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Original Article

Correlation between *APOE* gene polymorphisms and efficacy of trimetazidine in treating chronic heart failure secondary to non-ischemic cardiomyopathy: a population-based study in China

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Abstract: Objective: The aim of study was to explore association between *APOE* gene polymorphisms and efficacy of trimetazidine (TMZ) in treating chronic heart failure secondary to non-ischemic cardiomyopathy in a Chinese population. Methods: A total of 336 patients with chronic heart failure, secondary to non-ischemic cardiomyopathy, received three times daily oral administration of TMZ. After treatment, patients were assigned to effective and ineffective groups. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was performed to identify *APOE* gene polymorphisms. The heart rate variability (HRV) of patients was measured. Binary logistic regression analysis was employed to estimate risk factors influencing efficacy of TMZ. Results: Overall effective rate of the 336 cases was 76.6%. After treatment with TMZ, compared with the ineffective group, effective group had decreased brain natriuretic peptides (BNP), left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), and left ventricle end systole volume (LVESV), with increased left ventricular ejection fraction (LVEF). Frequencies of *APOE* ε4 allele and ε4/ε4 genotype were lower in the effective group than ineffective group. SDNN, SDANN, RMSSD and pNN50 were all reduced in patients with ε4 allele, compared to those with ε2 allele, ε2/ε4, or ε2/ε4 genotypes. Binary logistic regression analysis revealed that *APOE* ε4 allele, BNP level, LVEDD, LVEF, 6MWT, SDANN and pNN50, after treatment, were risk factors that decreased efficacy of TMZ. Conclusion: *APOE* ε4 allele may be associated with efficacy of TMZ in treating chronic heart failure secondary to non-ischemic cardiomyopathy.

Keywords: *APOE*, polymorphism, chronic heart failure secondary to non-ischemic cardiomyopathy, trimetazidine, heart rate variability

Introduction

Heart failure is a common syndrome resulting in substantial morbidity and mortality, with a prevalence of over 5.8 million in the United States and more than 23 million worldwide [1]. Heart failure is not a single entity but a complex multi-system clinical syndrome that may lead to impairment in respiratory, hematological, renal, vascular, endocrine, and musculoskeletal systems [2]. Interestingly, heart failure results from any structural or functional cardiac disorder, including ischemic or non-ischemic cardiomyopathy, which represents an increasingly common reason for recurrent hospital admissions [3, 4].

Trimetazidine (TMZ), 1-(2, 3, 4 trimethoxybenzyl) piperazine di-hydrochloride, is an effective and well tolerated anti-anginal agent, providing functional improvement and symptom relief in angina pectoris as well as cytoprotection during ischemia [5]. TMZ is a 3-ketoacyl coenzyme A thiolase (3-KAT) inhibitor that changes cardiac energy metabolism from free fatty acid oxidation to glucose oxidation through suppressing mitochondrial long-chain 3-KAT [6]. Several functional studies have also reported that TMZ showed clinical efficacy in treating a variety of clinical forms of ischemia, including angina pectoris [7, 8] and acute coronary syndromes [9]. Interestingly, TMZ facilitates the recovery of myocardial cells and promotes mitochondria

metabolism, thereby improving cardiac function in patients with heart failure [10]. It has been reported that inter-individual differences in drug metabolism, drug absorption, and drug response, ranging from no therapeutic benefit to life-threatening side effects, are affected by a variation in genes [11].

Apolipoprotein E (APOE) is involved in lipid metabolism as a ligand for several cell-surface receptors, including low density lipoprotein (LDL) receptor, LDL-receptor related protein, and very LDL (VLDL) receptor [12]. APOE is encoded by *APOE* gene, a polymorphic gene with three common variant alleles (*APOE* ε2, ε3 and ε4) [13]. *APOE* ε4 allele is associated with increased levels of cholesterol and higher risk of Alzheimer's disease, as well as coronary heart disease [14]. Given the association between *APOE* gene polymorphisms and heart disease, the present study explored association between *APOE* gene polymorphisms and efficacy of TMZ in treating chronic heart failure secondary to non-ischemic cardiomyopathy.

