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Original Article

The correlation between ApoE gene polymorphism and non-ischemic chronic heart failure

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Abstract: Objective: To investigate the correlation between the distribution of Apo lipoprotein E (ApoE) gene polymorphism and the severity of chronic heart failure (CHF) secondary to non-ischemic cardiomyopathy (NICM). Methods: One hundred and twenty patients with CHF due to NICM and one hundred and eighty patients with CHF due to ischemic cardiomyopathy (ICM) treated in our hospital from January 2013 to December 2016 were selected as NICM group and ICM group, respectively. The genomic DNA of the two groups was extracted for the genotyping of ε locus of ApoE gene by PCR-RFLP. The left ventricular end-diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF) were measured by color Doppler ultrasonography. Results: In NICM group, the ε4/4 and ε3/4 genotypes and the frequency of allele ε4 were significantly higher than those in ICM group ($P < 0.05$); ε2/3 and ε2/4 genotypes and frequency of allele ε2 were significantly lower than ICM group ($P < 0.05$); but there was no significant difference between both group in ε2/2 and ε3/3 genotypes and ε3 frequency ($P > 0.05$). Logistic regression analysis showed that ε4/4 genotype and allele ε4 were the influencing factors of CHF secondary to NICM ($P < 0.05$). Patients in the NICM group showed different genotypes and different features in both LVEF and LVEDD, the order of which is: LVEDD (ε4/4) > LVEDD (ε3/4) > LVEDD (ε3/3) > LVEDD (ε2/4) > LVEDD (ε2/3) > LVEDD (ε2/2) ($P < 0.05$); LVEF (ε4/4) < LVEF (ε3/4) < LVEF (ε3/3) < LVEF (ε2/4) < LVEF (ε2/3) < LVEF (ε2/2) (all $P < 0.05$). Conclusion: ApoE gene polymorphism is closely related to CHF secondary to NICM, and CHF is more severe in NICM patients with ε4/4 type ApoE gene.

Keywords: Non-ischemic cardiomyopathy, chronic heart failure, apolipoprotein E gene, polymorphism

Introduction

Non-ischemic cardiomyopathy (NICM) is cardiomyopathy that caused by non-coronary artery disease and myocardial ischemia, which mainly involves dilated cardiomyopathy, hypertrophic cardiomyopathy and so forth. Recently, the incidence of NICM has significantly increased up to 15% [1, 2]. Moreover, NICM can easy to induce cardiac dysfunction and malignant arrhythmia, therefore, it is one of the main causes for heart failure and sudden death [3, 4]. Unlike ischemic heart disease, it's difficult for most NICM patients to draw attention to occult attack. Once clinical manifestations are observed, the patients have already developed to a serious chronic cardiac dysfunction [1, 5]. However, recent studies have reported that there are significant differences in the degree cardiomyopathy and process of chronic heart failure (CHF) in patients with NICM, and the researchers have found that the distribution of gene polymorphism is closely related, among which ApoE gene polymorphism is the most concerned, especially ApoE ε polymorphism in exon 4 [6]. Moreover, studies have indicated that ApoE gene has obvious variability, and the population is mainly characterized by exon 4 polymorphism, which is the missense mutation of ApoE ε gene [7]. The synthesis of ApoE in the

body is controlled by three alleles at the same locus, namely ε2, ε3 and ε4, so the polymorphism of ApoE gene is composed of three homozygous (ε2/2, ε3/3, ε4/4) and three heterozygous (ε2/3, ε3/4, ε2/4). Therefore, some researchers believe that the accumulation of small phenotypic effect induced by ApoE gene polymorphism complicates the role of NICM in the occurrence and development of CHF, however, their specific correlation is still unclear [8].

Therefore, this study analyzed the incidence of CHF secondary to NICM and ApoE ε genotyping in Chinese Han patients in our hospital, so as to analyze the correlation between the pathogenesis of CHF secondary to NICM and ApoE ε gene polymorphism and to explore the relationship between ApoE ε gene polymorphism and the degree of heart failure.

Materials and methods

Subjects

All the patients in the study were the Han Chinese, no sibship between each other, and they all voluntarily signed the informed consent. The study was approved by the Ethics Committee of our hospital.

