., and the detection instrument was ABI7500 fluorescence quantitative PCR amplification instrument.Lipids include total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), apolipoprotein A (ApoA1), apolipoprotein B (ApoB). Beckman Coulter AU-5800 automatic biochemical analyzer was used for testing. All operations are strictly in accordance with the instructions[7, 8].

1.4.2 Carotid ultrasound EPIQ5 ultrasonic diagnostic instrument was used with probe frequency of 1 ~ 18MHz. Results and determination: carotid intimal thickness (IMT) was measured. Normal IMT: common carotid artery or its bifurcation IMT<1.0mm; Thickening of IMT: 1.0mm≤ common carotid artery or its bifurcation IMT<1.5mm; Plaque formation: IMT≥1.5mm in any carotid artery[9]. The 2338 patients who underwent carotid ultrasound were divided into four groups: normal group, thickening group, stable plaque group and unstable plaque group. Stable plaque group: smooth surface, homogeneous or strong echo in more than 50% of plaque area, followed by sound shadow. Unstable plaque group: surface not smooth,>50% plaque is heterogenous, low and irregular hypoechoic dark area or ulcerative plaque. If the subject has both stable and unstable plaques, they are classified as unstable plaques. All ultrasonic test results were determined by 2 professional ultrasound physicians.

1.5 Statistical methods The collected data and the clinical results have been statistically analyzed using IBM SPSS version 20.0 software. Quantitative data were expressed as mean values ± standard deviation (SD). Ranges and frequency of distributions were estimated for quantitative variables. Normally distributed data were compared using Student's *t* test for 2 groups and ANOVA test for more than 2 groups. The significance of differences between proportions was tested by the Chi square test (χ^2) or Fisher's exact tests. Correlation analysis of ApoE genotype/allele and blood lipid level using Spearman rank correlation analysis. Differences were considered significant with *p* value <0.05. Allele and genotype differences between groups and deviations from Hardy–Weinberg equilibrium were tested by Chi square test. Multivariate linear regression analysis was used to explore the risk factors of cardio-cerebrovascular complications of T2DM.

2. Results

2.1 General characteristics and biochemical variables of the study population

The course of diabetes, fasting blood glucose and HbA1C in the case group were significantly higher than those in the control group (*P* <0.05). The TC and LDL-c levels in the control group were lower than those in the case group, and the difference was statistically significant. The levels of TC and LDL-c in CHD group were higher than those in CI group (*P* <0.05).Demographic, clinical and biochemical data of enrolled subjects are summarized in Table 1.

2.2 Results of ApoE gene polymorphism detection

Six common ApoE genotypes E2/E2,E2/E3, E2/E4, E3/E3,E3/E4, E4/E4 and three alleles E2, E3 and E4 were detected by PCR-RFLP. Genotype E3/E3 was the most common (54.02%), followed by E3/E4 (20.53%), E2/E3 (19.63%), and E2/E4 was the least common (0.77%). E3 was the most common allele (74.10%), followed by E4 (13.15%) and E2 (12.75%).

2.3 ApoE genotype frequency and allele frequency distribution

Through H-W balance test, the distribution of ApoE genotype in the study subjects conforms to the law of genetic balance, indicating that the sample is representative (*P*>0.05). The results showed that the ApoE genotypes in both the case group and the control group had the highest frequency of E3/E3, while the frequencies of the remaining genotypes in the case group were E3/E4, E2/E3 and E2/E4 from high to low, while the frequencies of the remaining genotypes in the control group were E2/E3, E3/E4 and E2/E4. The ApoE allele frequency in the case group was E3, E4 and E2 from high to low, while the control group was E3, E2 and E4. There was a significant difference between the ApoE genotype and allele frequency between the case group and the control group. The E3/E4 genotype of the case group was significantly higher than that of the control group (*P*<0.05), while the E2/E3 genotype was significantly lower than that of the control group

(P<0.05). The E2 allele frequency in the case group was significantly lower than that in the control group (P<0.05), while the E4 allele frequency was significantly higher than the control group (P<0.05). In the case group, the frequency of E2/E3 and E3/E4 genotypes of CI group was lower than that of CHD group, while the frequency of E3/E3 genotype was higher than that of CHD group. Comparison of ApoE genotype and allele frequency in each group is presented in Table 2.

