Associations between *Apolipoprotein E* Gene Polymorphisms and Cardiovascular Complications of Uremic Patients on Maintenance Hemodialysis

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Keywords

Apolipoprotein E · Gene polymorphisms · Uremia · Maintenance hemodialysis · Cardiovascular complications

Abstract

This study is to investigate the associations between *apolipoprotein E* (ApoE) gene polymorphisms and cardiovascular complications of uremic patients on maintenance hemodialysis (MHD). Uremic patients on MHD (189, case group) and healthy people (165, control group) were recruited. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to test ApoE gene polymorphisms. Indicators of MHD and cardiovascular complications were observed. Compared with the control group, the case group had decreased frequency of $\epsilon 3/3$ genotype and $\epsilon 3$ allele and increased $\epsilon 4/3$ genotype and $\epsilon 4$ allele. The $\epsilon 4$ group had elevated adiponectin, serum creatinine, blood urea nitrogen, and total cholesterol but decreased HDL-C compared with other groups. The $\epsilon 3$ group had reduced complications. ApoE $\epsilon 3$ and $\epsilon 4$ alleles were related with car-

diovascular complications of the uremic patients on MHD. We concluded that ApoE gene polymorphisms were associated with susceptibility to infections in uremia, and that ApoE $\epsilon 3$ and $\epsilon 4$ alleles might correlate with cardiovascular complications of uremic patients on MHD.

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Introduction

Uremic illness is caused by the inadequate removal and accumulation of organic products that are normally cleared by kidney. It manifests through various symptoms such as vascular disease, anemia, hypertension, and other complications [1, 2]. As the end stage of chronic kidney disease, uremia is the common syndrome of nearly all end-stage kidney diseases instead of an independent kidney disease [3]. End-stage renal disease (ESRD) is considered one of the most common lethal diseases, and hemodialysis (HD), peritoneal dialysis, and kidney transplantation are 3 common treatment therapies [4]. Dialysis refers

to the process of molecules being diffused in solution across a semipermeable membrane [5]. As an effective treatment strategy, HD could clear many small molecule toxins and prolong the survival rate of uremic patients [6]. However, a high mortality rate among HD patients has been reported in spite of improvements in dialysis technology [7]. Recent studies have shown that compared with the general population, patients in the stage of uremia undergoing HD presented higher mortality rate and mainly resulted from cardiovascular disease, including coronary heart disease, heart failure, and peripheral vascular disease [8, 9]. And cardiovascular complications have been demonstrated as a main cause of morbidity and mortality in patients with kidney failure [10]. Interestingly, it has been reported that *apolipoprotein E* (ApoE) is a genetic risk factor for some cardiovascular diseases, such as myocardial infarction and coronary artery disease [11].

As a major human apolipoprotein, the main role of ApoE is to regulate intracellular cholesterol levels and lipid uptake into cells by interacting with the low-density lipoprotein (LDL) receptor [12]. ApoE ϵ 2, ApoE ϵ 3, and ApoE ϵ 4 are 3 polymorphic alleles of ApoE with a worldwide frequency of 8.4, 77.9, and 13.7% respectively [13, 14]. The 3 alleles code for 3 isomorphs of ApoE are E2, E3, and E4 [15]. ApoE is involved in the transportation of lipid and cholesterol in the central nerve system and blood circulation, and ApoE gene polymorphisms are responsible for a variety of diseases, such as cardiovascular disease, Alzheimer's disease, and type 2 diabetes [16]; it has been reported that ϵ 4 allele of ApoE was correlated with high-levels of total cholesterol (TC) and LDL cholesterol (LDLC) [17]. However, the effects of ApoE gene polymorphisms on the cardiovascular complications of uremic patients on maintenance hemodialysis (MHD) have been rarely reported. We conduct this study with the aim of exploring the association between ApoE gene polymorphisms and the cardiovascular complications of uremic patients on MHD.