Materials and methods

Study subjects

From March 2014 to March 2016, 336 patients with chronic heart failure, secondary to non-ischemic cardiomyopathy, were admitted to Inner Mongolia University Science and Technology of Baotou Medical School First Affiliated Hospital. These patients consisted of 206 males and 130 females, with a mean age of (53.8 ± 8.3) years, mean weight of (69.1 ± 9.5) kg, and disease duration ranging from 0.5 to 10 years. Among these 336 patients, there were 183 cases with primary cardiomyopathy and 123 cases with secondary cardiomyopathy including alcoholic myocardiopathy, drug-induced cardiomyopathy, and metabolic cardiomyopathy. At admission, patients were given a physical examination, X-ray, electrocardiogram (ECG), blood biochemical assay, coronary arteriongraphy or CT examination, and ultrasonic cardiogram (UCG). Patients with hypertension, coronary heart disease, or hypertension, combined with coronary heart disease, were recorded. Inclusion criteria: Patients diagnosed with chronic heart failure, secondary to non-ischemic cardiomyopathy, according to the American Heart Association (AHA) Guidelines on prevention, diagnosis, and treatment of acute and chronic HF; Patients receiving 8-week drug therapy (β-blocker (AstraZeneca Pharmaceutical Co. Ltd., Wuxi, Jiangsu, China), angiotensin-converting enzyme (ACE) inhibitors (Guangzhou Baiyunshan Pharmaceutical Co. Ltd., China), diuretics (Jiangsu Ruinian Qianjin Pharmaceutical Co. Ltd., China), digoxin (Shanghai Xinyi Pharmaceutical Co. Ltd., China), nitric acid ester (Shanghai Macklin Biochemical Technology Co. Ltd., China), and digitalis preparations (Guangzhou Baiyunshan Pharmaceutical Co. Ltd., China); Patients with cardiothoracic ratio (CTR) > 0.5 shown in chest X-ray images and with cardiac dilatation due to left ventricular dilatation; Patients with ventricular systolic dysfunction, diffusely distributed segmental wall-motion abnormalities shown in UCG, and left ventricular ejection fraction < 50%. Exclusion criteria: Patients with chronic heart failure secondary to non-ischemic cardiomyopathy; Patients with hypertrophic cardiomyopathy; Patients with systemic diseases, systematic diseases, and autoimmune diseases; Patients with severe mitral regurgitation. Protocol for this study was approved by the Ethics Committee of Inner Mongolia University Science Technology in Baotou Medical School First Affiliated Hospital. Written informed consent was obtained from each patient.

Treatment regimes

Initially, patients without contraindications to medicines used in basic treatments were given basic treatment, according to American College of Cardiology/American Heart Association (ACC/AHA) guidelines [15]. Each patient received oxygen inhalation and bed rest under continuous ECG monitoring. Each patient received thrice-daily oral administration of 20 mg TMZ (Servier, France). During treatment, a regular follow up was conducted for each patient.

Outcome measures

All patients were given a dynamic electrocardiogram (ECG) inspection, before and after treatment with TMZ. All patients underwent continuous ECG monitoring for 24 hours using a TLC-4000 holographic dynamic ECG analysis system (Contec Medical Systems, Beijing, China). Record-playback approach was performed for

data collection of dynamic ECG with technical guidance from professionals. Using automatic analysis and a man-machine dialogue, heart rate variability (HRV) was obtained after eliminating artifacts and interference. HRV: SDNN, standard deviation of NN intervals often calculated over a 24-hour period; SDANN, standard deviation of the average NN intervals calculated over short periods, usually 5 minutes; RMSSD, root mean square of successive differences, square root of the mean of the squares of successive differences between adjacent NNs; pNN50, the proportion of NN50 divided by total number of NNs. Venous blood was extracted from each patient and levels of brain natriuretic peptides (BNP) were measured according to manufacturer instructions (Biomedica, Austria). An Acuson Sequoia 512 ultrasound system (Acuson, Mountain View, CA, USA) was used to examine left ventricular end diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF), left ventricular end systolic diameter (LVESD), and left ventricle end systole volume (LVESV).

Six-minute walk test (6MWT): before and after treatment with TMZ, a 6MWT was performed indoors along a flat, straight, enclosed, and seldom traveled corridor that had 20-30 length and a hard surface. Distances that 100 patients walked in 6 minutes were measured.