2.4 Relationship between ApoE gene polymorphism and lipid metabolism

Comparison of blood lipid levels in patients with different ApoE genotypes showed that the levels of TC, LDL-c and ApoB in patients with E3/E4 genotype were significantly higher than those of E2/E3, E2/E4, E3/E3 genotypes(P<0.05). The levels of TC, TG, LDL-c and ApoB in patients with E2/E4 and E3/E3 genotypes were higher than those with E2/E3 genotype(P<0.05). Comparison of serum lipid levels among ApoE genotypes is presented in table 3.

2.5 Relationship between ApoE genotype and carotid atherosclerotic plaque

Normal group and thickening group were classified as non-plaque group. Stable plaque group and unstable plaque group were classified as plaque group. The genotype frequency of ApoE E3/E4 in the plaque group was higher than that in the non-plaque group, while the frequency of E2/E3 and E2/E4 genotypes was lower than that in the non-plaque group (P<0.05).Relationship between ApoE genotype and carotid atherosclerotic plaque is presented in table 4.

2.6 Correlation analysis of ApoE genotype, allele and blood lipid level

As shown in Table 5, ApoE genotype and ApoE allele were positively correlated with TC and LDL-c levels (P<0.01).

2.7 Multivariate linear regression analysis of the related factors of cardio-cerebrovascular complications of T2DM.

Taking the occurrence of complications as the dependent variable, age, gender, diabetes duration, BMI, SBP, DBP, HbA1C, fasting plasma glucose, TG, TC, LDL-c, HDL-c, carotid plaque and ApoE genotype as independent variables for multiple linear regression analysis. The results show that carotid plaque, diabetes duration and ApoE E3/E4 genotype are independent risk factors (P < 0.05). The related data were presented in table 6.

3. Discussion

Chronic complications of T2DM are the main cause of death and disability of diabetes mellitus, and macrovascular complications are the most common complications of T2DM. Diabetic macrovascular disease mainly refers to cardiovascular, cerebrovascular and peripheral vascular diseases. The main cause of cardio-cerebrovascular complications is atherosclerosis, which is one of the most common causes of human death. To date, some studies have shown that the interaction between T2DM and cardiovascular risk supports the progressive development of vascular injury, which leads to atherosclerosis [10]. Studies have pointed out that ApoE genotype is the main influencing factor for the development of atherosclerosis [11, 12], and differences in ApoE genotype can lead to differences in individual pathogenesis[13]. Therefore, the study on ApoE genotype polymorphism has been paid more and more attention.

ApoE is involved in the regulation of lipid metabolism in the body through various ways, and is an important internal factor affecting the level of body lipid[14]. The most common alleles of ApoE gene are E2, E3, E4, and there are 6 different ApoE phenotypes in the population: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, E4/E4, among which E3/E3 is the most common phenotype [15]. Studies at home and abroad have shown that ApoE gene polymorphism conform to the laws of genetics, but there are certain ethnic and regional differences, and there are differences in susceptibility to cardio-cerebrovascular diseases among ApoE individuals with different genotypes [16, 17]. Results from previous studies suggest that ApoE E4 allele has a variable significance in terms of predicting the risk of vascular events in different populations. In Finnish population, E4-bearing genotypes associated with increased risk for macro and micro vascular complications in T2DM patients both in men and women, in contrast to E2 phenotype which somehow protected from macroangiopathy and associated with lower plasma TC and LDL-C concentrations and lower plasma lipoprotein (a) levels[18]. In contrast, the E4 allele was not found to influence the risk for cardiovascular disease in Italian diabetic patients and no significant differences among different genotypes were identified[19]. However, in another Italian study, ApoE E4 allele was reported as a risk factor for CAD and has been associated with low ApoE concentrations[20]. In Greek patients with CAD, there was no significant association between E4 allele and risk for CAD or myocardial infarction (MI), though a negative association of E2 allele with MI was observed[21]. Also, E4 allele was not associated with an increased risk for cardiovascular disease (CVD) or ischemic vascular event (IVE) among Greek patients with CVD[22].In this study, the ApoE genotype of patients with T2DM and patients with cardio-cerebrovascular complications in the Affiliated Hospital of Xuzhou Medical University were statistically analyzed. The results showed that the ApoE genotypes in both the case group and the control group had the highest frequency