Subjects and Methods

Ethical Statement

This study was performed with the approval of the Clinical Ethical Committee of The First Affiliated Hospital of Anhui Medical University. Informed consent was obtained from all subjects.

Study Subject

A total of 189 uremic patients on MHD hospitalized in The First Affiliated Hospital of Anhui Medical University from June 2012 to June 2016 were recruited; 103 were males and 86 were females. Patient age ranged from 25 to 75 years with a mean age of 46.21 ± 9.40 years. All patients had a dialysis age of more than 3 months with no

or transfusion histories in the last 3 months, no acute infections in the last 1 month, and no other blood diseases except for renal anemia. Patients with tumors, severe heart failure, active hepatitis, and rheumatic diseases were excluded from this study. Among 189 uremic patients on MHD, their primary disease was as follows: 58 had chronic glomerulonephritis, 9 had pyelonephritis, 47 had diabetic nephropathy, 33 had benign arteriolar nephrosclerosis, 7 had polycystic kidney, 4 had gouty kidney, 15 had chronic interstitial nephritis, 2 had obstructive nephropathy, and 14 had unclear reasons. The inclusion criteria were as follows: (1) all patients were in accordance with the diagnostic criteria of uremia issued by the Kidney Branch of Chinese Medical Association [18]; (2) patients had no acute infections, surgeries, and trauma histories 2 months before treatment; (3) patients had no severe heart, brain, lung, and liver diseases; and (4) patients had no severe bleeding tendency. The exclusion criteria were as follows: (1) patients who received irregular HD during this study; (2) patients who were pregnant or just in their baby nursing period; and (3) patients with combined hypotension. An additional 165 healthy people during the same period were recruited as the control group, among whom 90 were male and 75 female with a mean age of 45.12 ± 9.09 years. There was no statistical significance between the 2 groups in terms of age and sex difference ($p > 0.05$).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Genomic DNA in the peripheral venous blood was prepared. Peripheral venous blood (3–5 mL) was collected from each subject, and 1% ethylene diamine tetraacetic acid (EDTA) anti-coagulated routine phenol method was adopted to extract DNA. DNA was diluted to the concentration of 10 ng/ μ L. The polymorphisms of ApoE gene site in fourth exon was detected by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). PCR primers were designed by Primer Premier 5.0 software and synthesized by Shanghai Sango Biotech Co., Ltd., (Shanghai, China). The forward primer: 5'-AACAACTGACCCCGGTGGCG-3'. The reverse primer: 5'-ATGGCGTGAGGCCGCGCTC-3'. The PCR amplification was conducted with a total reaction volume of 25 μ L. The PCR amplification conditions were as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 min, anneal at 65°C for 30 min, and extension at 72°C for 30 min; these conditions were repeated for 45 circles and at a 72°C extension for 10 min. The identified PCR amplification products were transferred into another 0.5 mL eppendorf tube, and shaken well after being added with 0.5 μ L of HhaI enzyme (10 U/ μ L). After being centrifuged for a while, the tube was placed in a water bath box for over 3 h at a constant temperature of 37°C. A volume of 10 μ L of enzymatic hydrolysates was collected to carry out electrophoresis (Mini 2D, Bio-Rad, Inc., Hercules, CA, USA) at 120 V for 90 min with 8% polyacrylamide gel electrophoresis (19:1). The ethidium bromide was used to stain and the result was observed under an ultraviolet lamp.

Related Factor Detection in Uremic Patients on MHD

The selected patients were treated with central venous catheters, and received vascular fistula surgeries after their blood vessels became mature. Those who had not received vascular fistula would receive long-term internal jugular vein catheters. All patients received HD with hemophane dialyzer (Tianjin HaidiNina International Trade Co., Ltd., Tianjin, China) and bicarbonate dialysate (dialysate flow rate: 500 mL/min, blood flow rate: 200–250 mL/min). Patients received HD 3 times a week, 4 h each time. The