Efficacy evaluation

TMZ-treated patients were classified into NYHA functional class II to class IV, according to New York Heart Association Functional Classification (NYHA-FC) [16]. According to cardiac function, TMZ treated patients were allocated into an effective group and an ineffective group. TMZ was considered as markedly effective if NYHA functional class was improved by more than two classes, in patients after 24 weeks of TMZ treatment; TMZ was considered as effective if NYHA functional class was improved by one class in patients after 24 weeks of TMZ treatment; TMZ was considered as ineffective if NYHA functional class did not improve in patients after 24 weeks of TMZ treatment. An effective group consisted of patients showing remarkable improvement and those showing improvement in NYHA functional class [Total effective rate = (markedly effective + effective)/total patients × 100%].

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay

A PCR-RFLP assay was performed to identify *APOE* gene polymorphisms. Isolation of leukocyte DNA was carried out using a blood genomic DNA isolation kit (non-centrifugal type, Takara Biotechnology (Dalian) Co., Ltd., China). Using Primer Premier 5 Design Program (Thermo Fisher Scientific Inc., Waltham, MA, USA), following repeated annealing and electrophoresis on polyacrylamide gel (Ruida Henghui Biotechnology Co., Ltd., Beijing, China), the primers were designed as 5'-TAAGCTTGGCACGGCTGTCAAGGA-3' (forward) and 5'-AGAATTCGCCCCGGCCTGGTACAC-3' (reverse). PCR reaction conditions were pre-denaturation at 94°C for 3 minutes and 32 cycles of denaturation at 94°C for 40 seconds, annealing at 58°C for 45 seconds and extension at 72°C for 55 seconds, followed by final extension at 72°C for 10 minutes. Single stranded DNA was maintained at 4°C and the PCR reaction was terminated. PCR products were digested by a restriction enzyme (Cfo 1) at 37°C for 6 hours, followed by electrophoresis on 8% polyacrylamide gel (Ruida Henghui Biotechnology Co., Ltd., Beijing, China). Afterward, the products were stained using ethidium bromide for 30 seconds and exposed to ultraviolet radiation for observation. The size of PCR products was 227 bp and different genotypes were cut into fragments of different sizes: ε2/ε2 contained two bands of 91 bp and 81 bp; ε2/ε3 contained 91 bp, 81 bp and 48 bp; ε2/ε4 contained 91 bp, 81 bp, 72 bp and 48 bp; ε3/ε3 contained 91 bp and 48 bp; ε3/ε4 contained 91 bp, 72 bp and 48 bp; ε4/ε4 contained 71 bp and 48 bp.

Statistical analysis

Data were analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Continuous data are expressed as mean ± standard deviation. t-test was used for comparisons between two groups when normality (and homogeneity of variance) assumptions were satisfied, otherwise rank-sum test was used. Categorical data are expressed as ratio and percentage and Chi-square test was performed for comparison between groups. Binary logistic regression analysis was employed to estimate risk factors influencing the efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy.

Table 1. Comparison of patients with chronic heart failure secondary to non-ischemic cardiomyopathy between effective and ineffective groups

Item	Effective group	Ineffective group	*P*
Gender (M/F)	63.3%/36.7%	55.0%/45.0%	0.184
Mean age (year)	53.7 ± 7.9	54.1 ± 9.3	0.705
Weight (kg)	68.6 ± 9.6	70.7 ± 9.2	0.086
Disease duration(year)	4.9 ± 1.9	5.2 ± 1.7	0.208
BNP level (pg/ml)			
Before treatment	3863.61 ± 751.23	3992.56 ± 679.35	0.172
After treatment	1532.44 ± 461.30	2492.38 ± 375.82	< 0.001
NYHA functional class			0.133
II	27.3%	17.5%	
III	48.5%	60.0%	
IV	24.2%	22.5%	
LVEDD (mm)			
Before treatment	63.18 ± 8.20	63.95 ± 7.33	0.453
After treatment	57.25 ± 6.49	61.96 ± 7.58	< 0.001
LVEF (mm)			
Before treatment	32.15 ± 4.07	31.47 ± 4.88	0.215
After treatment	41.39 ± 4.61	36.87 ± 5.13	< 0.001
LVESD (mm)			
Before treatment	52.06 ± 7.15	53.82 ± 7.96	0.062
After treatment	42.10 ± 6.03	46.79 ± 7.12	< 0.001
LVESV (mm)			
Before treatment	61.68 ± 9.32	62.91 ± 10.28	0.316
After treatment	56.79 ± 8.05	60.35 ± 9.64	0.001
Complication			
Hypertension	30.8%	40.0%	0.129
Heart disease	36.3%	46.3%	0.112
Hypertension & heart disease	5.5%	11.3%	0.074
Dyslipidemia	50.8%	57.5%	0.294
6MWT (m)			
Before treatment	192.65 ± 87.47	185.18 ± 92.30	0.511
After treatment	422.40 ± 112.69	246.37 ± 102.98	< 0.001
HRV			
SDNN (ms)			
Before treatment	78.67 ± 10.12	76.93 ± 12.51	0.207
After treatment	111.02 ± 10.96	106.59 ± 11.37	0.002
SDANN (ms)			
Before treatment	72.14 ± 14.63	71.52 ± 15.38	0.744
After treatment	103.42 ± 15.18	89.54 ± 11.66	< 0.001
RMSSD (ms)			
Before treatment	20.06 ± 2.61	19.46 ± 2.77	0.078
After treatment	25.75 ± 3.26	24.01 ± 3.05	< 0.001
pNN50			
Before treatment	8.43 ± 1.95	8.12 ± 1.63	0.199
After treatment	12.11 ± 2.30	10.23 ± 2.05	< 0.001

