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Different associations of apolipoprotein E polymorphism with metabolic syndrome by sex in an elderly Chinese population

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ABSTRACT

The metabolic syndrome (MetS) is characterized by a cluster of metabolic disorders including abnormal lipid and lipoprotein metabolism. Apolipoprotein E (ApoE) is involved in the regulation of the metabolism of cholesterol, lipoproteins, and triglycerides. The common *ApoE* polymorphism has been found to be associated with cardiovascular disease and diabetes. This study evaluated the *ApoE* genetic polymorphism and its relation to MetS defined by the modified National Cholesterol Education Program and International Diabetes Federation criteria in a population-based cross-sectional survey of an elderly Chinese population in Beijing, China. Genotypes of 937 men and 1385 women were included in the study. All participants were measured for blood pressure, anthropometric measurements, and fasting concentrations of glucose, triglycerides, cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. We applied a logistic regression model to derive adjusted odds ratios (ORs) and their 95% confidence intervals. In this Chinese population, the ε2, ε3, and ε4 allele frequencies were 8.3%, 83.4%, and 8.3% for men and 8.7%, 82.9%, and 8.4% for women, respectively. In men, concentrations of fasting triglycerides were higher among the APOE2 and E4 subjects; and a lower level of high-density lipoprotein cholesterol was observed in the APOE4 group. There were approximately linear associations of low-density lipoprotein cholesterol levels with APOE genotype groups in both men and women. We observed that the ε4 allele was associated with a significantly increased OR of MetS defined by the modified National Cholesterol

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Education Program criteria in men (OR, 1.75; 95% confidence interval, 1.17-2.63). In summary, our data show that common polymorphism of *ApoE* gene is associated with the presence of MetS in an elderly Chinese population.

1. Introduction

The metabolic syndrome (MetS) is characterized by a cluster of metabolic disorders and defined clinically by the presence of at least 3 primary metabolic abnormalities among central obesity, dyslipidemia (high triglycerides or low high-density lipoprotein [HDL]), hyperglycemia, and high blood pressure [1-3]. The MetS and its individual diagnostic component have been reported to be associated with increased risk of cardiovascular disease and type 2 diabetes mellitus in different populations [4,5].

Apolipoprotein E (Apo E) is associated with very low-density lipoproteins (VLDLs), chylomicrons, and HDLs, and acts as ligands for lipoprotein receptors involved in clearance of VLDL and chylomicrons. It can regulate the plasma levels of cholesterol, lipoproteins, and triglycerides (TGs) [6]. The human *ApoE* gene, located at 19q13.2, comprises 4 exons and 3 introns. The common polymorphisms of *ApoE*, Cys112Arg (rs429358) and Arg158Cys (rs7412), encode 3 common isoforms, Apo E2 (Cys112 and Cys158), Apo E3 (Cys112 and Arg158), and Apo E4 (Arg112 and Arg158), respectively; the gene $\varepsilon2/\varepsilon3/\varepsilon4$ variants comprise 6 genotypes: 3 homozygous ($\varepsilon2/\varepsilon2$, $\varepsilon3/\varepsilon3$, and $\varepsilon4/\varepsilon4$) and 3 heterozygous ($\varepsilon2/\varepsilon3$, $\varepsilon2/\varepsilon4$, and $\varepsilon3/\varepsilon4$) [7].

The *ApoE* polymorphism can influence lipid transportation and metabolism, as well as play an important role in regulation of immune response and cell signaling mechanisms [6]. Compared with $\varepsilon3/\varepsilon3$ individuals, carriers of the $\varepsilon2$ allele have lower plasma cholesterol and higher TG levels [8-10]. In contrast, carrying $\varepsilon4$ allele is associated with higher plasma levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) [8-10]. Various studies have demonstrated the association between *ApoE* polymorphism and increased risk of coronary artery disease [8-11]. In addition, *ApoE* polymorphism has been investigated as a risk factor of other chronic diseases, including diabetes and Alzheimer disease [12-14].

Given the importance of *ApoE* in cholesterol, lipid, and TG metabolism, it is biologically possible that polymorphisms of this gene may influence an individual's susceptibility to MetS. However, the association between *ApoE* polymorphism and MetS in Asians has been evaluated in very few studies [15-17]. In the present study, we evaluate *ApoE* genetic polymorphism, ($\varepsilon2/\varepsilon3/\varepsilon4$), in relation to MetS in a population-based survey of an elderly Chinese population in Beijing, China.

