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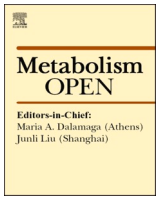

Sex-specific differences in the association between APOE genotype and metabolic syndrome among middle-aged and older rural Indians

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ABSTRACT

Background: Metabolic syndrome (MetS), characterized by elevated blood pressure, high blood glucose, excess abdominal fat, and abnormal cholesterol or triglyceride levels, significantly increases the risk of various non-communicable diseases. This study focuses on understanding the sex-specific association between Apolipoprotein E (APOE) polymorphism and MetS among middle-aged and older adults in rural southern India.
Methods: This cross-sectional study utilized data from the Centre for Brain Research-Srinivaspura Aging, Neuro Senescence, and COGnition (CBR-SANSCOG) study. Participants (n = 3741) underwent comprehensive clinical assessments and blood investigations, including APOE genotyping. MetS was defined using the National Cholesterol Education Program – Adult Treatment Panel III (NCEP ATP III) and the Consensus criteria. Statistical analyses, including chi-square tests, ANCOVA, and logistic regression, were conducted to explore the association of APOE genotype with MetS and its components, stratified by sex.
Results: Females carrying the APOE E4 allele had 1.31-fold increased odds of MetS (95 % CI: 1.02,1.69, p = 0.035) according to the NCEP ATP III criteria but not when the Consensus criteria were applied. The study also noted sex-specific differences in the association of APOE with various MetS components, including lipid levels and waist circumference.
Discussion: Our findings reveal a sex-specific association between the APOE E4 allele and MetS, with only females having an increased risk. This study contributes to the understanding of the genetic underpinnings of MetS and highlights the importance of considering sex-specific differences in MetS research and its prevention strategies. This study underscores the complexity of MetS etiology and emphasizes the need for further research to elucidate the role of genetic, environmental, and lifestyle factors in its progression, particularly in sex-specific contexts.

1. Introduction

Metabolic syndrome (MetS), also known as syndrome X, is a global, polygenic, and multifactorial metabolic disorder, which is characterized by elevated blood pressure, high blood glucose, excess abdominal fat, and abnormal cholesterol or triglyceride (TG) levels [1]. These factors individually increase the risk of cardiovascular diseases and together possess an additive effect. In addition, MetS has been linked to a significantly increased risk for other non-communicable diseases, such as type 2 diabetes, stroke, cancer, and all-cause mortality [2].

In the backdrop of India's rapidly growing economy, shifts in demography and lifestyle changes, there has been an almost 10 % increase in mortality due to non-communicable diseases over the last three decades [3] despite improvements in health infrastructure. In line with this trend, the prevalence of MetS has increased to around 30 %, at par with that in high-income countries [4]. Thus, there is an imminent need to better understand MetS and its associated factors among Indians to help develop strategies for its prevention.

A complex combination of environmental factors is involved in the pathogenesis of MetS; however, the influence of genetic factors has [5] been relatively understudied. Genetic predisposition is estimated to contribute to about half the risk for individual MetS components and 30 % to the overall MetS phenotype [6].

The apolipoprotein E (APOE) gene is a gene that codes for a multifunctional protein (APOE), which is known to play a key role in lipid transport and metabolism, in addition to regulation of immune

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responses and cellular signaling processes [7]. The APOE gene is located at chromosome 19, composed of 299 amino acids, and consists of four exons and three introns [8]. Three alleles are commonly present in humans, designated as ε2, ε3, and ε4, which code for three major isoforms E2, E3 and E4, respectively [9]. Therefore, polymorphism leads to six different genotypes, three homozygous (E2/2, E3/3, and E4/4) and three heterozygous (E2/3, E2/4, and E3/4).

Different APOE isoforms show different biological activities through their interactions with lipoprotein receptors, ultimately influencing the circulating levels of cholesterol [8]. Compared with E3-carrying individuals, E2 carriers have lower plasma total cholesterol, while E4 carriers have higher plasma levels of total cholesterol (TC) and low-density lipoprotein (LDL) [7]. As lipid abnormalities are an important component of MetS, it is likely that E4 carriers could have an increased risk for MetS. Various studies have demonstrated the association between APOE E4 and increased risk of Alzheimer's disease [10]. In addition, different APOE alleles have been investigated as risk factors for other diseases, including age-related macular degeneration [11], hearing loss [12], vision impairment [12], and severity of Lewy body pathology [13].