Note: M, male; F, female; BNP, brain natriuretic peptide; NYHA, New York Heart Association; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; LVESV, left ventricle end systole volume; 6MWT, 6 minute walk test; HRV, heart rate variability; SDNN, the standard deviation of NN intervals; SDANN, the standard deviation of the average NN intervals; RMSSD, root mean square of successive differences; pNN50, the proportion of NN50 divided by total number of NNs.

Table 2. Frequencies of *APOE* gene genotype and allele between effective and ineffective groups

	Effective group (n, %)	Ineffective group (n, %)	*P*
Genotype			
ε2/ε2	0 (0%)	0 (0%)	1
ε2/ε3	41 (16.0%)	8 (10%)	0.183
ε2/ε4	11 (4.3%)	4 (5.0%)	0.790
ε3/ε3	163 (63.7%)	44 (55.0%)	0.164
ε3/ε4	36 (14.1%)	18 (22.5%)	0.073
ε4/ε4	5 (2.0%)	6 (7.5%)	0.015
Allele			
ε2	52 (10.2%)	12 (7.5%)	0.318
ε3	403 (78.7%)	114 (71.3%)	0.051
ε4	57 (11.1%)	34 (21.3%)	0.001

Table 3. Frequencies of *APOE* gene genotypes between effective and ineffective groups

	Effective group (n, %)	Ineffective group (n, %)	*P*
ε2/X	41 (16.0%)	8 (10%)	0.183
ε2/ε4	11 (4.3%)	4 (5.0%)	0.790
ε3/ε3	163 (63.7%)	44 (55.0%)	0.164
ε4/X	41 (16.0%)	24 (30.0%)	0.006

Results

Cardiac function and prognosis after treatment with TMZ in effective group were better than ineffective group

Of the 336 patients, TMZ was markedly effective in 72 cases, effective in 184 cases, and ineffective in 80 cases. The total effective rate was 76.6%. There were no significant differences in gender, mean age, disease duration, and NYHA functional class between the effective and ineffective groups ($P > 0.05$). More patients with hypertension were found in the ineffective group than effective group ($P < 0.001$). Before treatment with TMZ, BNP level, LVEDD, LVEF, LVESD, LVESV, 6-min walk test (6MWT), and HRV did not significantly differ between the effective and ineffective groups ($P > 0.05$). After treatment with TMZ, LVEF was increased while BNP level, LVEDD, LVESD and LVESV were decreased in the effective group compared with ineffective group ($P < 0.05$); the 6MWT was longer and the HRV was better in the effective group than ineffective group ($P < 0.05$) (**Table 1**).

More ε4/X carriers existed in the ineffective group than effective group

As shown in **Table 2**, frequencies of *APOE* ε4 allele and ε4/ε4 genotype were lower in the effective group than ineffective group ($P < 0.05$). Furthermore, patients were classified as ε2 carriers, ε3/ε3 carriers, ε2/ε4 carriers and ε4 carriers. These results indicated that the ineffective group had more ε4/X carriers than effective group ($P < 0.05$, **Table 3**).