2. Materials and methods

2.1. Study population

The study was a population-based cross-sectional survey in an elderly population sample conducted from April 2001 to March 2002 in Wanshoulu Community of Haidian District, a metropolitan area representative of the geographic and economic characteristics in Beijing, China. Detailed study methods have been published elsewhere [18]. In brief, subjects were randomly selected based on a 2-stage stratified sampling approach. First, 9 residential communities or streets (≈300-600 households) were randomly selected from a total of 94 residential communities in the Wanshoulu area. Second, all individuals were chosen from the selected street; but only one participant was selected from each household. A total of 2680 subjects 60 years or older were selected, and interviews were completed for 2334 subjects (87.1%) including 943 men (83.5%) and 1391 women (89.7%).

A structured questionnaire was administered to all study participants and covered demographic characteristics, prior disease history, physical activity, tobacco and alcohol use, and family history of diseases. For leisure time physical activity, participants were asked to report their most common sports activities and to estimate the duration (hour per day) of the sports activities they participated in. Participants were also asked when they began to drink alcohol at least once a month for 6 months, the types of beverage they drank, how many liangs (=50 g) were drunk per day during the regular drinking period, and when they changed their drinking habits, ignoring any recent changes. In-person interview and physical examinations were conducted by trained nurses and physicians. Current weight, standing height, waist and hip circumferences, and resting blood pressure were measured using a standard protocol. Body mass index (BMI) was computed as weight in kilograms divided by the square of height in meters (kg/m^2). Resting blood pressure measurements were taken twice from the right arm of participants in a sitting position after 30 minutes of rest; the average of these measurements was used in analyses.

In addition to in-person interview, overnight fasting blood samples were obtained from participants to measure plasma concentrations of lipids and glucose. Plasma glucose was measured using a modified hexokinase enzymatic method. Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C, and TG were measured enzymatically with available reagents following the manufacturer's protocol. The study was approved by the Committee for Medical Ethics of the Chinese PLA General Hospital, and signed informed consent was obtained from all participants.

Two commonly used definitions of MetS were applied to define prevalence of MetS in this study. The first definition of MetS was based on the modified US National Cholesterol Education Program (NCEP) Adult Treatment Panel III [19,20], wherein MetS was identified when 3 or more of the following criteria were present: (1) *high blood pressure* defined as resting blood pressure at least 130/85 mm Hg or known treatment of hypertension; (2) *hypertriglyceridemia* defined as TG at least 1.7 mmol/L (150 mg/dL); (3) *low HDL-C* defined as fasting HDL-C less than 1.0 mmol/L in men and less than 1.3 mmol/L in women; (4) *hyperglycemia* defined as fasting glucose of at least 5.6 mmol/L; and (5) *central obesity* defined as waist circumference greater than 88 cm or greater than 102 cm in women and men, respectively.

The second definition of MetS was the International Diabetes Federation (IDF) definition [21]. To have MetS, participants were required to have *central obesity* defined by waist circumference at least 90 cm in men and at least 80 cm in women for Chinese population and to also have any 2 of the following 4 additional factors: *hyperglycemia* defined as fasting plasma glucose at least 5.6 mmol/L, high blood pressure, hypertriglyceridemia, and reduced HDL-C; and the criteria for the 3 latter factors were the same as those of the NCEP.

2.2. Assessment of ApoE polymorphisms

High–molecular-weight genomic DNA was extracted from whole blood by the standard proteinase K-phenol-chloroform method. Multiplex tetra-primer amplification refractory mutation system (multiplex T-ARMS) polymerase chain reactions (PCRs) were performed with a combination of 6 primers for the 2 single nucleotide polymorphisms in a single reaction tube as described [22] with minor modification. The typical genotyping results obtained from multiplex T-ARMS PCR were also confirmed by DNA sequencing (Invitrogen, Beijing, China). The laboratory staff were blind to the identity of the subjects and their MetS status. As quality control measures, 15 reactions that contained 1 water, 6 quality control DNA samples, and 8 blinded DNA samples, respectively, were carried out for testing the concordance rate. The test experiments were done in duplicate. *ApoE* genotyping of 6 quality control DNA samples and 8 blinded DNA samples were finally confirmed by DNA sequencing. The concordance rate of quality control samples between the results coming from multiplex T-ARMS PCR and DNA sequencing was 100%. DNA from blood samples donated by 12 subjects was not included in the study because of being accidentally lost during DNA purification. Genotyping data were obtained from 99.4% of men (n = 937) and 99.6% of women (n = 1385).