However, the prevalence of MetS and its individual components such as abdominal obesity, insulin resistance and lipid abnormalities differ by sex [14–18]. There has been conflicting evidence on the sex-specific risk for MetS, with some studies showing higher incidence in males [14,15], while the reverse is the case in others [16,17]. These sex-specific differences could be due to associated lifestyle related factors, such as physical inactivity, tobacco usage and alcohol usage [18]. Another possible mechanism could be via the differential effect of genetic factors such as APOE. Yet studies on the sex-specific associations for APOE with MetS are extremely limited. To the best of our knowledge, there are no studies that have investigated this association in the Indian population.

In the present study, we evaluate the sex-specific association between APOE polymorphism and MetS and its components among middle-aged and older adults from rural southern India. We hypothesize that there is a sex-specific association between APOE E4 and MetS in this population.

2. Methods

2.1. Study design and recruitment

This was a cross-sectional study among middle-aged and older adults belonging to an ongoing prospective aging cohort study in rural India, namely, the Centre for Brain Research - Srinivaspura Aging, Neuro Senescence and COGnition (CBR-SANSCOG) study. CBR-SANSCOG cohort participants are recruited through an area sampling strategy from the rural areas in Srinivaspura, located in the southern Indian state of Karnataka.

2.2. Participants

CBR-SANSCOG participants are individuals without dementia aged 45+ years and hail from a predominantly agrarian community. Exclusion criteria for the CBR-SANSCOG cohort are individuals diagnosed with dementia, severe psychiatric illness, terminal medical illness, and hearing or visual or locomotor impairment that could affect the study assessments. The details of the study protocol and recruitment strategy have been published as separate papers [19,20].

For the present study, we included CBR-SANSCOG cohort participants (n = 3741) who had complete data on their baseline clinical assessments and blood investigations (biochemical and APOE genotyping) that were conducted from January 1, 2018 to April 30, 2023. Participants with the APOE ε2/ε4 genotype were excluded because of the potentially opposite effects of ε2 and ε4 alleles on lipid levels.

2.3. Ethics clearance and informed consent

The CBR-SANSCOG study has obtained ethics clearance from the Institutional Human Ethics Committee of the Centre for Brain Research, Indian Institute of Science, Bangalore (CBR/42/IEC/2022–23). All participants provided written, informed consent before undergoing the study assessments.

2.4. Clinical assessments

2.4.1. Historical information

A structured questionnaire was administered to all study participants, which covered demographic characteristics, clinical history of prior diagnosis of medical conditions, such as diabetes mellitus, hypertension, dyslipidemia, cardiac illness as well as information on physical activity, tobacco usage, and alcohol usage.

2.4.2. Waist circumference

By using a non-stretchable standard measuring tape, the waist circumference was measured at the point where the line just above the uppermost lateral border of the right ilium intersects with the body's midaxillary line. The circumference was recorded with precision to the nearest 0.1 cm.

2.4.3. Blood pressure

Systolic and diastolic blood pressure of the participants were measured from all four limbs in both supine and standing positions to the nearest 2 mmHg using a mercurial sphygmomanometer (Diamond Deluxe BP apparatus, Industrial Electronic & Allied Products). Each participant's systolic/diastolic BP was taken to be average of the total available systolic/diastolic BP measurements.

2.5. Blood investigations

2.5.1. Biochemical investigations

A total volume of 15 ml of peripheral venous blood was drawn after overnight fasting using vacutainers, by skilled phlebotomists, for a range of tests, including biochemical, hematological, and genetic analyses in the CBR-SANSCOG study protocol. The plasma glucose level was determined using the hexokinase method. Serum TC, LDL, HDL and TG levels were estimated using colorimetric enzymatic methods.