APOE gene ε4 allele may be a risk factor influencing efficacy of TMZ

The number of patients with hypertension and NYHA functional class changed between the effective and ineffective groups in ε2 carriers, ε3/ε3 carriers, ε2/ε4 carriers and ε4 carriers ($P < 0.05$) but disease duration did not ($P > 0.05$). Before treatment with TMZ, BNP level, LVEDD, LVEF, LVESD, LVESV and 6MWT did not differ remarkably between the effective and ineffective groups in ε2 carriers, ε3/ε3 carriers, ε2/ε4 carriers and ε4 carriers ($P > 0.05$). After treatment with TMZ, there were significant differences in BNP level, LVEDD, LVEF, LVESD, LVESV and 6MWT between the effective and ineffective groups in ε2 carriers, ε3/ε3 carriers, ε2/ε4 carriers and ε4 carriers, respectively ($P < 0.05$). Besides, more patients with ε4 allele were found compared with those patients with other genotypes in the ineffective group ($P < 0.05$). NYHA functional class was enhanced, LVEF was lowered, BNP level, LVEDD, LVESD and LVESV were increased, and 6MWT was shortened in the ε4 carriers compared to ε2 carriers, ε3/ε3 carriers and ε2/ε4 carriers ($P < 0.05$, **Table 4**). These results reveal that *APOE* gene ε4 allele may be a risk factor influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy.

APOE gene ε4 allele may be a risk factor influencing the HRV of patients with chronic heart failure secondary to non-ischemic cardiomyopathy, after treatment with TMZ

As shown in **Table 5**, no significant differences were found in the HRV between the effective and ineffective group, before treatment with TMZ ($P > 0.05$). SDNN, SDANN, RMSSD and pNN50 were all increased in patients, after treatment with TMZ, and were all decreased in the ineffective group compared with effective

Table 4. Association between *APOE* gene genotypes and patients with chronic heart failure secondary to non-ischemic cardiomyopathy in the effective and ineffective groups

Item	ε2/X		*P*	ε2/ε4		*P*	ε3/ε3		*P*	ε4/X		*P*
	Effective group (n=41)	Ineffective group (n=8)		Effective group (n=11)	Ineffective group (n=4)		Effective group (n=163)	Ineffective group (n=44)		Effective group (n=41)	Ineffective group (n=24)	
Disease duration (year)	4.79 ± 2.10	5.07 ± 1.93	0.729	4.83 ± 1.85	5.11 ± 3.16	0.832	4.57 ± 2.28	5.06 ± 1.92	0.193	5.02 ± 2.16	5.39 ± 2.35	0.521
BNP level (pg/ml)												
Before treatment	3804.27 ± 805.63	3874.13 ± 715.22	0.821	3896.59 ± 757.38	3955.81 ± 1040.26	0.905	3784.62 ± 792.18	3851.78± 696.50	0.620	3951.47 ± 784.58	4077.34 ± 681.29	0.515
After treatment	1472.66 ± 423.23	2402.35 ± 636.82	< 0.0101	1616.32 ± 453.28	2634.16 ± 569.34	0.007	1128.32 ± 385.19	1997.25 ± 557.78	< 0.0001	1906.48 ± 421.86	3016.52 ± 657.62	< 0.0001
NYHA functional class			0.049			0.043			0.014			0.048
II	12	6		8	0		38	7		12	1	
III	14	1		2	3		70	34		38	10	
IV	15	1		2	1		25	3		20	13	
LVEDD (mm)												
Before treatment	63.05 ± 9.12	63.76 ± 8.13	0.839	63.45 ± 8.54	64.26 ± 7.39	0.870	62.57 ± 7.90	63.01 ± 6.96	0.702	64.02 ± 7.57	64.98 ± 8.25	0.635
After treatment	57.25 ± 7.06	62.91 ± 7.44	0.045	55.48 ±6.26	63.73 ± 5.07	0.031	55.18 ± 8.58	59.34 ± 6.79	0.003	59.05 ± 8.59	64.31 ± 7.43	0.015
LVEF (mm)												
Before treatment	31.95 ± 4.30	31.06 ± 4.69	0.600	32.04 ± 4.77	31.55 ± 5.10	0.865	32.85 ± 4.53	31.90 ± 4.17	0.211	31.39 ± 3.97	30.86 ± 4.28	0.616
After treatment	40.51 ± 4.58	36.82 ± 4.95	0.045	41.94 ± 3.92	36.72 ± 4.25	0.044	42.31 ± 4.19	37.86 ± 4.55	< 0.001	39.61 ± 4.81	35.23 ± 4.96	0.001
LVESD (mm)												
Before treatment	51.62 ± 7.29	53.44± 8.06	0.528	51.20 ± 5.83	53.07 ± 8.22	0.628	50.74 ± 7.50	52.95 ± 7.06	0.081	53.06 ± 8.00	54.97 ± 7.64	0.349
After treatment	40.53 ± 6.78	45.67 ± 5.08	0.048	40.03 ± 5.13	46.25 ± 3.68	0.046	42.40 ± 7.21	45.63 ± 6.74	0.008	44.26 ±7.33	48.09 ±6.95	0.042
LVESV (mm)												
Before treatment	61.63 ± 8.74	62.44 ± 7.82	0.809	61.27 ± 7.09	62.96 ± 8.12	0.700	60.95 ± 7.26	62.18 ± 7.92	0.329	62.59 ± 8.02	63.49 ± 7.30	0.654
After treatment	54.86 ± 6.38	60.11 ± 7.08	0.042	54.39 ±5.28	61.26 ± 5.06	0.042	55.16 ±6.54	58.39 ± 6.98	0.004	58.07 ± 7.11	62.89 ± 7.78	0.013
Complication												
Hypertension	9	3	0.350	2	1	0.770	58	18	0.515	10	10	0.145
Heart disease	7	2	0.596	2	0	0.360	71	23	0.303	13	12	0.144
Hypertension & heart disease	2	0	0.524	1	0	0.533	8	4	0.292	3	5	0.109
Dyslipidemia	28	5	0.749	3	2	0.409	71	19	0.964	28	20	0.183
6MWT (m)												
Before treatment	199.77 ± 82.59	185.65 ± 87.07	0.663	202.51 ± 79.54	190.43 ± 91.64	0.801	212.14 ± 92.50	196.59 ± 98.41	0.330	185.60 ± 87.52	171.16 ± 83.25	0.516
After treatment	432.61 ± 92.08	218.42 ± 84.31	< 0.011	421.94 ± 89.67	248.36 ± 87.22	0.005	487.25 ± 91.47	278.99 ± 89.10	< 0.001	378.21 ± 82.30	198.72 ± 90.14	< 0.001