Table 1 – Demographic, anthropometric, and plasma biochemical characteristics of the participants

	Men (n = 937)	Women (n = 1385)	*P* value
Age (mean ± SD)	69.0 ± 5.6	66.7 ± 6.0	<.01
Education (y) (%)			
0-6	27.3	51.0	
7-12	37.0	34.8	
≥13	35.7	14.2	<.01
Marital status (%)			
Married	92.8	78.6	
Unmarried/separated/divorced	0.4	0.5	
Bereft of spouse	6.8	20.9	<.01
Smoking status (%)			
Never	42.2	86.9	
Former	33.2	4.5	
Current	24.6	8.6	<.01
Current alcohol consumption (%)	30.0	5.1	<.01
Regular exercise (h/d) (%)			
<1	15.2	23.1	
1-3	57.8	56.3	
≥4	27.0	20.6	<.01
Hypertension (%)	47.2	50.4	.13
Diabetes mellitus (%)	15.7	15.4	.84
CHD (%)	32.8	36.4	.07
Stroke (%)	17.4	14.6	.07
Peripheral arterial disease (%)	14.7	23.1	<.01
Antihypertension medications[a] (%)	82.6	81.8	.74
Antidiabetic medications[b] (%)	66.4	70.8	.67
Lipid-lowering medications (%)	9.6	12.6	.03
Family history of CHD or stroke (%)	33.3	35.2	.35
ApoE genotype (n, %)			
ε2/ε2	10 (1.1)	10 (0.7)	
ε2/ε3	130 (13.9)	194 (14.0)	
ε2/ε4	5 (0.5)	27 (2.0)	
ε3/ε3	645 (68.8)	955 (69.0)	
ε3/ε4	144 (15.4)	193 (13.9)	
ε4/ε4	3 (0.3)	6 (0.4)	.08
ApoE allele (n, %)			
ε2	155 (8.3)	241 (8.7)	
ε3	1564 (83.4)	2297 (82.9)	
ε4	155 (8.3)	232 (8.4)	.86

Subjects with missing values were excluded from the analysis.
[a] Among subjects who reported to be diagnosed with hypertension.
[b] Among subjects diagnosed with diabetes.

Table 2 – Distributions of the *ApoE* genotypes by MetS status based on the modified NCEP and IDF criteria in Chinese elderly people

MetS definition	Men (%)		*P* value	Women (%)		*P* value
Modified NCEP	MetS (n = 205)	MetS-free (n = 732)		MetS (n = 631)	MetS-free (n = 754)	
ApoE genotype						
ε2/ε2	5 (2.4)	5 (0.8)		5 (0.8)	5 (0.7)	
ε2/ε3	29 (14.2)	101 (13.8)		93 (14.7)	101 (13.4)	
ε2/ε4	1 (0.5)	4 (0.5)		14 (2.2)	13 (1.7)	
ε3/ε3	125 (61.0)	520 (71.0)		425 (67.4)	530 (70.3)	
ε3/ε4	45 (21.9)	99 (13.5)		91 (14.4)	102 (13.5)	
ε4/ε4	0	3 (0.4)	.01	3 (0.5)	3 (0.4)	.90
ApoE genotype						
ε2 carriers	34 (16.7)	106 (14.6)		98 (15.9)	106 (14.3)	
ε3/ε3	125 (61.3)	520 (71.4)		425 (68.9)	530 (71.5)	
ε4 carriers	45 (22.0)	102 (14.0)	.01	94 (15.2)	105 (14.2)	.72
IDF	MetS (n = 325)	MetS-free (n = 609)		MetS (n = 748)	MetS-free (n = 635)	
ApoE genotype						
ε2/ε2	5 (1.5)	5 (0.8)		6 (0.8)	4 (0.6)	
ε2/ε3	46 (14.2)	84 (13.8)		100 (13.4)	94 (14.8)	
ε2/ε4	1 (0.3)	4 (0.7)		17 (2.3)	10 (1.6)	
ε3/ε3	219 (67.4)	425 (69.8)		519 (69.4)	435 (68.5)	
ε3/ε4	53 (16.3)	89 (14.6)		104 (13.9)	88 (13.9)	
ε4/ε4	1 (0.3)	2 (0.3)	.84	2 (0.3)	4 (0.6)	.76
ApoE genotype						
ε2 carriers	51 (15.7)	89 (14.7)		106 (14.5)	98 (15.7)	
ε3/ε3	219 (67.6)	425 (70.2)		519 (71.0)	435 (69.6)	
ε4 carriers	54 (16.7)	91 (15.1)	.70	106 (14.5)	92 (14.7)	.81