Table 1. The biochemical indicators between the case and control groups

Indicator	Case group (n = 189)	Control group (n = 165)	t	p value
Adiponectin, µg/mL	33.79±11.28	12.77±2.93	23.27	<0.001
Scr, µmol/mL	768.30±111.99	62.00±13.10	80.52	<0.001
BUN, mmol/mL	27.01±5.39	3.42±1.09	55.22	<0.001
Albumin, g/L	34.80±5.00	40.03±1.56	12.89	<0.001
TC, mmol/L	4.05±1.73	3.64±0.38	2.00	0.047
Triglyceride, mmol/L	2.75±0.76	1.47±0.31	20.22	<0.001
HDL-C, mmol/L	0.88±0.24	1.53±0.39	19.27	<0.001
LDL-C, mmol/L	3.27±0.83	2.48±0.67	9.83	<0.001

Scr, serum creatinine; BUN, blood urea nitrogen; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

venous blood was taken from each patient on the empty stomach before HD. Blood lipid was detected with the help of the Clinical Laboratory of The First Affiliated Hospital of Anhui Medical University. Adiponectin was detected by enzyme-linked immunosorbent assay (ELISA) kit (Huijia Biotechnology Co., Ltd., Xiamen, China); serum creatinine (Scr; kits were bought from Shanghai ShensuoYoufu Medical Diagnostic supplies Co., Ltd., Shanghai, China) and blood urea nitrogen (BUN; kits were bought from Shanghai Jianglai Bio-technology Co., Ltd., Shanghai, China) were tested by enzymic method; TC (kits were bought from Guangzhou Kangda Scientific Instrument Co., Ltd., Guangdong, China), triglyceride (kits were bought from Shanghai Bangjing Industrial Co., Ltd., Shanghai, China), high-density lipoprotein cholesterol (HDL-C) (kits were bought from Roche Diagnostic Co., Indianapolis, IN, USA), and LDL-C (kits were bought from Changchun Huili Biotech Co., Ltd., Jilin, China) were detected by the lipid method; albumin (kits were bought from Shanghai Bangyi Biotechnology Co., Ltd., Shanghai, China) was detected by ELISA. After HD, uremic patients on MHD could undergo hypotension due to excessive ultrafiltration and hypovolemia; additionally, patients might have hypertension due to sodium retention or renin-angiotensin elevation; furthermore, patients might present with coronary heart disease and chronic cardiac insufficiency owing to the changes of serum potassium and calcium. The cardiovascular complications of uremic patients on MHD after 4-week HD were observed, which included hypotension, hypertension, coronary heart disease, and chronic cardiac insufficiency [19]. The WHO standard [20] was adopted to diagnose hypotension and hypertension; Minnesota standard [21] was used to diagnose coronary heart disease; heart failure diagnosis standard of Framingham [22] was adopted to diagnose chronic cardiac insufficiency. Uremic patients on MHD were confirmed to have cardiovascular complications when they exhibited no less than one of the above symptoms.

Statistical Analysis

SPSS 22.0 statistical software was used to analyze data. Hardy-Weinberg equilibrium was used to test the genotype distribution of uremic patients on MHD. Measurement data were expressed as mean ± SD and tested by *t* test. Count data were presented as a percentage or ratio and detected by chi-square test or Fisher's exact probability test. Rank data were expressed by the rank-sum test. The

OR and 95% CI for relative risk were expressed by non-conditional logistic regression analysis. *p* < 0.05 was considered statistically significant.

Results

Adiponectin, Scr, BUN, Albumin, TC, Triglyceride and LDL-C are Higher Yet ALB and HDL-C Lower in the Case Group

ELISA was used to determine the expressions of Adiponectin, Scr, BUN, albumin, TC, triglyceride, HDL-C, and LDL-C. The results showed that Adiponectin, Scr, BUN, albumin, TC, triglyceride, HDL-C, and LDL-C in the case and control groups were considered significant (all *p* < 0.05). Adiponectin, Scr, BUN, TC, triglyceride, and LDL-C in the case group were notably higher than those of the control group, while albumin and HDL-C were lower than those of the control group (all *p* < 0.05; Table 1). It was concluded that Adiponectin, Scr, BUN, albumin, TC, triglyceride, and LDL-C might be increased, while albumin and HDL-C decreased in cardiovascular complications of uremic patients on MHD.