2.5.2. APOE genotyping

Whole blood samples were used to extract genomic DNA utilizing the Genomic DNA isolation kit from MACHEREY-NAGEL, Germany. This genomic DNA was then diluted to a concentration of 50 ng/ml with nuclease-free water for the analysis of APOE genotypes. Polymerase Chain Reaction (PCR) was conducted using the EmeraldAmp® GT PCR master mix by Takara Bio. Sci., Japan in a volume of 50 ml. The primers specifically targeting human APOE were: forward primer, 5′-CGCGGGCACGGCTGTCCAAGGA-3′, and reverse primer, 5′ GCCCCGGCCTGGTACACTGCCA-3'. After PCR, the products were resolved using 2 % agarose gel electrophoresis (AGE), trimmed to remove unnecessary segments, and then weighed. Gel extraction was carried out employing either the QIA Quick Gel Extraction Kit or the NucleoSpin Gel and PCR Clean-up Mini kit, in accordance with the guidelines provided by the manufacturer. The final PCR products were suspended in TE buffer and their sequences determined using Sanger sequencing by Applied Biosystems.

Based on the APOE genotype, participants were categorized into three distinct groups. The E2 carrier group included those with either ε2/ε2 or ε2/ε3 genotype, the E3 carrier group was composed of participants with the ε3/ε3 genotype, and the E4 carrier group comprised individuals with either the ε4/ε4 or ε3/ε4 genotype.

2.6. MetS definition

Two definitions were used to define MetS as follows.

(i) *National Cholesterol Education Program – Adult Treatment Panel III (NCEP ATP III) definition:* As per this definition, MetS is present, if three or more following criteria are fulfilled: (1) High blood pressure defined as systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg or on drug treatment for hypertension; (2) Hyperglycemia defined as fasting blood glucose ≥100 mg/dl or on drug treatment for diabetes; (3) Hypertriglyceridemia defined as TG ≥ 150 mg/dl or on drug treatment for elevated TG; (4) Low HDL defined as HDL <40 mg/dl for male and <50 mg/dl for female or on drug treatment for low HDL; and (5) Waist circumference of ≥102 cm in male or ≥ 88 cm in female [21].

(ii) *Consensus criteria*: We selected the cut offs specific to South Asian populations, wherein the waist circumference cut-offs are ≥90 cm in males or ≥ 80 cm in females; the rest of the criteria align with the NCEP ATP III criteria as mentioned above [2,21].

2.7. Statistical analysis

Demographic data were presented as means with standard deviation for continuous variables and as frequencies with percentages for categorical variables. The demographic characteristics by sex were compared using the Student's t-test and the Chi-square test for continuous and categorical variables, respectively. Body mass index (BMI), waist-hip ratio (WHR) and various metabolic components were compared by APOE genotypes using analysis of covariance (ANCOVA) model and further, Tukey post hoc tests were used to perform multiple comparisons by APOE genotype. The prevalence of APOE genotype by MetS status was compared using the Chi-square test. We examined the association between APOE and MetS utilizing multivariable binary logistic regression. APOE carrier status was the of the independent variable, whereas MetS status (as per the NCEP- ATP III and Consensus criteria) and its individual components were the dependent variables. The models were adjusted for age, years of education, marital status, physical inactivity, tobacco usage, alcohol usage, and history of cardiac illness. While examining the association between each component of MetS and the APOE genotypes, we adjusted for the other MetS components, in addition to the above-mentioned covariates. Statistical analysis was conducted using STATA (version 18.0), considering a p-value of less than 0.05 as significant. All analyses were stratified by sex.

3. Results

Out of the 6274 participants recruited into the CBR-SANSCOG study, 3741 participants met the inclusion criteria for the present study. Compared with people who were excluded, the study participants had higher income (high income: 70.8 % vs 76.0 %, p-value <0.001), higher job skills (high job skill: 66.7 % vs 74.2 %, p-value <0.001) and more educated (mean years of education: 4.1 years vs 4.56 years, p-value <0.001).