2.3. *Statistical analysis*

The χ^2 test was used to compare the distributions of *ApoE* alleles and genotypes between men and women. The exact χ^2 goodness-of-fit test was used to test for Hardy-Weinberg equilibrium of the genotypes. Characteristics of study participants were compared using the *t* test for continuous variables and χ^2 test for categorical variables. In addition, BMI, waist-to-hip ratio (WHR), and each component of MetS were compared by *ApoE* genotypes separately in both men and women using general linear model test with polynomial contrasts for linear trend and Tukey post hoc comparisons of the means. In these analyses, adjustment for age and BMI was made as appropriate (see footnotes to table for details). Unconditional logistic regressions were used to estimate multivariate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between *ApoE* genotypes and MetS according to sex. Potential confounders included in analyses were age, marital status, exercise, alcohol consumption, smoking status, BMI, and family history of coronary heart disease (CHD) or stroke. All statistical tests were based on 2-sided probability. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

3. Results

The distribution of selected demographic, anthropometric, and plasma biochemical characteristics among men and women is presented in Table 1. The average age of men and women was 69.0 and 66.7 years, respectively. There were significant differences between men and women in most of the factors. The allele distribution of *ApoE* genotypes among men and women is also summarized in Table 1. The most frequent genotype for subjects was ε3/ε3 (68.8% men, 69.0% women), followed by ε3/ε4 (15.4% men, 13.9% women), ε2/ε3 (13.9% men, 14.0% women), ε2/ε4 (0.5% men, 2.0% women), ε2/ε2 (1.1% men, 0.7% women), and ε4/ε4 (0.3% men, 0.4% women). The ε2, ε3, and ε4 allele frequencies were 8.3%, 83.4%, and 8.3% for men and 8.7%, 82.9%, and 8.4% for women, respectively. The frequencies of ε2, ε3, and ε4 alleles were consistent with Hardy-Weinberg equilibrium among both men and women.

In addition, Table 2 shows the frequencies of *ApoE* genotypes among participants with and without MetS defined by the modified NCEP and IDF criteria. Using the modified NCEP criteria, the frequencies of *ApoE* genotypes for men with MetS were significantly different from men who were MetS free; more men with MetS carried the ε4 allele than those without MetS. The frequencies of *ApoE* genotypes were similar among women with and without MetS based on the modified NCEP definition. Using the IDF definition, there was no difference in the frequencies of *ApoE* genotypes among subjects with and without MetS for both men and women.

To evaluate the effect of *ApoE* genotype, subjects in our study were subdivided into 3 groups: (1) ε3/ε3 subjects (APOE3 group), (2) subjects carrying ε2/ε2 or ε2/ε3 genotypes (ε2 carriers, APOE2 group), and (3) subjects carrying ε4/ε4 or ε3/ε4 (ε4 carriers, APOE4 group). Subjects with the ε2/ε4 genotype (n = 32) were excluded from the extra analyses because of the opposite effects of ε2 and ε4 alleles on lipid levels.

Table 3 – Comparison of clinical risk factor levels by *ApoE* genotypes stratified by sex in Chinese elderly people

	Men (n = 932)			P value	Women (n = 1358)			P value
	APOE2	APOE3	APOE4		APOE2	APOE3	APOE4	
BMI [a,b]	25.4 ± 3.2	25.3 ± 3.1	25.4 ± 3.1	.74	25.8 ± 3.7	25.9 ± 3.7	25.4 ± 3.7	.23
WHR [a,b]	0.90 ± 0.06	0.89 ± 0.05	0.89 ± 0.06	.25	0.84 ± 0.04	0.85 ± 0.06	0.85 ± 0.06	.24
Waist circumference [a,b]	90.5 ± 9.1	89.5 ± 9.1	90.5 ± 9.2	.32	86.0 ± 9.3	86.2 ± 9.3	85.6 ± 9.3	.69
Systolic blood pressure (mm Hg) [a,c]	132.9 ± 19.6	136.1 ± 17.8	135.5 ± 19.6	.89	138.9 ± 21.6	137.5 ± 21.6	137.2 ± 21.5	.64
Diastolic blood pressure (mm Hg) [a,c]	77.3 ± 10.2	77.6 ± 10.2	77.8 ± 10.2	.91	77.9 ± 10.4	76.7 ± 10.5	76.2 ± 10.4	.21
Triglycerides (mmol/L) [a,c]	1.53 ± 0.8 *	1.28 ± 0.8	1.51 ± 0.89 ‡	<.01	1.75 ± 1.1	1.65 ± 1.2	1.74 ± 1.1	.26
Total cholesterol (mmol/L) [a,c]	4.83 ± 2.3	5.19 ± 2.3	5.11 ± 2.3	.22	5.39 ± 1.00	5.49 ± 0.9	5.57 ± 1.0	.16
HDL-C (mmol/L) [a,c]	1.32 ± 0.2 †	1.30 ± 0.3	1.24 ± 0.2	.06	1.50 ± 0.4	1.43 ± 0.3	1.42 ± 0.42	.06
LDL-C (mmol/L) [a,c]	2.82 ± 0.8	3.20 ± 0.8 *	3.22 ± 0.7 †	<.01	3.14 ± 1.1	3.41 ± 1.2 *	3.47 ± 1.1 †	<.01
Fasting glucose (mmol/L) [a,c]	6.32 ± 1.7	6.01 ± 1.8	5.99 ± 1.7	.16	6.11 ± 2.0	6.20 ± 2.2	6.20 ± 2.0	.84