Electrophoresis Results of PCR Products Are Observed

PCR-RFLP was adopted to detect the genotypes of ApoE. Then 6 common genotypes of ApoE were identified: ε3/3 genotype containing 2 fragments, 91 bp and 48 bp; ε4/4 genotype containing 2 fragments, 72 bp and 48 bp; ε2/2 genotype containing 2 fragments, 91 bp and 83 bp; ε4/3 genotype containing 3 fragments, 91 bp, 72 bp and 48 bp; ε3/2 genotype containing 3 fragments, 91 bp, 83 bp, and 48 bp; ε4/2 genotype containing 3 fragments, 91 bp, 83 bp, and 48 bp (Fig. 1).

Fig. 1. Detection results of ApoE genotypes evaluated by PCR-RFLP. $\epsilon 2/2$: 91 bp and 83 bp; $\epsilon 3/3$: 91 bp, and 48 bp; $\epsilon 4/4$: 72 bp, and 48 bp; $\epsilon 3/2$: 91 bp, 83 bp, and 48 bp; $\epsilon 4/3$: 91 bp, 72 bp, and 48 bp; $\epsilon 4/2$: 91 bp, 83 bp, and 48 bp; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; ApoE, apolipoprotein E.

$\epsilon 3/3$ and $\epsilon 4/3$ Genotype Together with $\epsilon 3$ and $\epsilon 4$ Allele Might Correlate with Cardiovascular Complications of Uremic Patients on MHD

Hardy-Weinberg equilibrium was employed to test the genotype distribution of uremic patients on MHD. The results showed that the differences between actual genotype frequencies and theoretical genotype frequencies between the 2 groups had no statistical significance ($p > 0.05$) and that the genotype distribution had group representativeness of genetic equilibrium. Comparisons of frequencies of genotype and allele of ApoE between the 2 groups are shown in Table 2. $\epsilon 3/3$ and $\epsilon 4/3$ genotypes had the highest frequencies in both case and control groups, $\epsilon 2/2$ and $\epsilon 4/2$ presented as the second, and $\epsilon 4/4$ and $\epsilon 3/2$ as the lowest. Distribution frequencies of $\epsilon 3/3$ genotype and $\epsilon 3$ allele in the case group were remarkably lower than those of the control group (all $p < 0.05$), indicating that $\epsilon 3/3$ genotype and $\epsilon 3$ allele might correlate with cardiovascular complication for uremic patients on MHD. Distribution frequencies of $\epsilon 4/3$ genotype and $\epsilon 4$ allele in the case group were notably higher than those of the control group (all $p < 0.05$), suggesting that $\epsilon 4/3$ genotype and $\epsilon 4$ allele might also correlate with cardiovascular complication for uremic patients on MHD. Distribu-

tion frequency differences of other genotypes and alleles between the 2 groups had no statistical significance (all $p > 0.05$). The results showed that $\epsilon 3/3$ and $\epsilon 4/3$ genotype together with $\epsilon 3$ and $\epsilon 4$ allele might correlate with cardiovascular complications of uremic patients on MHD.

Adiponectin, Scr, BUN, and TC were Elevated, Yet HDL-C Reduced in the $\epsilon 4$ Group