Of our study participants, 48.33 % were male and 51.67 % were female. The mean age of males and females were 60.39 (±10.10) years and 57.50 (±9.70) years, respectively. APOE E3 was the most common genotype, with a frequency of 78.48 % among males and 75.69 % among females, whereas APOE E4 genotype was found in 14.16 % of males and 16.14 % of females (Table 1). The prevalence of MetS among males was 36.95 % and that among females was 40.14 % as per the NCEP ATP III criteria, whereas according to the Consensus criteria it was 44.75 % and 48.27 % among males and females, respectively. There were significant sex-specific differences in education, marital status, alcohol usage, diabetes mellitus, hypertension, cardiac illness, high blood pressure, hyperglycemia, hypertriglyceridemia, low HDL, high waist circumference and MetS (by both consensus and NCEP ATP III criteria) (Table 1).

Table 1
Demographic, anthropometric, and plasma biochemical characteristics of the participants of rural cohort.

Characteristics	Males (n = 1808) (%)	Females (n = 1933) (%)	p value
Age, mean (SD)	60.39 ± 10.10	57.50 ± 9.70	**<0.001**
Education			**<0.001**
Illiterate	481 (26.60)	1174 (60.73)	
Primary/Middle school	807 (44.63)	614 (31.76)	
High school/Diploma	376 (20.80)	129 (6.67)	
Graduate/Postgraduate	144 (7.96)	16 (0.83)	
Marital status			**<0.001**
Living without a partner	91 (5.03)	555 (28.71)	
Living with a partner	1717 (94.97)	1378 (71.29)	
Tobacco usage			0.157
No	1196 (66.15)	1236 (63.94)	
Yes	612 (33.85)	697 (36.06)	
Alcohol usage			**<0.001**
No	1542 (85.29)	1908 (98.71)	
Yes	266 (14.71)	25 (1.29)	
Diabetes mellitus			**<0.001**
Yes	446 (24.67)	299 (15.47)	
No	1362 (75.33)	1634 (84.53)	
Hypertension			**<0.001**
Yes	681 (37.67)	562 (29.07)	
No	1127 (62.33)	1371 (70.93)	
Cardiac illness			**<0.001**
Yes	1749 (96.74)	1917 (99.17)	
No	59 (3.26)	16 (0.83)	
High blood pressure			**<0.001**
Yes	852 (47.12)	741 (38.33)	
No	956 (52.88)	1192 (61.67)	
Hyperglycemia			**0.001**
Yes	811 (44.86)	767 (39.68)	
No	997 (55.14)	1166 (60.32)	
Hypertriglyceridemia			**0.008**
Yes	854 (47.23)	830 (42.94)	
No	954 (52.77)	1103 (57.06)	
Low HDL-C			**<0.001**
Yes	1193 (65.98)	1483 (76.72)	
No	615 (34.02)	450 (23.28)	
Body mass index (Asia Pacific criteria)			0.195
Underweight	207 (11.45)	245 (12.67)	
Normal	692 (38.27)	725 (37.51)	
Overweight	363 (20.08)	345 (17.85)	
Obesity	546 (30.20)	618 (31.97)	
High waist circumference (NCEP ATP-III criteria)			**<0.001**
Yes	84 (4.65)	471 (24.37)	
No	1724 (95.35)	1462 (75.63)	
High waist circumference (Consensus criteria)			**<0.001**
Yes	594 (32.85)	961 (49.72)	
No	1214 (67.15)	972 (50.28)	
Mets (NCEP ATP-III criteria)			**0.045**
Yes	668 (36.95)	776 (40.14)	
No	1140 (63.05)	1157 (59.86)	
Mets (Consensus criteria)			**0.031**
Yes	809 (44.75)	933 (51.73)	
No	999 (55.25)	1000 (51.73)	
APOE genotype			0.199
E2/E2	5 (0.28)	8 (0.41)	
E2/E3	128 (7.08)	150 (7.76)	
E3/E3	1419 (78.48)	1463 (75.69)	
E3/E4	245 (13.55)	291 (15.05)	
E4/E4	11 (0.61)	21 (1.09)	
APOE carriers			0.124
E2 carriers	133 (7.36)	158 (8.17)	
E3 carriers	1419 (78.48)	1463 (75.69)	
E4 carriers	256 (14.16)	312 (16.14)	

In males, there were no significant differences in the frequency of various APOE genotypes by MetS status (for both NCEP ATP III and Consensus criteria) as displayed in Table 2. On the other hand, among females with MetS as per the NCEP ATP III definition, the frequency of E4 carriers was higher (18.56 % vs 14.52 %, p-value- 0.032), and that of E2 was lower (7.09 % vs 8.90 %, p-value- 0.032). The APOE carrier

Table 2
Distributions of the ApoE genotypes by MetS status based on the modified NCEP and Consensus criteria.