[a] Mean ± SD are presented after adjustment of age.
[b] General linear model adjusted for age with polynomial contrasts for linear trend.
[c] General linear model adjusted for age and BMI with polynomial contrasts for linear trend.
* $P < .05$ for Tukey post hoc comparison between APOE2 vs APOE3.
† $P < .05$ for Tukey post hoc comparison between APOE2 vs APOE4.
‡ $P < .05$ for Tukey post hoc comparison between APOE3 vs APOE4.

Plasma concentrations of TG, total cholesterol, HDL-C, LDL-C, and fasting glucose, and levels of blood pressure, BMI, WHR, and waist circumference are presented in Table 3 by APOE groups for men and women. There were no significant differences in levels of blood pressure, BMI, WHR, waist circumference, and fasting glucose in men and women stratified by APOE groups. For men in the APOE3 group, the TG level was significantly lower by 18% to 19% than those who were among either the APOE2 or APO4 group. Compared with men within the APOE3 and E2 groups, the concentration of HDL-C was lower for the APOE4 group. There were positive and approximately linear associations between LDL-C levels and APOE groups among both men and women. The LDL-C concentrations were significantly higher by 13% to 14% for men in the APOE3 or E4 group than those in the APOE2 group. Similarly, women in the APOE2 group had significantly lower LDL-C by 8% to 10% than those who were in the APOE3 or E4 group. Concentrations of TG, total cholesterol, and HDL-C were similar for women across APOE groups. To minimize the potential confounding attributed to use of medications related to plasma levels of lipid and apolipoprotein, further analyses excluded subjects who reported using cholesterol-lowering medications (n = 264); results did not show substantial changes in TG, total cholesterol, HDL-C, and LDL-C concentrations and

Table 4 – Association between *ApoE* genotype and individual component of MetS among men and women

APOE genotype	Men			Women		
	APOE3	APOE2	APOE4	APOE3	APOE2	APOE4
High blood pressure [a]						
Yes/no	463/182	107/33	117/30	706/249	147/57	146/53
OR (95% CI)	1.00 (reference)	1.29 (0.83-2.00)	1.60 (1.02-2.52)	1.00 (reference)	0.90 (0.63-1.28)	1.03 (0.72-1.47)
Hyperglycemia (glucose ≥ 5.6 mmol/L) [a]						
Yes/no	317/328	71/69	78/69	485/469	94/110	108/91
OR (95% CI)	1.00 (reference)	1.25 (0.83-1.87)	1.42 (0.96-2.10)	1.00 (reference)	0.73 (0.51-1.05)	0.89 (0.63-1.27)
Hypertriglyceridemia [a]						
Yes/no	125/520	37/103	50/97	316/638	82/122	62/137
OR (95% CI)	1.00 (reference)	1.50 (0.97-2.33)	2.05 (1.36-3.10)	1.00 (reference)	1.43 (1.04-1.96)	0.96 (0.69-1.35)
Low HDL-C [a]						
Yes/no	103/542	20/120	30/117	339/615	67/137	85/114
OR (95% CI)	1.00 (reference)	0.85 (0.50-1.44)	1.22 (0.76-1.96)	1.00 (reference)	0.90 (0.65-1.25)	1.43 (1.05-1.97)
Central obesity NCEP [b]						
Yes/no	50/594	14/126	14/131	418/537	94/110	81/117
OR (95% CI)	1.00 (reference)	1.31 (0.69-2.46)	1.24 (0.66-2.33)	1.00	1.08 (0.79-1.48)	0.86 (0.63-1.19)
Central obesity IDF [b]						
Yes/no	331/313	77/63	77/68	737/218	164/40	149/49
OR (95% CI)	1.00 (reference)	1.13 (0.78-1.64)	1.03 (0.71-1.49)	1.00 (reference)	1.25 (0.85-1.84)	0.88 (0.61-1.26)
Central obesity China [b]						
Yes/no	442/203	103/37	110/36	704/251	155/49	147/51
OR (95% CI)	1.00 (reference)	1.27 (0.84-1.93)	1.42 (0.93-2.15)	1.00 (reference)	1.16 (0.81-1.67)	1.00 (0.70-1.42)