A total of 120 patients (67 males and 53 females, aged 44-68 years with average age of 58.9±12.8 years) with CHF secondary to NICM hospitalized and treated in our hospital from January 2013 to December 2016 were selected as NICM group. Inclusion criteria: All patients were in line with the NICM diagnostic criteria of *Recommendations on diagnosis and treatment of cardiomyopathies* which promulgated by the Chinese Society of Cardiology in 2007 [9]; patients with complete clinical data; patients underwent cardiac color Doppler ultrasonography. Exclusion criteria: Patients with serious sustained rapid ventricular arrhythmia, pericardial disease or congenital heart disease; patients suffered from severe lung, liver, kidney and other vital organ dysfunctions or systemic diseases.

A total of 180 patients (95 males and 85 females, aged 47-75 years with average age of 61.8±14.5 years) with CHF secondary to ischemic cardiomyopathy (ICM) concurrently hospitalized and treat in our hospital were selected as ICM group. Inclusion criteria: The patients were diagnosed with ICM; patients with complete clinical data; patients underwent cardiac color Doppler ultrasonography. Exclusion criteria: patients with serious sustained rapid ventricular arrhythmia, pericardial disease or congenital heart disease; patients suffered from severe lung, liver, kidney and other vital organs dysfunctions or systemic diseases.

ApoE ε genotyping detection

Genomic DNA extraction: Five-milliliter of fasting venous blood were drawn from the two groups of patients with EDTA anticoagulant in the early morning, then the leukocytes were obtained, and the sample genomic DNA was extracted by using the blood kit from German Qiagen company. Subsequently, the extracted DNA sample was stored in the refrigerator at -80°C for later detection.

PCR-RFLP genotyping: The ApoE ε gene was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in the study. According to the previous literature, the primers were designed and synthesized as the followings: the upstream primer, 5'-AACAACTGACCCCGGTGGTGGCG-3'; the downstream primer, 5'-ATGGCGCTGAGGCCG-CGCTC-3' [10]. The total volume of PCR reaction system was 40 μl, including 10X PCR Buffer 4 μl, $MgCl_2$ (50 mmol/L) 1.5 μl, dNTP (10 mmol/L) 1.0 μl, upstream and downstream primers each 1.0 μl, template DNA 2 μl, Taq DNA polymerase (5U) 0.4 μl, and ddH_2O 29.0 μl. PCR reaction condition: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 30 s, renaturation at 57°C for 1 min, extension at 70°C for 1 min, 30 cycles, and then extension at 72°C for 10 min.

ApoE-ε gene identification: The above-mentioned enzyme-digested products were observed and photographed after polyacrylamide gel electrophoresis and ethidium bromide dyeing. A mixture with PCR amplification products 25 μl, 10X enzyme digestion buffer solution 3 μl, ddH_2O 1 μl and Hhal 1 μl was incubate at 37°C for 3 to 5 h.

The size of ApoE alleles were ε2 (91, 83, 38), ε3 (91, 48, 38, 35) and ε4 (72, 48, 38, 35); the size of 3 homozygotes of ApoE genotype were ε2/2 (91, 83, 38), ε3/3 (91, 48, 35) and ε4/4 (72, 48, 38, 35); the size of 3 heterozygotes were ε3/2 (91, 83, 48, 38, 35) and ε4/2 (91, 83, 72, 48, 38, 35). After the PCR amplification

Table 1. Basic information

Group	Cases	Age (y)	Sex ratio (male: female)	BMI (kg/m^2)	Hypertension history (%)	Hyperlipidemia history (%)	Diabetes (%)
ICM	180	61.8±14.5	95:85	19.2±2.7	77 (42.8)	64 (35.6)	55 (30.6)
NICM	120	58.9±12.8	67:53	18.6±3.0	50 (41.7)	44 (36.7)	35 (29.2)
t/X^2		1.777	6.735	1.803	6.205	6.412	6.571
P		0.077	0.060	0.072	0.081	0.075	0.068

Table 2. Comparison of genotype frequencies of ApoE ε (n, %)