Mets definition	Males		p value	Females		p value
NCEP ATP-III criteria	Mets (n = 668) (%)	No Mets (n = 1140) (%)		Mets (n = 776) (%)	No Mets (n = 1157) (%)	
APOE genotype			0.327			**0.020**
E2/E2	0 (0.00)	5 (0.44)		4 (0.52)	4 (0.35)	
E2/E3	48 (7.19)	80 (7.02)		51 (6.57)	99 (8.56)	
E3/E3	517 (78.89)	892 (78.25)		577 (74.36)	886 (76.58)	
E3/E4	91 (13.62)	154 (13.51)		130 (16.75)	161 (13.92)	
E4/E4	2 (0.30)	9 (0.79)		14 (1.80)	7 (0.61)	
APOE carriers			0.948			0.032
E2 carriers	48 (7.19)	85 (7.46)		55 (7.09)	103 (8.90)	
E3 carriers	527 (78.89)	892 (78.25)		577 (74.36)	886 (76.58)	
E4 carriers	93 (13.92)	163 (14.30)		144 (18.56)	168 (14.52)	
Consensus criteria	**Mets (n = 809) (%)**	**No Mets (n = 999) (%)**		**Mets (n = 933) (%)**	**No Mets (n = 1000) (%)**	
APOE genotype			0.610			0.116
E2/E2	1 (0.12)	4 (0.40)		5 (0.54)	3 (0.30)	
E2/E3	59 (7.29)	69 (6.91)		77 (8.25)	73 (7.30)	
E3/E3	636 (78.62)	783 (78.38)		688 (73.74)	775 (77.50)	
E3/E4	110 (13.60)	135 (13.51)		148 (15.86)	143 (14.30)	
E4/E4	3 (0.37)	8 (0.80)		15 (1.61)	6 (0.60)	
APOE carriers			0.976			0.156
E2 carriers	60 (7.42)	73 (7.31)		82 (8.79)	76 (7.60)	
E3 carriers	636 (78.62)	783 (78.38)		688 (73.74)	775 (77.50)	
E4 carriers	113 (13.97)	143 (14.31)		163 (17.47)	149 (14.90)	

Note. Mets- Metabolic Syndrome, APOE- Apolipoprotein E.

status frequencies were similar among females with MetS and no MetS based on the Consensus criteria (Table 2).

We observed statistically significant differences in TC, LDL, HDL across different APOE carrier statuses in both sexes; TC and LDL were higher and HDL was lower in E4 and E3 carriers when compared to E2 carriers (Table 3).

The multivariable binary logistic regression analysis revealed a significant association between APOE genotype and MetS, as defined by the NCEP ATP-III criteria, among females, while no such association was observed among males. Females with APOE E4 were observed to have 1.31-fold increased odds of MetS (95 % CI: 1.02,1.69, p-value- 0.035). Furthermore, when MetS was defined by the Consensus criteria, we did not find any significant association between APOE genotype and MetS in either males or females as shown in Table 4.

Examination of the association between APOE E4 carrier status and individual components of MetS yielded mixed results. Females with the E4 genotype exhibited a 1.31-fold increase in the odds of hypertriglyceridemia (95 % CI: 1.01, 1.70, p-value- 0.042) and a 1.41-fold increase for low HDL (95 % CI: 1.02, 1.95, p-value- 0.040). However, there was a 0.72-fold decrease in the odds of having an increased waist circumference (95 % CI: 0.53, 0.99, p-value- 0.041), as per the NCEP ATP-III criteria. Among males, those with APOE E4 had a 0.70-fold decreased odd of elevated blood pressure (95 % CI: 0.52, 0.92, p-value- 0.012) (Table 4).

4. Discussion

Our study uncovered a sex-specific association with the APOE E4 allele, wherein females with APOE E4 had a 31 % higher chance of developing Metabolic Syndrome (MetS, as defined by the NCEP ATP III criteria) but this association was absent in males.