[a] Adjusted for age, education, marital status, exercise, alcohol consumption, smoking status, BMI, and family history of CHD or stroke.
[b] Adjusted for age, education, marital status, exercise, alcohol consumption, smoking status, and family history of CHD or stroke.

Table 5 – Association between *ApoE* genotypes and MetS defined by the modified NCEP and IDF definitions

APOE genotype	Men			Women		
	APOE3	APOE2	APOE4	APOE3	APOE2	APOE4
No. of components of MetS by modified NCEP						
≥1[a]						
Yes/no	535/110	122/18	132/15	865/90	186/18	179/20
OR (95% CI)	1.00 (reference)	1.41 (0.82-2.41)	1.84 (1.03-3.27)	1.00 (reference)	1.07 (0.63-1.84)	0.90 (0.54-1.51)
≥2[a]						
Yes/no	278/367	69/71	81/66	640/315	135/69	128/71
OR (95% CI)	1.00 (reference)	1.28 (0.88-1.85)	1.57 (1.09-2.26)	1.00 (reference)	0.99 (0.71-1.37)	0.88 (0.64-1.22)
≥3						
Yes/no	99/545	30/110	34/111	367/587	84/120	80/118
OR (95% CI)	1.00 (reference)	1.46 (0.92-2.31)	1.60 (1.03-2.51)	1.00 (reference)	1.13 (0.83-1.55)	1.08 (0.79-1.48)
≥4						
Yes/no	20/625	7/133	11/136	168/787	39/165	32/167
OR (95% CI)	1.00 (reference)	1.70 (0.70-4.14)	2.37 (1.10-5.11)	1.00 (reference)	1.10 (0.75-1.63)	0.88 (0.58-1.34)
MetS by modified NCEP[a]						
Yes/no	125/520	34/106	45/102	425/530	98/106	94/105
OR (95% CI)	1.00 (reference)	1.30 (0.84-2.02)	1.75 (1.17-2.63)	1.00 (reference)	1.16 (0.86-1.58)	1.12 (0.82-1.52)
No. of components of MetS by IDF						
≥1[a]						
Yes/no	552/93	123/17	134/13	868/87	186/18	180/19
OR (95% CI)	1.00 (reference)	1.27 (0.73-2.21)	1.73 (0.93-3.19)	1.00 (reference)	1.04 (0.61-1.79)	0.94 (0.56-1.59)
≥2[a]						
Yes/no	334/331	76/64	88/59	601/354	121/83	128/71
OR (95% CI)	1.00 (reference)	1.12 (0.77-1.62)	1.35 (0.94-1.95)	1.00 (reference)	0.87 (0.63-1.18)	1.06 (0.77-1.45)
≥3[a]						
Yes/no	104/541	32/108	43/104	288/667	64/140	73/126
OR (95% CI)	1.00 (reference)	1.51 (0.96-2.38)	2.05 (1.35-3.11)	1.00 (reference)	1.08 (0.78-1.51)	1.35 (0.98-1.86)
≥4[a]						
Yes/no	18/627	4/136	10/137	88/867	19/185	20/179
OR (95% CI)	1.00 (reference)	1.04 (0.34-3.15)	2.50 (1.12-5.61)	1.00 (reference)	0.98 (0.58-1.65)	1.09 (0.65-1.82)
MetS by IDF[a]						
Yes/no	219/425	51/89	54/91	519/435	106/98	106/92
OR (95% CI)	1.00 (reference)	1.10 (0.75-1.61)	1.12 (0.77-1.63)	1.00 (reference)	0.91 (0.67-1.24)	0.95 (0.70-1.30)

[a] Adjusted for age, education, marital status, exercise, alcohol consumption, smoking status, and family history of CHD or stroke.

the observed patterns with APOE groups in both men and women (data not shown).