Group	Cases	ε2/2	ε3/3	ε4/4	ε2/3	ε3/4	ε2/4
NICM Group	120	1 (0.8)	77 (64.2)	3 (2.5)	3 (2.5)	34 (28.3)	2 (1.7)
ICM Group	180	2 (1.1)	126 (70.0)	1 (0.6)	6 (3.3)	35 (19.4)	10 (5.6)
χ^2		8.045	5.236	11.445	8.363	9.896	10.531
P		0.055	0.061	0.001	0.041	0.012	0.002

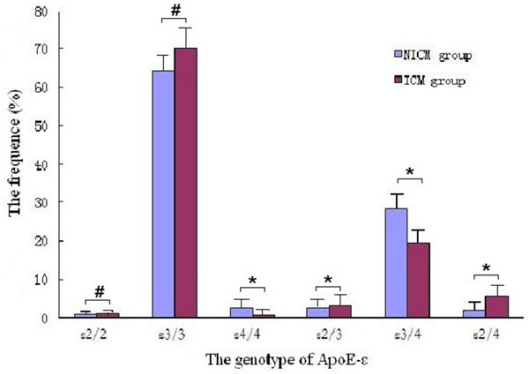

Figure 1. Comparison of ApoE ε genotype frequency in two groups. *P < 0.05, #P > 0.05.

products were digested with Hhal enzyme, the allele frequency and the ApoE ε2, ε3 and ε4 frequencies in the two groups were calculated.

Evaluation of degree of heart failure

Patients in both groups have applied heart color Doppler on admission, which was operated and measured by two experienced heart ultrasound physicians. Every patient's left ventricular end-diastolic diameter (LVEDD) value and left ventricular ejection fraction (LVEF) value were recorded. The average values of LVEDD and LVEF from the two physicians were used as the indicators for the evaluation of severity of patients' left heart failure.

Statistical methods

The statistical software SPSS 19.0 was used for statistics analysis. Measurement data were expressed by mean ± standard deviation; between-group comparisons were conducted by t test; comparison of LVEDD and LVEF of patients in the same group with different genotype was undertaken by one-way ANOVA. Enumeration data were expressed by percentage; between-group comparisons were conducted by X^2 test. Influencing factors of CHF were examined by Logistic regression analysis. And the difference was statistically significant when P < 0.05.

Results

Basic information

The difference of age, sex ratio, disease course and body mass index (BMI) between the two groups was not statistically significant (all P > 0.05, **Table 1**).

Comparison of genotype frequency and allele frequency

The genotype frequencies of ε4/4 and ε3/4 in NICM Group were significantly higher than those in ICM Group (both P < 0.05), while the ε2/3 and ε2/4 genotype frequencies were obviously lower than those in ICM Group (both P < 0.05). As for the genotype frequencies of ε2/2 and ε3/3, the between-group differences of them had no statistical significance (both P > 0.05). See **Table 2** and **Figure 1**.

The patients in NICM Group had an apparently higher allele frequency of ε4 and an obvious lower frequency of ε2, compared with those in ICM Group (both P < 0.05). But no statistical significance was found in the between-group comparison of ε3 frequency (P > 0.05). See **Table 3** and **Figure 2**.

Table 3. Comparison of allele frequencies of ApoE ε (n, %)

Group	Cases	ε2	ε3	ε4
NICM Group	120	7 (2.9)	191 (79.6)	42 (17.5)
ICM Group	180	20 (5.6)	293 (81.4)	47 (13.1)
X^2		9.908	4.241	8.335
P		0.011	0.094	0.042

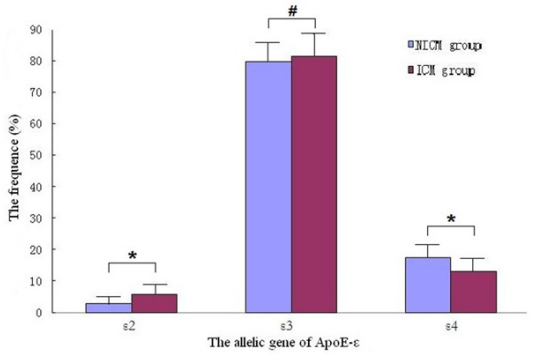

Figure 2. Comparison of ApoE ε allele frequency in two groups. *P < 0.05, #P > 0.05.