of E3/E3. The frequencies of other genotypes in the case group were E3/E4, E2/E3 and E2/E4 from high to low, while those in the control group were E2/E3, E3/E4 and E2/E4. The comparison results between the case group and the control group showed that although the E3 allele frequency was the highest in both groups, the E2 and E4 allele frequency was significantly different, which showed that the E4 allele frequency in the case group was significantly higher than that in the control group, suggesting that E4 allele might be a risk factor for cardio-cerebrovascular diseases. The case group was divided into two types: coronary heart disease and cerebral infarction. ApoE is one of the important parameters for the occurrence of cardiovascular diseases[23]. Studies have suggested that ApoE E4 allele is an independent risk factor for T2DM and coronary heart disease [24], but the correlation between ApoE and cerebral infarction is controversial. Some studies have pointed out that ApoE E4 allele is the genetic marker of cerebral infarction[25, 26], and other studies have suggested that ApoE E3/E3 and E3/E4 genotypes have protective effects on cerebral infarction in Chinese males rather than females [27]. In fact, ApoE gene polymorphism can affect lipid metabolism in a variety of ways, thus promoting or delaying the occurrence of cardio-cerebrovascular diseases[28]. In order to further clarify the ApoE gene polymorphism may play a role in cardio-cerebrovascular disease, blood lipid levels in patients with different phenotypes of ApoE in this study were compared. The results showed that the TC and LDL-c levels of patients with ApoE E2 allele were significantly reduced, while the TC and LDL-c levels of patients with ApoE E4 allele were significantly increased, suggesting that in this study, ApoE E2 allele has a certain protective effect, and ApoE E4 allele may increase the incidence of cardio-cerebrovascular complications, which is basically consistent with the results of previous studies[24,29,30].

Atherosclerosis is an important risk factor for cardio-cerebrovascular diseases, and LDL-c is a key factor for the occurrence and development of atherosclerosis [31]. In this study, the frequency of ApoE E3/E4 genotype in plaque group was significantly higher than that in non-plaque group.This suggests that the correlation between ApoE and carotid atherosclerotic plaque may be caused by the influence of E3/E4 genotype on lipid LDL-c, which further leads to carotid atherosclerosis and plaque formation. It is consistent with other scholars research on ApoE E3/E4 genotype and carotid plaque[32, 33]. The results of this study showed: E3/E4 genotype and E4 allele frequency of the case group were higher than of the control group. Regression analysis showed that ApoE E3/E4 genotype was significantly correlated with cardio-cerebrovascular complications. This suggests that the ApoE E3/E4 genotype and T2DM patients carrying E4 allele have a higher risk of cardio-cerebrovascular complications than other genotypes. E4 allele may be a risk factor for cardio-cerebrovascular complications in T2DM patients, and its mechanism may be related to the effect of ApoE gene on lipid metabolism.

This study shows that ApoE gene polymorphism does affect lipid metabolism. ApoE E2 allele has a certain protective effect, however E4 allele may be a risk factor for cardio-cerebrovascular complications in T2DM patients. ApoE E4 allele and E3/E4 genotype are significantly associated with the occurrence of carotid plaque and cardio-cerebrovascular complications, which has certain guiding significance for the early identification and prevention of the risk of complications in T2DM patients. ApoE polymorphisms seem to be very good candidates in studying the interplay between genetic and acquired risk factors.However, cardio-cerebrovascular diseases is the outcome of combined action of multiple factors. In the follow-up research, multi-center research should be carried out to increase the sample size of the research subjects for in-depth research. Future large-scale studies involving patients that will elucidate the pathophysiological pathways of cardio-cerebrovascular complications may lead to new insights and treatments for diabetes.

Table 1 Demographic, clinical and biochemical data of the study population

	CHD	CI	Control
Sex(male/female)	280/270	300/290	600/598
Age(years)	65.20±6.93	67.73±9.47	64.71±7.26
SBP(mmHg)	129.95±14.18	134.96±14.92	129.07±14.68
DBP(mmHg)	79.50±9.96	81.81±8.73	81.35±7.75
Diabetes duration(years)	13.25±9.00	12.59±8.18	6.63±3.99*Δ
Fasting plasma glucose (mmol/L)	9.40±3.83	8.60±2.67	8.41±2.49*Δ
HbA1C(%)	8.88±2.41	8.83±2.16	8.70±2.07*Δ
TG(mmol/L)	1.97±0.93	2.14±1.01	1.80±1.26
TC(mmol/L)	5.16±1.42	4.56±1.21*	3.51±1.40*Δ
LDL-c(mmol/L)	3.04±1.11	2.58±1.02*	2.30±1.00*Δ
HDL-c(mmol/L)	1.18±0.29	1.12±0.30	1.20±0.35
ApoA1(g/L)	1.04±0.26	1.02±0.19	0.98±0.19
ApoB(g/L)	0.96±0.30	0.83±0.25	0.81±0.27
BMI(kg/m^2)	25.89±2.73	25.45±2.74	26.16±3.91