We also examined the associations of individual components of MetS (ie, high blood pressure, hyperglycemia, hypertriglyceridemia, low HDL-C, central obesity) with APOE groups in men and women (Table 4). Compared with men within the APOE3 group, men in the APOE4 group were significantly more likely to have high blood pressure (OR = 1.60; 95% CI, 1.02-2.52) and hypertriglyceridemia (OR = 2.05; 95% CI, 1.36-3.10). Men in the APOE2 group also demonstrated greater likelihood of hypertriglyceridemia than men in the APOE3 group, although this association was not statistically significant. Compared with the APOE3 group, the APOE4 group was marginally associated with increased likelihood of hyperglycemia in men. The APOE groups were not associated with the odds of low HDL-C and central obesity in men.

Compared with women in the APOE3 group (Table 4), significantly higher likelihood of hypertriglyceridemia was seen among women in the APOE2 group (OR = 1.43; 95% CI, 1.04-1.96), whereas women in the APOE4 group had increased likelihood of low HDL-C (OR = 1.43; 95% CI, 1.05-1.97). The presence of high blood pressure, hyperglycemia, and central obesity by different cutoff points did not differ significantly by APOE groups in women after adjustment for age and other potential confounders.

Further analyses on the associations between APOE groups and MetS prevalence based on the modified NCEP and IDF criteria are shown in Table 5. The adjusted OR for MetS defined by the modified NCEP criteria was 1.75 (95% CI, 1.17-2.63) comparing men in the APOE4 group to men in the APOE3 group. However, using the IDF definition, the ORs for MetS were not significantly different when comparing men in either the APOE2 or APOE4 group to men in the APOE3 group. In addition, when using either the modified NCEP or the IDF criteria to define MetS components, we found positive associations between having more MetS components and the APOE4 group in men.

For women, there were no associations of APOE genotypes with the likelihood of MetS defined by the modified NCEP and IDF definitions (Table 5). Similarly, we did not find associations between APOE groups and the number of MetS components defined by different criteria among women. Additional analyses excluding subject who reported use of cholesterol-lowering medications did not appreciably change the likelihood estimates presented in Tables 4 and 5.

4. Discussion

Given the importance of Apo E in lipid and lipoprotein metabolism, it is conceivable that polymorphism in the *ApoE* gene might serve as a potential determinant of MetS, which is characterized by a cluster of metabolic disorders including abnormal lipid and lipoprotein metabolism [1-3]. To our knowledge, this study is the first to assess the associations between the common *ApoE* polymorphisms and MetS in a large population-based survey in an elderly Chinese population. We found that the ε4 allele was significantly associated with MetS prevalence defined by the modified NCEP definition in men, whereas MetS prevalence defined by the modified NCEP or IDF criteria was not associated with *ApoE* polymorphism in women.

The *ApoE* gene is biosynthesized in the liver, brain, spleen, kidney, and other tissues; and the common polymorphism of *ApoE* has been frequently studied in different populations. The distributions of ε2, ε3, and ε4 alleles in our study population were in Hardy-Weinberg equilibrium in both men and women and were similar to previous reports in the Chinese population [11,23].

Determined by different structures that arise from different *ApoE* (ε2, ε3, and ε4) alleles [7,24], Apo E isoforms show different biological activities through their interactions with lipoprotein receptors, ultimately influencing the circulation levels of cholesterol. Because of different receptor binding activities [24], Apo E4 is associated with higher and E2 with lower levels of total plasma cholesterol and LD-C compared with the E3 [8-10]. Results of previous studies examining the relationship between *ApoE* polymorphism and plasma TG levels have been inconsistent, which in part may be due to variation by sex and study design including young participants [8,23,25,26]. Generally consistent with previous work on associations of APOE groups with plasma lipid levels [8,23,25,26], we found approximately linear associations of APOE genotypes with the concentrations of fasting LDL-C in study participants. In addition, in men, concentrations of fasting TG were higher among the APOE2 and E4 subjects; and lower HDL-C was observed in the APOE4 group.