Logistic regression analysis of the risk factors of CHF secondary to NICM

In the Logistic regression analysis, group was set as a dependent variable (ICM Group = 0, NICM Group= 1). ApoE ε genotypes (ε2/2 = 0, ε3/3 = 1, ε4/4 = 2, ε2/3 = 3, ε3/4 = 4, ε2/4 = 5) and alleles (ε2 = 0, ε3 = 1, ε4 = 2) were set as independent variables. The results indicated that genotype ε4/4 and allele ε4 were risk factors of CHF secondary to NICM (both P < 0.05). See **Table 4**.

Comparison of degree of heart failure

No matter what genotype the NICM patients had, there were differences in both LVEDD and LVEF. The order of frequencies was: LVEDD (ε4/4) > LVEDD (ε3/4) > LVEDD (ε3/3) > LVEDD (ε2/4) > LVEDD (ε2/3) > LVEDD (ε2/2) (P < 0.05); LVEF (ε4/4) < LVEF (ε3/4) < LVEF (ε3/3) < LVEF (ε2/4) < LVEF (ε2/3) < LVEF (ε2/2) (both P < 0.05). See **Table 5** and **Figure 3**.

Discussion

The study has reported that about one-third NICM patients have the genetic basis, meanwhile, the onset of NICM is highly related to genetic mutation, among which ApoE gene has been the biggest concern in clinical study at present [11]. As ApoE plays an important role in metabolism of triglyceride and cholesterin in the body and it participates in the process of immunoregulation, activation of lipase, neuranagenesis and so on, the polymorphism of ApoE gene is considered to be associated with the pathogenesis of NICM [12]. Molecular genetics study suggests that the polymorphism of ApoE gene influences the level of different isomers of AopE by inheritance patterns, so as to have an effect on the occurrence of CHF secondary to NICM. Previous studies proved that isomer ApoE4 coded by ε2 allele could improve binding efficiency between ApoE and its receptor to enhance aerobic oxidation of glucose, inhibit oxidation of free fatty acids and reduce the use of free fatty acids on cardiac muscle and then could decrease intracellular acidosis caused by the accumulation of hydrogen ions and lactic acid, protect oxidation of mitochondria and inhibit cloudy swelling of mitochondria triggered by calcium ions [13, 14]; while the function of ε4 allele was opposite to it [15, 16].

Foreign scholars confirmed that the frequency of the ε4 allele was significantly increased in patients with dilated cardiomyopathy among Caucasian and Asian populations, so they thought that ε4 allele was a risk factor for the onset of dilated cardiomyopathy, conversely, ε2 allele had an obvious protective effect on dilated cardiomyopathy [17-19]. However, there has been little research on the relationship between ApoE gene polymorphism and CHF secondary to NICM so far, and no definite conclusion on this issue [20, 21]. Therefore, to provide evidence-based basis for clinical prevention and treatment, this study investigated the relationship between ApoE genotypes and allele frequencies of NICM patients with secondary CHF through the analysis of ApoE gene polymorphism distribution in NICM patients with secondary CHF among Chinese Han patients in our hospital.

The results of this study verified that ε4/4 and ε3/4 genotypes were predominant genotypes in patients with CHF secondary to NICM, whereas ε2/3 and ε2/4 genotypes were predominant genotypes in patients with CHF secondary to ICM; moreover, the frequency of ε4 allele was obvious higher among patients with CHF

Table 4. Logistic regression analysis about the risk factors of secondary chronic heart failure

Variable	β	SE	Wald value	OR value	95% CI	P
Constant	-0.002	2.674	12.341			0.796
Genotype						
ε2/2	0.237	0.814	7.004	1.268	(0.883, 1.625)	0.087
ε3/3	1.064	0.356	9.036	2.898	(1.967, 3.589)	0.066
ε4/4	1.532	0.625	6.235	4.627	(2.799, 6.437)	0.012
ε2/3	0.842	1.027	10.364	2.322	(1.724, 3.013)	0.095
ε3/4	1.106	0.714	5.175	3.021	(2.138, 3.958)	0.075
ε2/4	1.136	0.558	6.029	3.114	(1.902, 4.214)	0.059
Allele						
ε2	0.278	0.641	8.571	1.321	(0.421, 2.054)	0.074
ε3	1.127	0.420	7.538	3.087	(0.842, 4.986)	0.106
ε4	1.468	0.622	9.416	4.339	(2.986, 5.723)	0.028