Note: BNP, brain natriuretic peptide; NYHA, New York Heart Association; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; LVESV, left ventricle end systole volume; 6MWT, 6-minute walk test.

Table 5. Comparison of HRV of patients with chronic heart failure secondary to non-ischemic cardiomyopathy between the effective and ineffective groups

HRV	ε2/X		*P*	ε2/ε4		*P*	ε3/ε3		*P*	ε4/X		*P*
	Effective group (n=41)	Ineffective group (n=8)		Effective group (n=11)	Ineffective group (n=4)		Effective group (n=163)	Ineffective group (n=44)		Effective group (n=41)	Ineffective group (n=24)	
SDNN (ms)												
Before treatment	77.67 ± 9.30	76.84 ± 9.68	0.820	78.61 ± 10.15	76.46 ± 9.63	0.720	79.47 ± 10.28	77.46 ± 11.54	0.264	79.89 ± 12.13	74.41 ± 10.61	0.071
After treatment	112.37 ± 9.33	105.08 ± 8.21	0.045	112.29 ± 5.81	105.13 ± 4.94	0.048	112.70 ± 12.09	108.19 ± 9.66	0.023	109.82 ± 9.15	104.97 ± 8.68	0.040
SDANN (ms)												
Before treatment	72.74 ± 9.48	71.81 ± 10.05	0.803	72.93 ± 9.94	71.27 ± 10.22	0.781	73.16 ± 10.43	72.06 ±9.63	0.529	70.97 ± 9.08	69.28 ± 10.41	0.495
After treatment	102.45 ± 10.17	89.07 ± 9.82	< 0.001	103.84 ± 10.55	88.92 ± 9.16	0.027	104.67 ± 9.50	90.35 ± 10.62	< 0.001	100.47 ± 10.72	87.31 ± 11.15	< 0.001
RMSSD (ms)												
Before treatment	19.63 ± 2.35	18.96 ± 2.50	0.469	20.11 ± 1.95	19.43 ± 2.61	0.592	20.49 ± 2.66	19.74 ± 2.03	0.084	18.87 ± 2.53	18.14 ± 1.90	0.225
After treatment	26.07 ± 2.49	24.16 ± 2.07	0.048	25.95 ± 1.06	24.34 ± 1.32	0.029	26.19 ± 1.87	24.96± 2.58	0.001	23.96 ± 2.14	21.49 ± 2.28	< 0.001
pNN50												
Before treatment	8.78 ± 1.90	8.05 ±1.76	0.320	8.51 ± 2.04	8.11 ± 1.87	0.738	9.03 ± 1.77	8.62 ± 1.64	0.168	7.93 ± 1.60	7.06 ± 1.52	0.102
After treatment	11.94 ± 1.53	10.52 ± 1.38	0.019	12.39 ± 1.30	10.82 ± 1.01	0.049	13.14 ± 1.95	12.19 ± 1.76	0.004	11.07 ± 1.44	9.53 ± 1.98	0.001