While prior studies have established a link between APOE E4 and MetS [22,23], the results have been varied. Of note, a longitudinal study in Germany found that E4 carriers had higher odds of developing Mets [24]. A small Serbian study which showed that individuals with APOE E4 had a significantly increased odds of having MetS (as per the International Diabetes Federation criteria) than those without [25]. Another study among non-diabetic, cardiovascular patients from Italy showed similar results, with E4 allele having a positive association with MetS [26]. In contrast, a study from Thailand did not find an association between APOE E4 and MetS [23]. Another study among Indians aged 45 years and below with acute myocardial infarction did not show any association [27]. Interestingly, a small study from Brazil among individuals with morbid obesity (mean age of 41 years) demonstrated a negative association between APOE E4 and MetS. Possible reasons for the diversity in these findings could be differences in the age groups in the populations studied, dissimilar inclusion and exclusion criteria and the use of different criteria to diagnose MetS. However, none of these studies looked at the sex specific association of APOE with MetS.

Findings from the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study among participants with a mean age of 56.5 years showed no such association, wherein MetS was defined by the Consensus criteria [28]. Interestingly, our study also found no association when the Consensus criteria was used (the primary difference between the Consensus and NCEP ATP III criteria is in terms of the waist circumference cut-offs, wherein the former has cut-offs tailored for Asian populations), highlighting the differing conclusions that can be derived when using diverse criteria to diagnose the same condition, even when the change is as small as changing one component out of five. This was also reflected in the disparity in the prevalence of MetS – 38.6 % and 46.6 % identified by the NCEP ATP III and Consensus criteria, respectively.

Some studies have been conducted exclusively in the male population with conflicting findings. For example, a small study among young Croatian males revealed no association between any APOE genotype and the prevalence MetS [29], which was in line with our findings, whereas another study among middle-aged American males found an increased risk of MetS among those with APOE E4 [22]. We found no studies in a female only cohort.

Contrary to our results, a study conducted in China, found an association between the APOE E4 allele and MetS in males, but not in females, based on the NCEP ATP III criteria [7]. This discrepancy may stem from the significantly different genetic backgrounds of South Indians compared to the Chinese population. Additionally, the older age profile of the Chinese cohort may have impacted how APOE influences the various aspects of MetS. Interestingly, a recent study in animal models revealed a sex-specific association between APOE E4 and metabolic disturbance only in male mice on high fat diets [30].

In line with earlier investigations exploring the connection between APOE isoforms and lipids parameters [8], our findings confirm that LDL and TC levels are higher in E4 compared to E2 carriers, across both sexes. In India, studies have shown a higher prevalence of dyslipidemia in individuals carrying the E4 allele [31], with specific genotypes like E3/E4 being linked to lower HDL and higher LDL levels in coronary artery disease (CAD) patients [32], and increased TG levels in Type 2 diabetes mellitus (T2DM) patients [33]. Even though there is no major

Table 3
Comparison of clinical risk factor levels by APOE genotypes stratified by sex.