Table 5. Comparison of degree of heart failure of patients with NICM in different genotype

Genotype	NICM Group	
	LVEDD (mm)	LVEF (%)
ε2/2	54.1±3.1	51.1±4.3
ε3/3	56.7±2.3	35.2±3.3
ε4/4	58.6±3.2	26.8±2.4
ε2/3	54.9±2.4	48.6±3.2
ε3/4	57.8±3.3	31.1±2.7
ε2/4	55.6±2.2	42.3±2.9
F	9.247	10.002
P	0.032	0.011

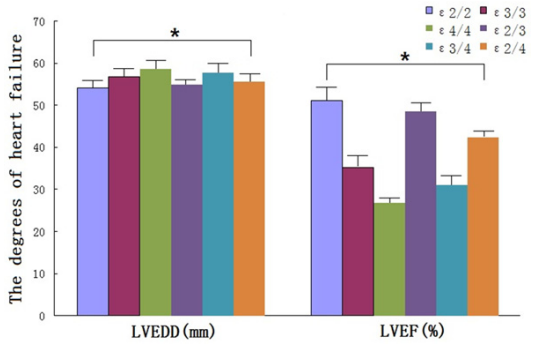

Figure 3. Comparison of degree of heart failure of patients with NICM in different genotype. *P < 0.05.

secondary to NICM, but the frequency of ε2 allele was obvious higher among patients with CHF secondary to NICM. These results suggested that ε4 allele and genotypes of ε4/4 and ε3/4 were susceptible genes for patients with CHF secondary to NICM, which deserved concern and further study. In addition, the frequency of ε3 allele was highest among Chinese Hanpatients in our hospital, indicating that ε3 allele might play a minor role in the pathologic process of CHF in patients with cardiomyopathy, which consisted with the previous study [22].

Logistic regression analysis was adopted to further prove that ε4/4 genotype and ε4 allele were risk factors of CHF secondary to NICM, in the meanwhile, the degree of heart failure in NICM patients with ε4/4 genotype was more serious while the degree of heart failure in NICM patients with ε2/2 genotype was minor, which was also confirmed by the analysis of clinical manifestations. However, due to the complicated pathogenesis of NICM, the risk factors of CHF secondary to NICM are also more complex. In other words, ApoE ε genotypes and alleles are only an important part among various related genes associated with the onset of NICM.

However, only one gene polymorphism cannot distinctly explain pathogenesis of CHF secondary to NICM. So, whether there are other vital related gene polymorphisms involved in the pathogenesis of CHF secondary NICM is worth further research. At the same time, the sample size of this study is relatively small, so the conclusions of this study need to be confirmed through further expanding sample size with multicenter.

In conclusion, ApoE gene polymorphism is closely related to CHF secondary to NICM, especially, the degree of CHF would be more serious when the NICM patients carrying ε4/4 ApoE genotype.

Disclosure of conflict of interest

None.

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References

[1] Kayvanpour E, Sedaghat-Hamedani F, Amr A, Lai A, Haas J, Holzer DB, Frese KS, Keller A, Jensen K, Katus HA and Meder B. Genotype-phenotype associations in dilated cardiomyopathy: meta-analysis on more than 8000 individuals. Clin Res Cardiol 2017; 106: 127-139.

[2] Peng S, Wu PS. A meta-analysis of the clinical curative effect of trimetazidine in the treatment of chronic heart failure secondary to non-ischemic cardiomyopathy among Chinese population. Clinical Focus 2011; 26 : 1848-1855.

[3] Li L, Bainbridge MN, Tan Y, Willerson JT and Marian AJ. A potential oligogenic etiology of hypertrophic cardiomyopathy: a classic single-gene disorder. Circ Res 2017; 120: 1084-1090.

[4] Mazurek S and Kim GH. Genetic and epigenetic regulation of arrhythmogenic cardiomyopathy. Biochim Biophys Acta 2017; 1863: 2064-2069.

[5] Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythm 2016; 32: 398-403.

[6] Traversy MT, Vandal M, Tremblay C, Tournissac M, Giguere-Rancourt A, Bennett AD and Calon F. Altered cerebral insulin response in transgenic mice expressing the epsilon-4 allele of the human apolipoprotein E gene. Psychoneuroendocrinology 2017; 77: 203-210.