Note: HRV, heart rate variability; SDNN, the standard deviation of NN intervals; SDANN, the standard deviation of the average NN intervals; RMSSD, root mean square of successive differences; pNN50, the proportion of NN50 divided by total number of NNs.

Table 6. Logistic regression analysis for risk factors influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy

Variable	B	S.E,	Wals	Sig.	Exp (B)	95% CI	
						Low	High
BNP level (after treatment)	0.006	0.001	26.342	0.000	1.006	1.004	1.009
LVEDD (after treatment)	0.102	0.047	4.589	0.032	1.107	1.009	1.215
LVEF (after treatment)	-0.214	0.085	6.331	0.012	0.807	0.683	0.954
LVESD (after treatment)	0.083	0.072	1.360	0.244	1.087	0.945	1.250
6MWT (after treatment)	-0.019	0.005	15.834	0.000	0.981	0.972	0.990
SDNN (after treatment)	-0.039	0.036	1.215	0.270	0.961	0.896	1.031
SDANN (after treatment)	-0.071	0.029	5.811	0.016	0.931	0.879	0.987
MSSD (after treatment)	-0.123	0.124	0.982	0.322	0.884	0.694	1.128
pNN50 (after treatment)	-0.668	0.226	8.731	0.003	0.513	0.329	0.798
ε4 allele	-0.217	0.085	6.576	0.010	0.805	0.682	0.950

Note: TMZ, trimetazidine; 95% CI, 95% confidence interval.

group ($P < 0.05$). At the same time, it was found that more patients with ε4 allele were found in the ineffective group ($P < 0.05$). SDNN, SDANN, RMSSD and pNN50 were all reduced in ε4 carriers compared to the ε2 carriers, ε3/ε3 carriers and ε2/ε4 carriers after treatment with TMZ ($P < 0.05$).

APOE gene ε4 allele and hypertension confirmed as risk factors influencing efficacy of TMZ

With the efficacy of TMZ as an independent variable, and BNP level (after treatment), LVEDD (after treatment), LVEF (after treatment), LVESD (after treatment), 6MWT (after treatment), SDNN (after treatment), SDANN (after treatment), MSSD (after treatment), pNN50 (after treatment), and ε4 allele as dependent variables, logistic regression analysis was performed to estimate risk factors influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy. It was found that ε4 allele, BNP level (after treatment), LVEDD (after treatment), LVEF (after treatment), 6MWT (after treatment), SDANN (after treatment), and pNN50 (after treatment) were confirmed as risk factors influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy ($P < 0.05$, **Table 6**).

Discussion

Chronic heart failure is a major health problem commonly resulting from ischemic or non-ischemic cardiomyopathy [17, 18]. Clinically, statins and coronary artery bypass grafts (CABG) have achieved a well-established role in treatment of ischemic cardiomyopathy [19, 20]. As for patients with non-ischemic cardiomyopathy, benefits from β-blockade and renin-angiotensin system blockade therapies have been obtained, very gradually [21, 22]. Application of TMZ in the management of ischemic or non-ischemic cardiomyopathy has recently been reported. Researchers have supported its functional role in increasing myocardial blood supply to improve heart failure [10]. Not surprisingly, in this study, 100 patients hospitalized for chronic heart failure secondary to non-ischemic cardiomyopathy yielded a 78% total effective rate. Due to existence of inter-individual differences in response to oral TMZ, this present study investigated association between *APOE* gene polymorphisms and efficacy of TMZ in chronic heart failure secondary to non-ischemic cardiomyopathy.