Clinical factors	Males			p value[f]	Females			p value[f]
	APOE2	APOE3	APOE4		APOE2	APOE3	APOE4	
BMI[a]	23.22 ± 4.05	23.33 ± 4.14	22.88 ± 3.62	0.485	22.72 ± 3.99	23.39 ± 4.38	23.15 ± 4.32	0.150
WHR[a]	0.96 ± 0.10	0.96 ± 0.07	0.99 ± 0.60	0.133	0.90 ± 0.09	0.89 ± 0.09	0.89 ± 0.09	0.463
Waist circumference[a]	85.34 ± 12.00	85.33 ± 9.99	87.58 ± 51.80	0.313	79.52 ± 10.43	79.80 ± 10.96	79.28 ± 11.02	0.696
Systolic blood pressure[b]	122.16 ± 18.09[c]	125.28 ± 19.71	121.10 ± 16.46	**0.003**	118.35 ± 18.47	120.40 ± 18.56	121.70 ± 19.16	0.070
Diastolic blood pressure[b]	77.14 ± 10.70	77.52 ± 11.35	75.87 ± 10.64	0.134	74.37 ± 12.02	74.32 ± 10.70	74.65 ± 10.69	0.597
Triglycerides[b]	177.15 ± 113.64	169.62 ± 111.71	182.28 ± 122.22	0.156	165.35 ± 99.63	154.28 ± 87.03[d]	172.88 ± 108.00	< **0.001**
Total cholesterol[b]	162.39 ± 39.32[c]	172.76 ± 38.22	173.82 ± 41.08[e]	**0.009**	177.46 ± 43.52[c]	189.76 ± 39.42	197.57 ± 10.38[e]	< **0.001**
HDL[b]	40.22 ± 10.38[c]	37.83 ± 11.86	36.23 ± 11.32[e]	**0.004**	44.44 ± 9.62[c]	42.82 ± 10.97	41.60 ± 11.66[e]	**0.032**
LDL[b]	93.03 ± 25.16[c]	105.46 ± 31.20	105.86 ± 33.04[e]	< **0.001**	102.08 ± 33.31[c]	119.37 ± 32.75	121.65 ± 36.17[e]	< **0.001**
Fasting glucose[b]	123.18 ± 75.23	115.55 ± 52.59	117.63 ± 59.98	0.278	107.55 ± 39.38	106.13 ± 40.27	109.23 ± 40.38	0.310

Note. BMI- Body Mass Index, WHR- Waist-hip ratio, HDL- High density lipoprotein, LDL- Low density lipoprotein.
(Bold values represent p values < 0.05).
[a] ANCOVA model adjusted for age.
[b] ANCOVA model adjusted for age and body mass index.
[c] p value < 0.05 for Tukey post hoc comparison between APOE2 vs APOE3.
[d] p value < 0 .05 for Tukey post hoc comparison between APOE3 vs APOE4.
[e] p value < 0 .05 for Tukey post hoc comparison between APOE2 vs APOE4.
[f] p value from ANCOVA model.

Table 4
Association between ApoE genotype and individual component of MetS among males and females.

Outcome	Males			Females		
	APOE3	APOE2	APOE4	APOE3	APOE2	APOE4
High blood pressure[b]	1.00 (Reference)	0.77 (0.53,1.12)	0.70 (0.52,0.92)*	1.00 (Reference)	0.94 (0.65,1.35)	1.12 (0.85,1.46)
Hyperglycemia[b]	1.00 (Reference)	1.20 (0.82,1.76)	0.92 (0.69,1.23)	1.00 (Reference)	0.88 (0.62,1.26)	1.18 (0.91,1.54)
Hypertriglyceridemia[b]	1.00 (Reference)	1.15 (0.77,1.70)	1.30 (0.97,1.74)	1.00 (Reference)	1.29 (0.91,1.84)	1.31 (1.01,1.70)*
Low HDL[b]	1.00 (Reference)	0.73 (0.49,1.06)	1.36 (1.00,1.85)	1.00 (Reference)	0.88 (0.59,1.30)	1.41 (1.02,1.95)*
High waist circumference (NCEP ATP III criteria)[b]	1.00 (Reference)	1.34 (0.61,2.95)	0.87 (0.43,1.74)	1.00 (Reference)	0.72 (0.47,1.11)	0.72 (0.53,0.99)*
High waist circumference (Consensus criteria)[b]	1.00 (Reference)	1.15 (0.77,1.74)	0.83 (0.60,1.14)	1.00 (Reference)	1.29 (0.91,1.83)	0.88 (0.68,1.14)
Mets (NCEP ATP III criteria)[a]	1.00 (Reference)	0.95 (0.65,1.38)	0.99 (0.75,1.31)	1.00 (Reference)	0.82 (0.58,1.16)	1.31 (1.02,1.69)*
Mets (Consensus criteria)[a]	1.00 (Reference)	1.01 (0.70,1.45)	1.01 (0.77,1.32)	1.00 (Reference)	1.23 (0.88,1.72)	1.22 (0.95,1.56)

Note. HDL- High density lipoprotein, NCEP ATP III- National Cholesterol Education Program – Adult Treatment Panel III, MetS- Metabolic syndrome.
[a]p value < 0.05.
[a] Logistic regression model adjusted for age, education, marital status, physical activity, tobacco usage, alcohol usage, and cardiac illness.
[b] Each component of MetS was adjusted for other components along with gender, age, marital status, physical activity, tobacco usage, alcohol usage, and cardiac illness.

difference between E3 and E4 protein binding to LDL receptors, there is a preferential association of E4 with TG-rich lipoprotein particles. This could lead to increased competition at the LDL receptor, resulting in decreased uptake of LDL and increased cholesterol in the bloodstream [34].