Patients were divided into 3 groups according to the associations between different genotypes and uremic patients on MHD: $\epsilon 2$ group, patients with $\epsilon 2/2$ and $\epsilon 4/2$ genotypes; $\epsilon 3$ group, patients with $\epsilon 3/3$ and $\epsilon 3/2$ genotypes; and $\epsilon 4$ group, patients with $\epsilon 4/3$ and $\epsilon 4/4$ genotypes. Age, sex, body-mass index, albumin, triglyceride, and LDL-C concentrations had no obvious differences in different ApoE genotypes (all $p > 0.05$). Adiponectin, Scr, BUN, and TC in the $\epsilon 4$ group were higher than those in the other groups, while HDL-C in the $\epsilon 4$ group was lower than those in the other groups (all $p < 0.05$). The $\epsilon 3$ group had a higher level of HDL-C and lower level of adiponectin, Scr, BUN, and TC than those in the $\epsilon 2$ group (all $p < 0.05$; Table 3). The results revealed that $\epsilon 4$ could accelerate the expression of Adiponectin, Scr, BUN, and TC, while it could decelerate the expression of HDL-C.

$\epsilon 4$ Is More Likely to Have Cardiovascular Complications of Uremic Patients on MHD

A total of 106 individuals were diagnosed with uremia on MHD; of these cases, 19 exhibited one kind of cardiovascular complication, 57 exhibited 2 kinds of cardiovascular complications, and 30 exhibited 3 kinds of cardiovascular complications. In other words, 33 uremic patients on MHD were diagnosed with hypotension, 54 patients with hypertension, 65 patients with coronary heart disease, and 71 patients with chronic cardiac insufficiency. Uremic patients on MHD in the $\epsilon 4$ group were more likely to have complications of hypotension, hypertension, coronary heart disease, and chronic cardiac insufficiency when compared with patients in other groups (all $p < 0.05$). Patients in the $\epsilon 3$ group had less chance to have the above complications than patients in the $\epsilon 2$ and $\epsilon 4$ groups (all $p < 0.05$; Table 4). These findings revealed that $\epsilon 4$ was more likely to have complications of hypotension, hypertension, coronary heart disease, and chronic cardiac insufficiency of uremic patients on MHD.

$\epsilon 3$ and $\epsilon 4$ Genotypes Correlate with Cardiovascular Complication of Uremic Patients on MHD

Binary logistic regression was conducted with cardiovascular complication as an independent variable. The genotypes in the $\epsilon 3$ and $\epsilon 4$ groups were correlated

Table 2. Frequencies of genotype and allele of ApoE in the case and control groups

ApoE	Type	Case group (n = 189)	Control group (n = 165)	χ^2	OR (95% CI)	p value
Genotype	$\epsilon 2/2$	21	16	Ref		
	$\epsilon 3/2$	6	5	0.13	0.914 (0.236–3.540)	0.897
	$\epsilon 3/3$	58	108	2.46	0.409 (0.198–0.845)	0.014
	$\epsilon 4/3$	63	20	2.11	2.400 (1.054–5.462)	0.035
	$\epsilon 4/4$	11	5	0.82	1.676 (0.484–5.801)	0.413
Allele	$\epsilon 4/2$	30	11	1.52	2.078 (0.804–5.368)	0.128
	$\epsilon 2$	78	48	Ref		
	$\epsilon 3$	185	241	3.65	0.472 (0.314–0.710)	<0.001
	$\epsilon 4$	115	41	2.12	1.726 (1.040–2.864)	0.034

ApoE, *apolipoprotein E*.**Table 3.** Associations between ApoE genotype and the baseline characteristics of uremic patients on MHD in the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ groups

Indicator	$\epsilon 2$ group (n = 51)	$\epsilon 3$ group (n = 64)	$\epsilon 4$ group (n = 74)	p value
Age, years	46.49±10.46	45.69±8.89	46.47±9.18	0.862
Gender				0.962
Male	28	34	41	
Female	23	30	33	
BMI, kg/m ²				0.142
<18.5	14	21	11	
≥18.5–≤24.9	17	15	24	
>25–<29.9	11	18	24	
≥30	9	10	15	
Adiponectin, µg/mL	32.65±9.51	28.65±8.94*	39.03±12.03*,#	<0.001
Scr, µmol/mL	763.78±123.04	723.76±91.62*	809.93±105.73*,#	<0.001
BUN, mmol/mL	26.61±5.67	24.36±4.73*	29.58±4.53*,#	<0.001
Albumin, g/L	34.36±5.64	35.43±4.98	34.57±4.54	0.460
TC, mmol/L	3.00±0.34	2.50±0.47*	6.11±0.55*,#	<0.001
Triglyceride, mmol/L	2.68±0.86	2.84±0.76	2.71±0.69	0.462
HDL-C, mmol/L	0.84±0.06	1.13±0.21*	0.69±0.11*,#	<0.001
LDL-C, mmol/L	3.20±0.94	3.38±0.83	3.23±0.76	0.460