After TMZ treatment, LVEF was increased while BNP level, LVEDD, LVESD and LVESV were decreased in the effective group compared with ineffective group. 6MWD was longer and HRV was better in the effective group than ineffective group. These findings indicate that LVEF, BNP level, LVEDD, LVESD, LVESV, 6MWD and HRV could act as indicators to evaluate efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy. Patients with a marked improvement in their LVEF, following β-blockade treatment, exhibited an excellent prognosis [23]. BNP level is a clinical measure in diagnosing heart failure,

with higher BNP levels predicting worse prognosis [24]. Cetin et al. found significant differences in LVEF, BNP level, LVEDD, LVESD and LVESV between pre-treatment and post-treatment when in-group comparisons were performed [25], largely consistent with this present study. Patients with chronic heart failure, with lower exercise capacity at hospital discharge, have higher risk of readmission to the hospital, suggesting 6MWD as an independent predictor useful in assessing exercise capacity and prognosis [26]. HRV is the physiological phenomenon of variation in the time interval between heartbeats. It has been reported that reduced HRV is related with increased arrhythmic risk in patients following myocardial infarction, poor prognosis, and sudden cardiac deaths [27]. In previous studies, TMZ has been commonly investigated for its significant role in improving cardiac function and physical tolerance, thereby facilitating treatment of left ventricle dysfunction as well as remodeling in patients with ischemic heart disease [28, 29]. Fortunately, Isser and his team thought that TMZ, when added to optimal medical therapy, improves LVEF, exercise capacity, and quality of life in patients with stable non-ischemic heart failure [30].

Importantly, frequencies of *APOE* ε4 allele and ε4/ε4 genotype were lower in the effective group than ineffective group, suggesting that ε4 allele might be associated with efficacy of TMZ in treating chronic heart failure secondary to non-ischemic cardiomyopathy. *APOE* polymorphism is a genetic determinant of plasma levels of cholesterol and may affect risks of atherosclerosis and coronary heart disease [31, 32]. Lehtimäki et al. detected *APOE* phenotypes and plasma cholesterol in 1,564 subjects, finding that serum concentrations of total cholesterol and low density lipoprotein cholesterol (LDL-C) altered, according to *APOE* phenotype, and there were increases in the two variables of the order of ε2/2 less than ε3/2 less than ε4/2 less than ε3/3 less than ε4/3 less than ε4/4 [33]. Interestingly, increased plasma LDL-C levels have been proposed to be linked with higher risk of coronary heart disease, independently of non-lipid and lipid risk factors [34]. With insight from the National Health and Nutrition Examination Survey III (NHANES-III), Doran et al. supported the prognostic value of LDL-C measurement on long-term mortality in cardiovascular events [35]. As shown in this present study, NYHA functional class was enhanced, LVEF was lowered, and BNP level, LVEDD, LVESD and LVESV were increased, while 6MWD was shortened in ε4 carriers compared to the ε2 carriers, ε3/ε3 carriers and ε2/ε4 carriers. These results suggest that *APOE* gene ε4 allele may be a risk factor influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy. Besides, both Thayer et al. and Christensen et al. proposed a clinically relevant inverse relationship between plasma total cholesterol, LDL-C, and measurement of HRV, which might explain the fact that SDNN, SDANN, RMSSD and pNN50 were all reduced in ε4 carriers with high concentrations of plasma total cholesterol and LDL-C, compared to the ε2 carriers, ε3/ε3 carriers and ε2/ε4 carriers [36, 37]. According to logistic regression analysis, *APOE* ε4 allele was confirmed as a risk factor influencing efficacy of TMZ in treating patients with chronic heart failure secondary to non-ischemic cardiomyopathy.

In conclusion, this present study provides evidence that *APOE* gene polymorphisms may be associated with efficacy TMZ in chronic heart failure secondary to non-ischemic cardiomyopathy in a Chinese population. Consequently, *APOE* ε4 allele is a risk factor influencing efficacy of TMZ in treating chronic heart failure secondary to non-ischemic cardiomyopathy. Considering high regional susceptibility to the *APOE* gene, further studies with larger sample sizes performed in other geographic areas are necessary to strengthen the association between *APOE* gene polymorphisms and efficacy TMZ in patients with ischemic or non- ischemic heart disease.

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Disclosure of conflict of interest

None.

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