[7] Vaisi-Raygani A, Rahimi Z, Nomani H, Tavilani H and Pourmotabbed T. The presence of apolipoprotein epsilon4 and epsilon2 alleles augments the risk of coronary artery disease in type 2 diabetic patients. Clin Biochem 2007; 40: 1150-1156.

[8] Cho KW, Lee J and Kim Y. Genetic variations leading to familial dilated cardiomyopathy. Mol Cells 2016; 39: 722-727.

[9] Cardiovascular disease branch of Chinese Medical Association, editorial board of Chinese Journal of Cardiology, Chinese suggested cardiomyopathy diagnosis and treatment group. The diagnosis and suggested treatment of cardiomyopathy. Chinese J Cardiol 2007; 35: 5-16.

[10] Prada D, Colicino E, Power MC, Weisskopf MG, Zhong J, Hou L, Spiro A 3rd, Vokonas P, Brenan K, Herrera LA, Schwartz J and Baccarelli AA. APOE epsilon4 allele modifies the association of lead exposure with age-related cognitive decline in older individuals. Environ Res 2016; 151: 101-105.

[11] Bartels K, Li YJ, Li YW, White WD, Laskowitz DT, Kertai MD, Stafford-Smith M, Podgoreanu MV, Newman MF and Mathew JP. Apolipoprotein epsilon 4 genotype is associated with less improvement in cognitive function five years after cardiac surgery: a retrospective cohort study. Can J Anaesth 2015; 62: 618-626.

[12] Zhang X, Xie J, Zhu S, Chen Y, Wang L and Xu B. Next-generation sequencing identifies pathogenic and modifier mutations in a consanguineous Chinese family with hypertrophic cardiomyopathy. Medicine (Baltimore) 2017; 96: e7010.

[13] Tully PJ, Peres K, Berr C and Tzourio C. The APOE epsilon 4 polymorphism does not predict late onset depression: the three-city study. Neurobiol Aging 2016; 40: 191, e199-110.

[14] Jurkovicova D, Goncalvesova E, Sedlakova B, Hudecova S, Fabian J and Krizanova O. Is the ApoE polymorphism associated with dilated cardiomyopathy? Gen Physiol Biophys 2006; 25: 3-10.

[15] Ulasova E, Perez J, Hill BG, Bradley WE, Garber DW, Landar A, Barnes S, Prasain J, Parks DA, Dell'Italia LJ and Darley-Usmar VM. Quercetin prevents left ventricular hypertrophy in the Apo E knockout mouse. Redox Biol 2013; 1: 381-386.

[16] McRobb L, Handelsman DJ and Heather AK. Androgen-induced progression of arterial calcification in apolipoprotein E-null mice is uncoupled from plaque growth and lipid levels. Endocrinology 2009; 150: 841-848.

[17] Lopes LR, Rahman MS and Elliott PM. A systematic review and meta-analysis of genotype-phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. Heart 2013; 99: 1800-1811.

[18] Svobodova H, Kucera F, Stulc T, Vrablik M, Amartuvshin B, Altannavch T and Ceska R. Apolipoprotein E gene polymorphism in the Mongolian population. Folia Biol (Praha) 2007; 53: 138-142.

[19] Marian AJ, van Rooij E and Roberts R. Genetics and genomics of single-gene cardiovascular diseases: common hereditary cardiomyopathies as prototypes of single-gene disorders. J Am Coll Cardiol 2016; 68: 2831-2849.

[20] Sun S, Fu J, Chen J, Pang W, Hu R, Li H, Tan L and Jiang Y. ApoE type 4 allele affects cognitive function of aged population in Tianjin city, China. Am J Alzheimers Dis Other Demen 2015; 30: 503-507.

[21] Chouinard-Watkins R and Plourde M. Fatty acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is it contributing to higher risk of cognitive decline and coronary heart disease? Nutrients 2014; 6: 4452-4471.

[22] Zhang J, Xuemei Z, Fan P, Liu R, Huang Y, Liang S, Liu Y, Wu Y and Bai H. Distribution and effect of apo E genotype on plasma lipid and apolipoprotein profiles in overweight/obese and non-obese Chinese subjects. J Clin Lab Anal 2012; 26: 200-205.