We observed that females with APOE E4 but not males had an increased odds for hypertriglyceridemia and low HDL. A previous study revealed that female with APOE E4 had higher levels of TG and lower levels of HDL [35]. A potential explanation for these divergent findings could lie in the interplay between APOE and estrogen. Estrogen is known to up-regulate APOE gene expression, increasing APOE mRNA via the estrogen receptor alpha-mediated pathway. This suggests that estrogen modulates APOE expression through both transcriptional and post-transcriptional mechanisms [36,37].

There was no association seen between hyperglycemia and APOE status in our study though APOE E4 has been shown to increase insulin resistance [22]. However, a very recent large study showed that there was no association between APOE and hyperglycemia or the prevalence of type 2 diabetes [38]. For waist circumference (WC), we observed decreased odds for elevated WC according to the NCEP ATP III criteria in females who were E4 carriers, although the confidence interval was large. Sex-specific associations have been observed between APOE and WC in an older US population, wherein female E4 carriers with a family history of diabetes had higher WC than their male counterparts [39], which was contrary to our finding.

Considering blood pressure, we found that male subjects with the E4 variant of APOE exhibited lower odds for elevated blood pressure than those with E3. Prior research on the relationship between blood pressure and APOE status has produced mixed outcomes [40,41]. The interaction between blood pressure and the APOE genotype is multifaceted, influencing lipid levels and other metabolic factors like uric acid, thereby impacting atherosclerosis. Furthermore, the age group of the study participants could also affect how various APOE genotypes influence blood pressure [42], thereby, contributing to the diverse findings in this area.

These sex-specific associations may have significant implications for public health. Since, males and females have different susceptibility to developing MetS, it could help influence health policy decisions in terms of targeted surveillance and prevention measures, which is especially valuable in limited resource settings, such as rural India. Sex-specific influences of APOE are also evident in other diseases like Alzheimer's disease [43], coronary artery disease [44]and osteoporosis [45], where the impact is more pronounced in female E4 carriers, which could point us towards the requirement for multimorbidity screening in this vulnerable group.

While the large sample size and robust assessments are strengths of our study, its cross-sectional design limits the ability to infer causality. Further, since our sample included rural-dwelling individuals aged 45 years and above from southern India, our findings may not be the generalizable across the entire Indian subcontinent. Another limitation

could be unaccounted lifestyle and environmental confounding factors that could potentially interact with genetic predispositions to influence MetS.

This study underscores the complexity of etiology of MetS and the need for further research to understand the interplay of genetic, environmental, and lifestyle factors in its development and progression. It also highlights the importance of considering sex-specific differences and the choice of diagnostic criteria in MetS research.

5. Conclusion

Our study reveals a significant sex-specific association between APOE E4 and MetS among aging adults in a rural Indian population, with a significant susceptibility observed only in females. Our findings emphasize the necessity of considering sex differences in MetS research and management, suggesting the potential for personalized healthcare strategies based on genetic risk factors. By focusing on a rural Indian cohort, the study not only contributes to the global understanding of MetS but also sheds light on the need for targeted prevention strategies that consider genetic predispositions and sex-specific risks in resource-limited settings. Further research is encouraged to deepen the understanding of the complex interactions between genetics, lifestyle, and environmental factors in the development of MetS, particularly in underserved populations.

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CRediT authorship contribution statement

Shilna Azhuvalappil: Writing – original draft, Conceptualization. **Raghav Prasad:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Pravin Sahadevan:** Software, Methodology, Formal analysis. **Hitesh Pradhan:** Data curation. **Pooja Rai:** Writing – review & editing. **Jonas S. Sundarakumar:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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