*vs CHD group , *P*<0.05 Δ vs CI group , *P*<0.05

SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c hemoglobin A1C,TG triglycerides, TC total cholesterol, LDL-c low density lipoprotein cholesterol, HDL-c high density lipoprotein cholesterol,ApoA1 apolipoprotein A, ApoB apolipoprotein B,BMI body mass index

Table 2 Comparison of ApoE genotype and allele frequency in each group (%)

Group	n	Genotype frequency						Allele frequency		
		E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	E2	E3	E4
CHD	550	5 (0.91%)	94 (17.09%)	19 (3.45%)	262 (47.63%)	165 (30.00%)	5 (0.91%)	123 (11.18%)	783 (71.18%)	194 (17.64%)
CI	590	2 (0.33%)	47 (7.96%)*	31 (5.25%)	378 (64.07%)*	128 (21.69%)*	4 (0.68%)	82 (6.95%)*	931 (78.90%)*	167 (14.15%)*
Control	1198	12 (1.00%)	318 (26.54%)*Δ	49 (4.09%)	623 (52.00%)Δ	187 (15.61%)*Δ	9 (0.75%)	391 (16.32%)*Δ	1751 (73.08%)Δ	254 (10.60%)*Δ

*vs CHD group , *P*<0.05 Δvs CI group , *P*<0.05

Table 3 Comparison of serum lipid levels among ApoE genotypes ()

Genotype	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	ApoA1 (g/L)	ApoB (g/L)
E2/E3	3.29±0.97	0.86±0.32	1.20±0.27	1.60±0.66	0.92±0.16	0.58±0.13
E2/E4	4.24±1.16*	2.14±0.80*	1.21±0.33	2.36±0.94*	0.98±0.27	0.78±0.25*
E3/E3	4.67±1.31*	1.84±1.64*	1.19±0.34	2.66±1.04*	1.02±0.22	0.86±0.30*
E3/E4	5.64±1.31*#Δ	2.00±1.04	1.17±0.29	3.40±0.88*#Δ	1.06±0.22	1.04±0.19*#Δ

The data presented are only for patients with type 2 diabetes in this study.

*vs genotype E2/E3 , *P*<0.05

vs genotype E2/E4 , *P*<0.05

Δvs genotype E3/E3, *P*<0.05

Table 4 Relationship between ApoE genotype and carotid atherosclerotic plaque

Carotid plaque	E2/E3	E2/E4	E3/E3	E3/E4
Non- plaque group（n=684）	213 (31.14%)	57 (8.33%)	327 (47.80%)	87 (12.72%)
plaque group （n=1654）	272 (16.44%)	10 (0.60%)	847 (51.21%)	525 (31.74%)
P	<0.01	<0.01	0.134	<0.01

The data presented are only for patients with type 2 diabetes in this study.

Table 5 Correlation analysis of ApoE genotype, allele and blood lipid level

blood lipid levels	ApoE genotype		ApoE allele	
	r	*P*	r	*P*
TC	0.360	<0.01	0.332	<0.01
TG	-0.024	0.732	-0.054	0.433
HDL-c	-0.037	0.594	-0.034	0.621
LDL-c	0.360	<0.01	0.333	<0.01

The data presented are only for patients with type 2 diabetes in this study.

Table 6 Regression analysis of the related factors of cardio-cerebrovascular complications of T2DM

variable	β	SE	β'	t	*P*	95%CI
diabetes duration	0.032	0.004	0.462	8.916	0.00	0.850-1.176
carotid plaque	0.373	0.071	0.320	5.242	0.00	0.614-1.629
ApoE E3/E4	0.192	0.060	0.169	3.212	0.00	0.826-1.211

Abbreviations

T2DM:Type 2 diabetes mellitus; Apolipoprotein E :ApoE; CHD :coronary heart disease; CI: cerebral infarctio; IMT:intimal thickness;SBP: systolic blood pressure; DBP:diastolic blood pressure; HbA1c: hemoglobin A1C;TG:triglycerides; TC: total cholesterol; LDL-c: low density lipoprotein cholesterol; HDL-c:high density lipoprotein cholesterol; ApoA1: apolipoprotein A; ApoB:apolipoprotein B; BMI:body mass index.

Declarations

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Authors'contributions

LK and WL organized the study. YG participated in selection of patients. LK and YG made the genetic analysis, laboratory investigations and interpretation of data. BS made the statistical analysis. LK was a major contributor in writing the manuscript. BS revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the Affiliated Hospital of Xuzhou Medical University(reference number: 2020–154). Written informed consent was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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