ApoE, *apolipoprotein E*; MHD, maintenance hemodialysis; Scr, serum creatinine; BMI, body-mass index; BUN, blood urea nitrogen; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

* Indicates differences when compared with the $\epsilon 2$ group.

Indicates differences when compared with the $\epsilon 3$ group.

with the cardiovascular complications of uremic patients on MHD (all $p < 0.05$). Uremic patients on MHD with higher serum levels of adiponectin, Scr, BUN, and TC as well as lower levels of HDL-C could more easily be affected by cardiovascular complications (Table 5). We could conclude that $\epsilon 3$ and $\epsilon 4$ genotypes might correlate with cardiovascular complication of uremic patients on MHD.

Discussion

It is reported that ESRD patients have a high risk rate of mortality [23]. However, a recent study reported that despite dialysis treatment, ESRD patients still had a high rate of death with cardiovascular disease as the main cause [24]. It has been shown that ApoE gene polymorphisms were involved in a variety of diseases, including

Table 4. Incidence analysis of the cardiovascular complications of uremic patients on MHD in the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ groups with different ApoE genotypes

Complications	$\epsilon 2$ group (<i>n</i> = 51)	$\epsilon 3$ group (<i>n</i> = 64)	$\epsilon 4$ group (<i>n</i> = 74)	<i>p</i> value
Hypotension				<0.001
Yes	7	2*	24*, #	
No	44	62	50	
Hypertension				<0.001
Yes	13	4*	37*, #	
No	38	60	37	
Coronary heart disease				<0.001
Yes	12	6*	47*, #	
No	39	58	27	
Chronic cardiac insufficiency				<0.001
Yes	21	6*	44*, #	
No	30	58	30	

ApoE, *apolipoprotein E*; MHD, maintenance hemodialysis.

* Indicates differences when compared with the $\epsilon 2$ group.

Indicates differences when compared with the $\epsilon 3$ group.

Table 5. Risk factors of cardiovascular complication for uremic patients on MHD confirmed by Logistic regression analysis

	B	SE	Wald	<i>p</i> value	OR	Lower	Upper
$\epsilon 3$ group	-1.636	0.803	4.148	0.042	0.195	0.04	0.94
$\epsilon 4$ group	5.129	1.98	6.713	0.01	168.816	3.487	8,173.253
Adiponectin, $\mu\text{g/mL}$	0.015	0.025	0.355	0.551	1.015	0.966	1.066
Scr, $\mu\text{mol/mL}$	0.001	0.005	0.009	0.924	1.001	0.99	1.011
BUN, mmol/mL	-0.08	0.116	0.472	0.492	0.924	0.736	1.159
TC, mmol/L	-0.672	0.535	0.576	0.209	0.511	0.179	1.458
HDL-C, mmol/L	-2.466	2.064	1.428	0.232	0.085	0.001	4.852

95.0% CI for EXP(B); Scr, serum creatinine; BUN, blood urea nitrogen; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; MHD, maintenance hemodialysis.

cardiovascular disease [16]. Thus, this study was supposed to explore the associations between ApoE gene polymorphisms and the cardiovascular complications of uremic patients on MHD.