Several studies have examined the relationship of *ApoE* polymorphism with MetS in different populations. In a cross-sectional study with 552 cardiovascular patients without diabetes and lipid-lowering therapy, carrying ε4 allele was found to be positively associated with MetS prevalence; and ε4 carriers were more likely to have high blood pressure by the NCEP MetS criteria [27]. Similarly, Sima et al [28] found that the frequency of the ε4 allele was higher in the MetS group than in the control group; and *Apo* ε4/ε3 genotype was correlated with high levels of TG, glucose, and obesity, whereas it was negatively correlated with HDL-C. Later, a small-scale survey study conducted in an Indian population also showed that carriers of ε4 allele had higher occurrence of dyslipidemia [29]; and there was a positive association between the combination of *Apo* ε4/ε4 and angiotensin-converting enzyme deletion/deletion genotype and NCEP MetS prevalence [15]. In another recent study of young Asian Indian patients with acute myocardial infarction, no associations were found between *ApoE* polymorphism and MetS defined by either the NCEP or the IDF definition [16]. However, none of these studies examined the associations by sex because of limited sample size. It has been suggested that the association of cardiovascular disease with *ApoE* polymorphism may vary by sex [30-32]. Our results provide evidence that carrying the ε4 allele was associated with higher likelihood of MetS in men, particularly when using the modified NCEP MetS criteria. The absence of a significant association between *ApoE* polymorphisms and MetS among women in the present study further adds to the extant evidence that sex modifies the effect of the *ApoE* on cardiometabolic risk factors.

Several definitions of MetS, including the modified NCEP and IDF definitions, have been approved by national or international organizations for research and/or clinical purpose; however, there is no a universally accepted definition. The IDF definition requires central obesity as a requisite for diagnosis of MetS and proposes ethnicity-specific cutoff points for waist circumference [21], which bring a higher prevalence of MetS than the NECP in the Chinese population [33]. It was reported that the agreement between the IDF and the NCEP definitions for identifying subjects with MetS was poor in men and moderate in women, with many lean subjects with hypertension and/or dyslipidemia undetected by the IDF definition in Asian populations [33]. This may partially explain the lack of association between APOE genotypes and IDF-defined MetS among men in our study.

It is possible that the *ApoE* polymorphisms may have separate effects on individual components of MetS. Our study showed that the ε2 allele was significantly associated with hypertriglyceridemia after adjustment for age and other confounders in both men and women, which can be explained by slower clearance rate of VLDL and remnants for subjects carrying the ε2 allele [7,34]. Consistent with previous results [8,32], we found a positive association between the ε4 allele and a higher likelihood of hypertriglyceridemia in men. These findings suggest that Apo E4 may more significantly influence plasma lipase activity and delay circulating triglyceride clearance in men [25,32]. Moreover, our study showed the tendency of positive associations between ε4 allele and high blood pressure and hyperglycemia in men. Our findings were consistent with previous studies linking low HDL-C level with the ε4 allele in women [26,32]. Although the biological mechanisms underlying the observed difference by sex are not known, one potential explanation may involve the relation between *ApoE* polymorphism and sex-hormone levels. It has been shown that *ApoE* ε4 is associated with lower testosterone levels in men [35]. Several studies have found that reduced testosterone levels are associated with higher total cholesterol, LDL-C, and TG levels; androgen deficiency is emerging as a risk factor of MetS in men [36]. Interestingly, in women, it is known that the production androgen, the precursor of estrogen, declines after the menopause. The association of *ApoE* with testosterone and dehydroepiandrosterone has been investigated in postmenopausal women without hormone replacement therapy; however, results were inconsistent, with strong [37] and null [38] association between *ApoE* ε4 allele and higher levels of testosterone and dehydroepiandrosterone in postmenopausal women. Overall, our results indicate that a complex interaction between sex,

ApoE, and MetS may exist; and further studies are needed to elucidate a mechanism.

As in any cross-sectional study of this kind, the strengths and weakness of the study need to be taken into account when considering the findings. The strengths of this study include the population-based study design and relatively large sample size of elderly participants, which enabled sex-specific analyses and more stable estimates of association. However, several limitations should be considered when evaluating our results. The cross-sectional study design limits our ability to identify causal relationship. Whether similar associations are seen for *ApoE* polymorphisms and risk of developing the MetS remains to be investigated. Among the limitations, the lack of response among eligible subjects has the potential for selection bias; but no significant differences were detected between subjects with complete and those with incomplete data. Moreover, it is unlikely that there was a difference in participation of subjects by *ApoE* polymorphism. Because of the nature of self-reported information, misclassification of prior medical history was unavoidable. However, it is unlikely that biased recall of medical history would be related to genetic polymorphism; and thus, this would not differentially affect the comparisons. A further concern was that fasting concentrations of HDL-C, TG, and glucose were measured only once, which might have led to random errors.

In summary, we found a significant association between APOE groups and MetS prevalence and increasing number of MetS components in elderly men within a Chinese population. No such association was seen in women. These findings suggest that *ApoE* polymorphism may have an etiologic role in MetS; it is critically important to develop different public health strategies for men and women to prevent, detect, and treat MetS in an elderly population in China. Prospective studies are needed to confirm this hypothesis.

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