First, our experiment results showed that adiponectin, Scr, BUN, TC, triglyceride, and LDL-C of the case group were notably higher than those of the control group, while albumin and HDL-C were lower than those of the control group. Consistent with our study, a previous study showed that plasma adiponectin depended on kidney function, which markedly increases among patients with kidney impairment [25]. And elevated BUN and creatinine indicate the impairment of kidney function [26]. Besides, another study revealed that low HDL-C, high

TC, and triglyceride levels can lead to renal damage, and demonstrated that the increased levels of LDL-C were independently related to chronic kidney disease in Japanese men [27].

Second, our study also found that adiponectin, Scr, BUN, and TC of the $\epsilon 4$ group were higher than other groups, while HDL-C of the $\epsilon 4$ group was lower than that in other groups. The $\epsilon 3$ group had an opposite trend when compared with the $\epsilon 4$ group. A higher expression of ApoE is evident in adipocytes [28]. One study focused on ApoE polymorphisms on Scr in healthy people revealed that ApoE4 carriers (ApoE4/3 and ApoE4/4) tended to have the lowest Scr level [29]. Besides, elevated levels of Scr and BUN were found in glomerulosclerosis, and ApoE is as-

sociated with the progression of glomerulosclerosis [30]. Previous studies have indicated a higher level of TC and LDL-C and lower level of HDL-C in individuals with the $\epsilon 4$ allele than non-carriers [31, 32]. Besides, we also found that the distribution frequencies of genotype $\epsilon 3/3$ and allele $\epsilon 3$ in the case group were remarkably lower than those of the control group, whereas the distribution frequencies of genotype $\epsilon 4/3$ and allele $\epsilon 4$ in the case group were notably higher than those of the control group. The genotypes in the $\epsilon 4$ group were verified as the risk factor of cardiovascular complication for uremic patients on MHD. It was proven that high plasma concentration of LDL-C increases the risk for cardiovascular disease and HDL-C was beneficial for cardiovascular systems and kidney function and by extracting tissue cholesterol [33, 34]. Another study suggested that $\epsilon 4$ increased the level of LDL-C and reduced the level of high density lipoprotein (HDL) in the high-fat intake population [35]. Consistent with our study, Dekroon et al. [36] also reported that through altering the components progression of metabolic syndrome, $\epsilon 4$ allele may exert a high risk on cardiovascular disease. Huebbe et al. [37] showed that $\epsilon 4$ allele is a major casual risk factor for cardiovascular events and or late-onset Alzheimer's disease. A study provided evidence that $\epsilon 3$ allele could function as a protective factor in patients with Alzheimer's disease who are from Vitória, Espírito Santo [38]. However, the ApoE- $\epsilon 3$ allele protection in diseases is yet to be explored. Besides, the regression analysis also indicated that ApoE $\epsilon 3$ and $\epsilon 4$ alleles were related to cardiovascular complications for uremic patients on MHD.

Moreover, this study also concluded that uremic patients on MHD in the $\epsilon 4$ group were more likely to have complications of hypotension, coronary heart disease, and chronic cardiac insufficiency, while patients in the $\epsilon 3$ group had less chance to have the above complications.

Evidence was provided by a previous study that elevated plasma lipid was the main risk for cardiovascular disease and ApoE polymorphisms influence the lipid composition in plasma and the progression of cardiovascular disease through other biochemical pathway [11]. Zhang et al. [35] observed that $\epsilon 4$ allele exerted a high risk on Chinese coronary heart disease patients. In another study, it has been shown that $\epsilon 4$ allele increases the risk of cognitive decline, stroke, hypertension, and coronary disease [39].

Conclusions

In conclusion, our study provides evidence that ApoE gene polymorphisms had effect on cardiovascular complications of uremic patients on MHD and ApoE $\epsilon 4$ genotypes and allele may be associated with cardiovascular complications of uremic patients on MHD. These findings provide clinical significance for preventing and treating cardiovascular complications of uremia MHD patients. However, the sample size in our study was small and a larger sample size would be needed to further explore the effect of ApoE gene polymorphisms on cardiovascular complications of uremic patients on MHD.

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Disclosure Statement

All authors have no conflicts of interest to disclose.

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